Molecular Pathways

Transcription-induced DNA double strand breaks: both oncogenic force and potential therapeutic target?

Michael C. Haffner, Angelo M. De Marzo, Alan K. Meeker, William G. Nelson*, Srinivasan Yegnasubramanian*

Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine, Baltimore, MD

*, Corresponding Authors: W.G.N., bnelson@jhmi.edu; S.Y., syegnasu@jhmi.edu

Running title: Targeting transcription-induced DSB for cancer therapy
Abstract

An emerging model of transcriptional activation suggests that induction of transcriptional programs, for instance by stimulating prostate or breast cells with androgens or estrogens, respectively, involves the formation of DNA damage, including double strand breaks (DSB), recruitment of DSB repair proteins, and movement of newly activated genes to transcription hubs. The DSB can be mediated by the class II topoisomerase TOP2B, which is recruited with the androgen receptor (AR) and estrogen receptor (ER) to regulatory sites on target genes and is apparently required for efficient transcriptional activation of these genes. These DSB are recognized by the DNA repair machinery triggering the recruitment of repair proteins such as PARP1, ATM and DNA-PK. If illegitimately repaired, such DSB can seed the formation of genomic rearrangements like the TMPRSS2-ERG fusion oncogene in prostate cancer. Here we hypothesize that these transcription induced TOP2B mediated DSB can also be exploited therapeutically and propose that, in hormone dependent tumors like breast and prostate cancers, a hormone cycling therapy, in combination with topoisomerase II poisons or inhibitors of the DNA repair components PARP1 and DNA-PK, could overwhelm cancer cells with transcription-associated double strand breaks. Such strategies may find particular utility in cancers, like prostate cancer, that show low proliferation rates, where other chemotherapeutic strategies that target rapidly proliferating cells have had limited success.
Background

The prevailing reductionist view of transcription has been challenged by mounting recent evidence suggesting that induction of transcription involves profound and dynamic changes in genome organization and structure. In this emerging view, hundreds of transcription factors and cofactors are present at locally high concentrations in “transcription factories” in the nucleus (1). Induction of widespread transcriptional changes, for example during hormonal-stimulation of hormone-responsive cells, can result in large-scale alterations in genome organization arising from movement of different target gene loci and distal regulatory elements to these transcriptional hubs, establishing cell type specific expression patterns (1-4).

In addition, recent reports have demonstrated that efficient induction of transcriptional programs by a wide array of nuclear hormone receptors and other transcription factors involves the recruitment of several factors involved in DNA damage and repair (5-12). For instance, unbiased proteomics approaches as well as candidate protein analyses have demonstrated that proteins such as Ku70, Ku80, PARP1, DNA-PK and TOP2B can be associated with transcription factors including AR and USF-1 and can bind to regulatory elements of target genes upon activation of transcription (9, 12). Inhibition of the catalytic function of several of these proteins results in an impaired induction of transcriptional programs suggesting a crucial role for DNA repair in transcriptional regulation. Among these, the class II topoisomerase, TOP2B, is especially interesting since it has been shown by numerous recent reports to be associated with multiple nuclear hormone receptors and transcription factors and has also been shown to be bound to promoters and enhancers of newly transcribed genes (5, 6, 11, 12). Interestingly, TOP2B catalytic activity was required for efficient transcriptional initiation and may be involved in the establishment of complex chromosomal conformational changes during activation of transcription (5, 6, 12, 13).

The two human class II topoisomerases, TOP2A and TOP2B, can resolve DNA topological constraints by passing one DNA segment through a transient DNA double strand break in another segment in an ATP dependent fashion (14). In addition to being able to resolve superhelical tension introduced by under- or over-winding of the DNA double helix, the class II topoisomerases are uniquely able to resolve higher order topological constraints such as tangles and knots. TOP2A and TOP2B are encoded by separate genes and although they show a high degree of sequence homology, they
differ significantly in their expression patterns and cellular functions (14, 15). Whereas TOP2A is almost exclusively expressed in proliferating cells, where it is required for resolution of topological constraints arising during replication and chromosomal segregation, TOP2B can be expressed throughout the cell cycle in dividing and non-dividing cells (14, 16, 17). Given the recent findings of its association with transcription factors and its catalytic activity at gene regulatory sites, we can speculate that TOP2B may be involved in resolving topological constraints arising from movement of chromosomal segments during transcriptional induction. Such a role for TOP2B in transcriptional initiation would be analogous to the requirement of TOP2A in resolving topological constraints associated with DNA replication and chromosomal segregation.

