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Review Article

The structure of the nucleus in normal and neoplastic prostate cells: untangling the role of type 2 DNA topoisomerases

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Abstract: Donald S. Coffey, a pioneer in the study of the structural basis of mammalian genome organization, was fascinated by DNA topoisomerases, chemo-mechanical enzymes that could catalyze changes in DNA structure. Work initiated in his laboratory and carried on with his influence and inspiration has led to the elucidation of specific roles for each of the two type 2 topoisomerases in DNA replication, RNA transcription, and androgen action in prostate cells. TOP2A principally acts in DNA synthesis elongation to prevent tangling of daughter DNA molecules during genome replication and mitotic segregation; TOP2B is required for androgen-stimulation of target gene transcription. DNA double-strand breaks inflicted by TOP2B upon androgen exposure appear responsible for the generation of *TMPRSS2-ERG* and other gene fusions, often found in complex chained rearrangements termed chromoplexy, in prostate cancer cells. TOP2B-mediated genome damage may also provide an avenue for improving prostate cancer treatment via timed androgen administration in conjunction with ionizing radiation, with TOP2-targeted drugs, or with DNA repair inhibitors.

Keywords: DNA topoisomerases, prostate cancer, normal prostate, structure of the nucleus

Introduction

In tribute to the life and scientific career of Donald S. Coffey, Ph.D., and with special thanks for his considerable talents as a mentor, this brief review will highlight the durable impact he has had on each of us as we explored the contributions of DNA topoisomerases to androgen action and to prostate cancer.

Ronald Berezney and Donald S. Coffey published their landmark paper describing the isolation of a “nuclear protein matrix” from mammalian cell nuclei in 1974 [1]. Despite containing just 0.03% of nuclear DNA, the resultant structure retained the size and shape of the cell nucleus, with readily visible nuclear pore complexes, a nuclear lamina, and a residual nucleolus. Subsequently, a similar “protein scaffold” was identified in metaphase chromosomes [2, 3]. Both the nuclear matrix and chromosome scaffold appeared to organize genomic DNA into topologically-constrained loop-domains of 50-200 kB [3-8]. These sub-structures responsible for genome organization were felt to critically affect cell and genome function, as misshapen cancer cell nuclei were found to contain equivalently abnormal nuclear matrix structures [9].

For the nuclear matrix, the revelation that the structure housed discrete sites for both replication and transcription has propelled ongoing research for more than four decades. Newly replicated DNA was first reported to be associated with the nuclear matrix by Berezney and Coffey in 1975 [10], a finding subsequently confirmed by a number of studies [11-14]. Isolated nuclear matrix structures were even able to continue synthesizing genomic DNA from *bona fide* replication forks when provided nucleic acid precursors *in vitro* [15-17]. Actively transcribing genes were also discovered to be associated with nuclear matrix [18-20]. Subsequently, residual nuclear structures were found to contain transcription factories capable

of continuing hnRNA transcript synthesis [21, 22]. The nuclear matrix was further ascertained to be the site of transcriptional regulation by ligand-dependent *trans*-acting factors like the androgen and estrogen receptors [23, 24].

From his unique scientific perspective, Coffey was instinctively fascinated not only with the structural underpinnings of biologic processes but also with the chemo-mechanical properties of the structures themselves. Not surprisingly, the discovery and characterization of DNA topoisomerases, enzymes which catalyzed changes in DNA structure but not in nucleotide sequence or chemistry, readily attracted his attention [25]. Type 2 DNA topoisomerases were recognized to be significant components of both the nuclear matrix and the chromosome scaffold, and to interact with genomic DNA at or near loop-domain attachment sites [26-31]. These enzymes, capable of creating transient double-strand breaks in the backbone of a substrate DNA molecule to allow passage of another DNA double helix via ATP hydrolysis, were assumed to generally manage higher order DNA organization as part of the nucleus and chromosome substructures. However, subsequent (and ongoing) studies, aided and/or inspired by Coffey, have revealed exciting new functions of type 2 topoisomerases in both DNA replication and regulated RNA transcription, unmasking key roles for the enzymes in the pathogenesis of prostate cancer.

TOP2A and DNA replication

Mammalian cells contain two type 2 topoisomerases, TOP2A and TOP2B, encoded by distinct genes. Each enzyme acts as a homodimer to catalyze double-strand DNA passage thought transient double-strand DNA breaks [32]. TOP2A is stereotypically expressed by proliferating cells, while TOP2B expression shows no such restriction [33-35]. The presence of TOP2A in the nuclear matrix of proliferating cells, and the location of the fixed sites for DNA replication, hinted strongly that the enzyme might play some sort of key role in replicative DNA synthesis. The topological demands of DNA replication are considerable. Using template base-pairing for the duplication of parental genome sequences, the creation of two full copies of 23 separate DNA sequences at a total length of some 3,088,286,401 bases would require fully unraveling a least 295,528,799 helical turns (10.45 bp/helical turn) in parental duplex DNAs via helicase action. When the template DNA is additionally wrapped more than 29,167,149 times (1.7 turns/histone octamer) around more than 17,157,147 nucleosomes (180 bp/nucleosome), the topological complexity becomes even greater.