Using an elegant assay that allows site specific labeling of DNA double strand breaks (DSB), Ju et al. showed that during transcriptional activation by the estrogen receptor (ER), transient DSB are generated at regulatory elements of ER regulated genes (6). This finding was further independently corroborated for other signaling pathways including the androgen receptor (AR) (Figure 1) (5, 6, 12). Induction of these transient DSBs tracked with the recruitment of TOP2B to these sites and targeted depletion of TOP2B dramatically reduced DSB generation, suggesting that these transcription associated DSBs were mediated by TOP2B. Interestingly, these double strand breaks were recognized by the DSB repair machinery. The DSB recognition proteins Ku70 and Ku80 for instance have been shown to bind together with nuclear hormone receptors at target sites (6, 8-10, 12). Furthermore, the DSB repair associated protein PARP1 and the kinases DNA-PKcs and ATM were also found to be associated with newly transcribed genes in different model systems (Figure 1) (5, 6, 9, 12). It is likely that other DNA repair proteins are also associated with transcriptional complexes. Future efforts to comprehensively characterize the repair proteins and pathways involved in transcriptional initiation are critically needed.

According to the conventional view, the transient double strand break induced by TOP2 enzymes during their catalytic cycle are extremely fleeting, and are not easily isolatable unless the TOP2-DNA cleavage complex is stabilized by TOP2 poisons such as etoposide (18, 19). The TOP2 transient double strand break is thought to be masked, evading recognition, recruitment, and activity of the double strand break repair machinery. However, in these recent reports, the TOP2B-mediated DSBs associated with transcriptional induction were persistent on the order of one to several hours, and were apparently recognized by the DSB repair machinery (5, 6, 20). The exact
biochemical mechanisms by which transient DSBs that are induced during the catalytic cycle of TOP2B can be converted into frank and persistent breaks is not well understood. However, several potential hypotheses can be generated based on accumulating evidence. Reactive oxygen species (ROS), such as those generated by intrinsic nuclear hormone signaling (11, 21) or other transcription factor signaling, or by inflammatory cells often seen associated with cancer lesions in vivo (22), can covalently trap TOP2 enzymes on DNA, stabilizing the TOP2-DNA cleavable complex (23). Furthermore, several dietary constituents, especially bioflavonoids, have been shown to mimic TOP2 poisons in stabilizing the TOP2-cleavage complex (14, 24). Collision of these DNA-TOP2-complexes with the replication fork or with transcription complexes (25, 26) followed by proteasomal degradation of TOP2B (27) can result in the conversion of the covalent TOP2B-DNA cleavage complex into frank double strand breaks (Figure 1).

More generally, it is becoming evident that efficient induction of transcriptional programs may involve, and perhaps require, generation of many forms of DNA damage, including DNA cytosine deamination (28, 29), base damage by local reactive oxygen species produced by hormone stimulation (11), LINE1-ORF2 endonuclease activity (7), and TOP2B mediated DSB (5, 6), at transcriptional regulatory sites. These DNA damage events apparently recruit the appropriate classes of DNA repair proteins, including those involved in base-excision repair (11, 28, 29), and non-homologous end-joining (6, 12) (7). The precise role of DNA damage and DNA repair proteins in transcriptional regulation is largely unknown.

Regardless of their role in transcriptional regulation, it is clear that the generation of DNA damage, particularly the generation of double strand breaks, during induction of transcriptional programs, may represent a critical vulnerability for cells. DNA double strand breaks play a major role in carcinogenesis: on one hand, unresolved DSBs can lead to cell cycle arrest, senescence and apoptosis, and on the other hand, if illegitimately repaired, such transcription-induced DSBs can seed the formation of genomic rearrangements, amplifications, and deletions (Figure 1) (30-32).

Structural genomic alterations can be found in the majority of tumors and certain genomic rearrangements are pathognomonic for specific malignancies (32-34). For instance, in the case of prostate cancer, androgen regulated genes are frequently fused to oncogenic transcription factors of the ETS family. The most prominent of these prostate cancer-specific recurrent rearrangements involves the androgen regulated gene
TMPRSS2 and the ETS family member ERG, occurring in >50% of prostate cancer cases (35, 36). These rearrangements bring ETS transcription factors under the transcriptional control of the androgen receptor (AR), resulting in an androgen-driven and prostate cancer-specific over-expression of an ETS fusion oncogene. The non-random and often lineage-specific nature of recurrent rearrangements suggests a controlled underlying mechanism involved in their formation (5, 7, 37).

For such a rearrangement to occur, the DNA loci involved would likely need to: i) be subject to double strand break formation, ii) at least transiently be in close spatial proximity, and (iii) become subject to illegitimate repair abnormally joining the two gene loci together. As described above, the induction of transcriptional programs could facilitate each of these steps, and therefore stimulation of transcriptional processes could be involved in generating these structural rearrangements (5, 7, 38). For instance, stimulation of androgen receptor signaling in prostate cells by dihydrotestosterone (DHT) can cause the TMPRSS2 and ERG gene loci to come in close proximity (7, 37), perhaps as a part of larger-scale genome reorganization to facilitate movement of induced genes (and possibly by-standers such as ERG) to transcriptional hubs (1, 2, 4). Concurrently, induction of androgen signaling induces DSB at precise TMPRSS2-ERG rearrangement junction sites. These DSB at the TMPRSS2 and ERG genes can be mediated by TOP2B as a consequence of DHT-induced androgen receptor signaling in prostate cells or by other nuclease activities that can be induced by severe exogenous genotoxic insults applied during DHT-stimulation of prostate cells (5, 7). Broken DNA ends can become illegitimately repaired by double strand break repair machinery, including the error-prone non-homologous end-joining pathway, to create de novo TMPRSS2-ERG gene fusions (5, 7). Therefore, at least in the case of recurrent rearrangements in prostate cancer, dysregulation of events that would normally occur during induction of transcriptional programs might predispose prostate cells to developing prostate cancer-specific recurrent gene fusions involving androgen receptor target genes. Whether dysregulation of transcriptional mechanisms may be involved in creating lineage-specific recurrent rearrangements in other cancers is still unknown. This hypothesis is certainly worth investigating further given the recent findings showing a general involvement of DNA damage and repair in lineage-specific transcriptional programs induced by ER, RAR, AP1, and insulin (6, 11, 12).

The development of these transcription-associated DSB may be exacerbated in the setting of actively replicating neoplastic cells compared to terminally differentiating...
normal cells. To illustrate this potentially generalizable principle, we can review the recent evidence in prostate neoplasia. It is interesting that the prostate cancer TMPRSS2-ERG gene rearrangements seem to appear first, often subclonally, in luminal cells of premalignant lesions called prostatic intraepithelial neoplasia (PIN), and are apparently selected for during subsequent progression to invasive carcinoma (5, 39). Given that they are almost never seen in normal prostate cells (5, 39), we can raise the question of whether there are any special factors that create a predilection for PIN cells and not normal prostate cells to initially develop these rearrangements. PIN luminal cells may harbor somewhat dysregulated androgen signaling, which is known to promote both caricaturized differentiation pathways as well as proliferation/survival pathways in neoplastic cells (40, 41). The proliferating cells in PIN lesions likely need to re-establish androgen-receptor transcriptional programs, involving DNA damage, chromosomal reorganization, and DNA repair, after every cell division, increasing the chances that these cells generate gene rearrangements that are then subject to selection. In contrast, androgen signaling in normal cells induces terminal differentiation with suppression of proliferation, so that de novo transcriptional programs only need to be established once, thus limiting the chance of creating transcription-related genome damage. In this regard, in human prostate tissues, AR and TOP2B, which were rarely both present in normal prostate cells at high levels, often showed robust co-expression in PIN luminal cells (5), possibly because of an ongoing requirement for TOP2B in re-establishing androgen-receptor transcriptional programs after cell division in PIN, but not in normal cells. This co-expression of AR and TOP2B in the PIN cells may predispose these cells to formation of transcription-related TOP2B-mediated double strand breaks and formation of rearrangements. Of course, because they can lose checkpoint pathways that would normally induce senescence or cell death in response to persistent genome insults, neoplastic cells are also more prone than are normal cells to surviving and proliferating despite persistent genome damage.

Since such processes are likely ongoing in cancer cells, the mechanisms described here may be more generally involved in generating an ongoing TOP2B or transcription-induced genomic instability (TIN). This transcription-associated TIN model would be somewhat distinct and complementary to other forms of genomic instability that are typically thought to be connected to errors in DNA replication (31, 42, 43). Indeed, a combination of both TIN and replication-related genome damage may create a “perfect
storm of genomic instability leading to the high rate of genomic deletions, amplifications, and rearrangements observed in many cancers.