With the type 1 DNA topoisomerase TOP1, thought to act in concert with helicases in front of the replicating fork to reduce 'over-winding' associated with parental strand separation, TOP2A has been provisionally assigned a number of different possible functions in DNA replication. These include the further reduction of 'over-winding' in front of replication forks, to resolution of 'pre-catenanes' behind the replication forks, to prevention of replicated daughter DNA molecule tangling at the terminus of replication, to chromatid condensation in preparation for mitosis [36]. TOP2A action at the site of DNA replication, *i.e.*, at or near the replication fork itself, would be necessary for enzyme function during the elongation phase of DNA synthesis.

To test whether what is now known as TOP2A is located at replication forks, the proximity of the enzyme to newly-replicated DNA was mapped in mammalian cells [37]. To do so, the propensity for the anti-cancer drug teniposide to trap TOP2 in covalent linkage with each 5'-phosphate of a cleaved double-strand DNA substrate was exploited. Upon teniposide treatment, TOP2-linked DNA could be isolated from the remainder of genomic DNA via K^+SDS precipitation. When radiolabeled thymidine was administered for just 90 seconds, the incorporated radiolabel was selectively recovered among DNA covalently linked to TOP2. At longer labeling times, or after a prolonged subsequent exposure to excess unlabeled thymidine, the selective recovery of incorporated label linked to TOP2 disappeared. These data indicated close proximity of TOP2 to replication forks; further analyses revealed direct covalent attachment of TOP2 to the ligated segment of the lagging strand behind the fork.

The identification of TOP2A action immediately behind the replication fork strongly implicated the enzyme in DNA synthesis elongation. Several more recent studies have buttressed this model, arguing even more strongly that

some fraction of parental helical turns pass through the replication forks as 'pre-catenanes' that can only be resolved by type 2 topoisomerases [36, 38]. Another mechanistic possibility is that TOP2A might facilitate nucleosome assembly/re-assembly on daughter DNA molecules [39].

The contributions of TOP2A to mitotic chromosome condensation and segregation have also been progressively better understood [40]. The enzyme, a critical component of the chromosome scaffold, acts to compact chromosomes by shortening chromatid lengths as condensins and KIF4 reduce the radii of the arms [41]. This chromosome structure role appears to be independent of any decatenation activity needed to ensure mitotic segregation [42]. One speculation is that TOP2A might prevent daughter DNA tangling during DNA synthesis elongation by resolving precatenanes behind the replication fork rather than by decatenating compacted chromosome arms [37]. Remarkably, a recent Hi-C analysis of genomic DNA organization in the compacted chromosome created for mitosis essentially fully validated a model for chromosome structure originally proposed more than three decades earlier by Pienta and Coffey [43, 44].

TOP2B and transcription

Like DNA replication, RNA transcription creates a number of DNA topology challenges [45]. Looping of DNA at gene transcription promoters and/or movement of genes across cell nuclei to transcription hubs or factories risks significant double-strand tangling problems that could be resolved only by type 2 topoisomerases. Transcription elongation tends to produce over-winding in front of the RNA polymerase complex and under-winding behind, amenable to mitigation by type 1 and type 2 topoisomerases. Finally, RNA-DNA hybrid sequences produced during transcription could be prone to R-loop structure formation [46].

Work on TOP2 expression in normal prostate tissues in the Coffey laboratory in the 1980's disclosed the likely existence of two distinct type 2 topoisomerase enzymes, one selectively present in proliferating cells, and another that could be detected in differentiated prostate epithelial cells [33, 47]. Eventually, the gene for TOP2B was cloned [48]. Until recently, specific mammalian cell requirements for TOP2B (versus TOP2A) were difficult to discriminate. To ascertain how TOP2B might contribute to differentiated prostate cell phenotypes, a potential role for the enzyme in executing androgen-regulated gene expression was evaluated [49]. In prostate cancer cells that had been starved of androgens, dihydrotestosterone stimulation resulted in androgen receptor (AR) recruitment of TOP2B to specific binding elements (AREs) in regulatory regions of androgen target genes, such as *KLK3* and *TMPRSS2*. Surprisingly, TOP2B enzyme activity was required for efficient activation of the entirety of the AR transcription program. At the AR target gene *TMPRSS2*, TOP2B was needed for assembling a looped transcription promoter/enhancer conformation. In these studies, TOP2B was found to be essential for the initiation step of regulated gene expression. Additional studies in other systems have also hinted at TOP2B facilitation of transcription elongation, particularly for long genes [50].