Clinical Translational Advances

Generation of DNA strand breaks is one of the major mechanisms of action for cancer chemotherapeutic drugs. Most of the existing DSB inducing chemotherapeutics in clinical use require cell division and therefore only efficiently target highly proliferative cells (44). Furthermore, breaks are not only induced specifically in tumor cells of the target tissue but also nonspecifically at other organ systems, resulting in many treatment-associated side effects.

The above described finding that DSBs can be generated during transcription opens several new avenues for developing more targeted therapeutic approaches. Therapeutic strategies exploiting this general concept will be illustrated using breast and prostate cancer as primary examples. Breast and prostate carcinoma are both typically hormone-dependent neoplasias and for the most part respond well to initial hormone ablative therapies (45). Unfortunately, a majority of tumors eventually escape hormone ablation, resulting in clinical relapse of the disease. It is, however, interesting that the resistant cancer cells can still express the androgen and/or estrogen receptor, suggesting that these cells still depend on AR and ER signaling (45). The hormone dependence of most primary tumors can be recapitulated in many cell line models of breast and prostate cancer, which require the presence of estrogen or androgen for proliferation. Long-term culture of these cell lines under hormone depleted conditions results in the development of clones that are resistant to hormone deprivation that can grow in the absence of estrogen or androgens.

Paradoxically, such preclinical models of castrate-resistant prostate cancer cells respond to low dose androgen treatment with significant growth inhibition (46). The clinical relevance of this finding is supported by anecdotal case reports in the literature indicating that substantial and prolonged responses can be achieved by testosterone replacement therapy in patients with castrate resistant disease (47-49). Similarly, hormone deprived breast cancer cells can respond to estrogen with growth cessation and apoptosis and high dose diethylstilbestrol can delay disease progression in patients with metastatic breast cancer (50, 51).
While the precise mechanisms behind this paradoxical anti-tumor response of hormone-ablated breast and prostate cancers to hormone replacement therapy are unclear, we can formulate some hypotheses based upon the recent findings regarding AR and ER induced transcriptional programs. Long-term hormone withdrawal from hormone ablative therapy of prostate and breast cancers may reset transcriptional programs that are mediated by nuclear hormone receptors. Upon hormone stimulation, these programs may become reinitiated, a process that could result in the induction of TOP2B-mediated DSBs (5, 6) as well as DNA damage from other pathways (7, 12). To exploit this, we would propose a cyclic administration of either androgens or estrogens in patients that have undergone hormone ablative therapies for prostate and breast cancer respectively. In such a hormone cycling protocol for breast cancer for instance, patients undergoing aromatase inhibition therapy would be treated with short intermittent doses of estrogen. In the case of prostate cancer, pharmacological castration using a gonadotropin-releasing hormone agonist or a CYP17 antagonist could be combined with cyclic administration of androgens. It is worth noting that a recent clinical trial of rapid androgen cycling in men with prostate cancer established the feasibility of such an approach (52). We hypothesize that during such a “hormone cycling” regime, DSBs would be specifically generated in the prostate and breast or other androgen and estrogen target tissues respectively.

Repair of these transcription-induced DNA lesions appears to at least partly depend on the activity of well characterized repair enzymes like PARP1 and DNA-PK (5, 6, 12). This opens up exciting therapeutic opportunities in which these repair proteins can be targeted by inhibitors that are already in clinical development in order to overwhelm the cell with DSB that are specifically induced by reactivating nuclear hormone signaling.

Poly(ADP-ribose) polymerase 1 (PARP1) participates in DNA damage repair and has been shown to be specifically recruited to sites of active transcription (Figure 1) (6, 9, 12). Genetic as well as pharmacological experiments crippling PARP1 function have provided strong evidence for the important role of PARP1 in the repair of DSBs (53). PARP inhibitors are currently being tested in clinical trials in combination with a variety of genotoxic chemotherapeutics but also show great promise as single agents in tumors lacking BRCA1 and BRCA2 (53-55). Novel preclinical findings showing that PTEN-deficient cells are especially sensitive to PARP1 inhibition raised further interest in other synthetic lethal combination therapies (56). A variety of highly potent PARP inhibitors...
are currently under clinical investigation and show favorable side effect profiles (53). A combination therapy of “hormone cycling” with PARP1 inhibition could be advantageous in patients that failed conventional hormone ablative therapies and could be of particular relevance in tumors that show genetic alterations that induce synthetic lethality in combination with PARP inhibition.

DNA-dependent protein kinase (DNA-PK) plays a critical role in the cellular response to DSBs because it associates with them and initiates repair (57). Inhibition of DNA-PKcs has been shown to sensitize cancer cells to chemotherapy- and radiation-induced double strand breaks (57, 58). While clinical use of most of the existing DNA-PK inhibitors (59) have been limited by general safety and specificity concerns (57), new compounds with more favorable risk profiles could be used in future trials testing such inhibitors in the context of hormone cycling.

A pharmacologically unexplored, but potentially interesting target is tyrosyl-DNA phosphodiesterase, TDP2/TTRAP. TDP2 cleaves the 5’ phosphotyrosyl-DNA topoisomerase bond that is generated during the TOP2 cleavage process and thereby restores 5’-phosphate termini at TOP2 cleavage sites, allowing subsequent repair of TOP2-mediated DSBs (60). Loss of TDP2 renders cells highly sensitive to the cytotoxic effects of TOP2 poisons. Therefore, inhibition of TDP2 could synergize with hormone cycling strategies in inducing apoptosis due to overwhelming TOP2B induced DSBs (61).

Given the evidence that activation of transcriptional programs involves TOP2B-mediated DSBs, a combination of a topoisomerase targeting drug together with “hormone cycling” therapy may be particularly effective. Class II topoisomerases have for a long time been at the center of oncology drug development; topoisomerase II targeting drugs have successfully been used clinically in a variety of cancers (62). TOP2 poisons, such as etoposide, have long been thought to be effective only in highly proliferative tumors, because only tissues with a high proliferation fraction would express high levels of TOP2A (14, 62). The new insights in the role of TOP2B in transcriptional regulation, however, suggests that TOP2 targeted drugs may be effective even in cancers showing low proliferation fractions if transcriptional programs can be cyclically induced. An ongoing clinical trial (ClinicalTrials.gov identifier: NCT01084759) is currently testing the safety and efficacy of testosterone cycling in combination with oral etoposide in prostate cancer patients with rising PSA undergoing androgen ablative therapy (63).
Concluding Remarks
As demonstrated by recent reports, efficient activation of transcriptional programs in response to stimuli such as hormone exposure involves the generation of DNA damage/double strand breaks, recruitment of DNA repair proteins, and large-scale genome reorganization to allow movement of activated genes and regulatory loci to transcriptional hubs. The DNA double strand breaks, if illegitimately repaired, can seed formation of genomic rearrangements and perhaps contribute to ongoing genomic instability. While these transcription-related DSBs pose a risk for generation of oncogenic genomic rearrangements and perhaps ongoing genomic instability, we may be able to take advantage of these processes to target certain cancers. For instance, for breast and prostate cancer, it may be possible to use a “hormone cycling” strategy to induce these DSBs repetitively and possibly couple such a strategy with pharmacological agents, such as TOP2 poisons, or PARP and/or DNA-PK inhibitors, in order to overwhelm the cancer cell with breaks, ultimately leading to cell death. Such a strategy would be analogous to the largely successful approach of using TOP1 and TOP2 poisons to overwhelm cancer cells with replication-associated DSBs mediated by TOP1 and TOP2A.

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**Figure legends**

**Figure 1.** An emerging model suggests that efficient induction of transcriptional programs can involve the generation of DNA double strand breaks and perhaps other DNA damage, repair of these breaks via recruitment of DNA repair proteins, and large scale movement of genes and regulatory elements to transcriptional hubs, in which transcription (co)factors are present at high local concentration. These events are illustrated for induction of transcriptional programs by AR. Upon transcriptional activation, AR associates with TOP2B and binds to AR target sites. DNA repair proteins including Ku70, Ku80, PARP1, ATM and DNA-PK and possibly other DNA repair enzymes also get recruited to these sites. TOP2B can mediate DSB in associated DNA. Reactive oxygen, perhaps generated during AR signaling, dietary carcinogens, and collision with the replication or transcription forks can transform these transient breaks into frank double strand breaks. Persistence of these breaks can result in induction of cellular senescence and apoptosis. Illegitimate repair of these breaks can lead to the formation of structural rearrangements. Such pathways are likely at work for other induced transcriptional programs, including those mediated by ER, RAR, and AP1.
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