TOP2B and the generation of androgen-regulated fusion genes in prostate cancer

Fusions between androgen-regulated genes, like *TMPRSS2*, and putative oncogenes, like *ERG*, have been detected in the majority of human prostate cancers [51]. As such, the recruitment of TOP2B by AR to AREs in prostate cells triggered by androgen exposure prompted an astonishing hypothesis: could TOP2B cleavage of double-strand DNA near ARE sites lead to fusion translocations involving and rogen-regulated genes? Prolonged trapping of TOP2 in its 'cleavable complex' conformation promotes proteasome-mediated degradation of enzyme protein, removal of the covalently-linked enzyme tyrosine from DNA ends by TDP2, and DNA repair via non-homologous end-joining (NHEJ), a pathway well-understood to be activated by anti-cancer TOP2 'poisons' like etoposide, doxorubicin, and mitoxantrone [52-54]. Using a variety of experimental techniques, including an adaptation of the K^+SDS precipitation assay, TOP2B cleavable complexes could be readily trapped near ARE sites in *TMPRSS2* in prostate cells *in vitro* that were concordant with translocation sites mapped in prostate cancer cases *in vivo* [49, 55].

Even more compellingly, gene fusions could be created via androgen stimulation of prostate

cancer cells *in vitro*, a phenomenon that required TOP2B catalytic activity [49]. The generality of this mechanism for androgen-induced translocation was furthered demonstrated by analyzing integration of exogenous DNAs which contained androgen induced TOP2B catalytic sites along with selectable markers [49]. In cells expressing AR, stable integration of the exogenous DNA plasmids containing androgen-induced TOP2B catalytic sites was more efficient than that of control sequences, unless TOP2B expression was disrupted. Since the initial report of TOP2B-mediated cleavage and TMPRSS2-ERG gene fusions, new data have emerged underscoring an even broader generality for the TOP2B-mediated genome breakage as a source of somatic cancer genome alterations [56], including in the generation of complex genome rearrangements referred to as chromoplexy [55, 61].

Androgen-stimulated TOP2B activity and prostate cancer treatment

Anti-cancer drug trapping of TOP2 cleavable complexes has become a mainstay treatment tactic for many different human cancers, often used in combination with other chemotherapy drugs or with radiation therapy [57]. To ascertain whether prostate cancer cells could be selectively sensitized to killing by TOP2 poisons, ionizing radiation, or other anti-cancer drugs, prostate cancer cells were assessed for the generation of DNA damage in response to androgen stimulation using comet and H2A.X focus formation assays [58]. The results indicated that AR promoted recruitment of TOP2B to the genome in such a way as to trigger widespread DNA double-strand breaks. In addition, when the administration of ionizing radiation was timed to the peak appearance of androgen-stimulated TOP2B-mediated DNA breaks, a synergistic effect on prostate cancer cell killing was evident *in vitro* and *in vivo*. These findings have profound and direct implication for prostate cancer treatment. Rather than delivering radiation fractions only when castrate levels of androgens are achieved via luteinizing hormone-releasing hormone (LHRH) analog administration, fractions of radiation can be administered shortly after a pulse of androgen stimulation in the context of androgen deprivation therapy so that the androgen-induced, TOP2B-mediated DNA damage can sensitize to each fraction of ionizing radiation. This would augment the therapeutic index of radiation therapy by selectively radiosensitizing AR-positive prostate cancer cells but not the surrounding normal pelvic tissues [58]. Similar strategies for systemic prostate cancer treatment, featuring timed androgen dosing along with TOP2 poisons or with inhibitors of DNA double-strand break repair, are also under development [35]. The somewhat unexpected benefits of high-dose androgen treatment in the setting of androgen deprivation therapy (bipolar androgen therapy, BAT) for men with castration-resistant prostate cancer (CRPC) are consistent with a mechanism by which prostate cancer cells are killed via androgen-stimulated genome damage mediated by TOP2B [59, 60].

Conclusions and reflections

As both a biological scientist and an engineer, Donald S. Coffey provided a unique perspective on human genome function in normal and neoplastic human cells, considering with wonder how 2.2 meters of DNA could be packed inside a cell nucleus with a diameter of 6 microns, replicated within half a day, and differentially used to create the hundreds of different types of cells in the human body. Throughout his career-long involvement in the Brady Urological Institute, as its Research Director, and the Sidney Kimmel Comprehensive Cancer Center, as its Deputy Director, he channeled his wonderment into better understanding how prostate cancer and other cancers arise and how they might be treated. Nonetheless, his most durable contributions over half a century of work are not limited to the transformative scientific observations he published. He possessed a unique ability to infect all who interacted with him with wonder, nurturing the creative forces of generations of researchers.

With this in mind, in reviewing our evolving understanding of type 2 DNA topoisomerase functions in DNA replication, RNA transcription, steroid hormone action, cancer-associated DNA translocations, and cancer treatment, each of us is humbled and amazed by the prescience of Coffey's early insights, inspired by his memory in our own pursuit of deeper understanding, and grateful we were able to enjoy his influence.

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Disclosure of conflict of interest

None.

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