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Topoisomerase II α in Chromosome Instability and Personalized Cancer Therapy

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Abstract

Genome instability is a hallmark of cancer cells. Chromosome instability (CIN), which is often mutually exclusive from hypermutation genotypes, represents a distinct subtype of genome instability. Hypermutations in cancer cells are due to defects in DNA repair genes, but the cause of CIN is still elusive. However, because of the extensive chromosomal abnormalities associated with CIN, its cause is likely a defect in a network of genes that regulate mitotic checkpoints and chromosomal organization and segregation. Emerging evidence has shown that the chromosomal decatenation checkpoint, which is critical for chromatin untangling and packing during genetic material duplication, is defective in cancer cells with CIN. The decatenation checkpoint is known to be regulated by a family of enzymes called topoisomerases. Among them, the gene encoding topoisomerase II α (TOP2A) is commonly altered at both gene copy number and gene expression level in cancer cells. Thus, abnormal alterations of TOP2A, its interacting proteins, and its modifications may play a critical role in CIN in human cancers. Clinically, a large arsenal of topoisomerase inhibitors have been used to suppress DNA replication in cancer. However, they often lead to the secondary development of leukemia because of their effect on the chromosomal decatenation checkpoint. Therefore, topoisomerase drugs must be used judiciously and administered on an individual basis. In this review, we highlight the biological function of TOP2A in chromosome segregation and the mechanisms that regulate this enzyme's expression and activity. We also review the roles of TOP2A and related proteins in human cancers, and raise a perspective for how to target TOP2A in personalized cancer therapy.

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CONFLICTS OF INTEREST

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Keywords

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INTRODUCTION

Chromosome instability (CIN) is one of the hallmarks of cancer cells¹ and is often mutually exclusive from hypermutation genotypes. Hypermutations in cancer cells are due to defects in DNA mismatch repair genes, including *MLH1* and *MSH2*;² however, the cause of CIN is still elusive. Most solid tumor cells with CIN are aneuploidy, indicating that abnormal mitosis is involved in the CIN phenotype. The process of mitosis is precisely regulated. The sequence of events is divided into six stages (prophase, prometaphase, metaphase, anaphase, and telophase) corresponding to the completion of one set of activities and the start of the next. During mitosis, the pairs of chromatids condense and attach to spindles that pull the sister chromatids to opposite sides of the cell, each cell receives chromosomes that are alike in composition and equal in number to the chromosomes of the parent cell. The decatenation and mitotic checkpoints govern sister chromosome segregation. Topoisomerase II α (TOP2A) is a key player in the decatenation checkpoint; when the decatenation checkpoint is defective, chromosomal mis-segregation is observed. CIN can provide these evolving cell subclones with a mechanism that causes unremitting genomic and mutational plasticity. Furthermore, the decatenation checkpoint deficiency can cause additional chromosome imbalances in cancer cells, increasing tumour aggressiveness (Figure 1).

In this review, we discuss the biological function of TOP2A in chromosome segregation and CIN. We also assess the mechanisms that may regulate this enzyme's expression and activity and shed light on the roles of TOP2A in human cancer progression and personalized cancer therapy.

CHROMOSOME INSTABILITY AND CANCER

CIN involves the unequal distribution of DNA to daughter cells upon mitosis, resulting in the loss or gain of chromosome during cell division, and ultimately, aneuploidy. Aneuploidy can be driven by genetic alterations that promote inaccurate chromosome segregation, which often causes whole chromosomes to be lost or gained. This condition is termed whole chromosome instability. In addition to numerical chromosomal abnormalities, cancer cells can show changes in chromosome structure, such as deletions of chromosome arms and amplifications of large chromosome regions. This condition is termed structural or segmental chromosomal instability.³

Recently, CIN was recognized as a distinct feature of most aggressive cancer types (Figure 2). In normal organisms, the integrity of the chromosome number and structure are essential to maintaining survival. Aneuploidy, in the form of large or local chromosomal changes, is more evident and unique in human cancer. Aneuploidy is distinct from polyploidy; in polyploidy, cells contain more than two complete sets of chromosomes but always have an

exact multiple of the haploid number, so the chromosomes remain balanced. Cancer cells not only have numerous gene mutations but are also characterized by aneuploidy and CIN.²

Whether CIN is a driver or passenger of cancer is a subject of debate in cancer research. Most researchers believe that CIN is an early event in tumor formation that causes the deletion or inactivation of tumor suppressor genes through defects in genome maintenance and destabilization of the nucleotide sequence;^{1,4,5} other researchers argue that CIN is a side effect of neoplastic growth and cell division, during which cancer cells frequently lose and gain chromosomes.⁶

To better define the role of CIN in cancer, it is important to understand the molecular basis of tumorigenesis. Genome alterations in cell subclones confer advantages on outgrowth and lead to the subclones' dominance in the local tissue environment. CIN is a cell-autonomous feature that provides evolving cell subclones with a mechanism that causes unremitting genomic and mutational plasticity, leading to increased cell survival, cell proliferation, and tumorigenesis.⁷ In essence, CIN drives tumor progression by accelerating the gain of oncogenic loci and the loss of tumor suppressor loci. Therefore, other subclones that have chromosomal alternations but do not have this kind of gain and loss will not acquire tumor-promoting features. Finally, multistep tumor progression can proceed because of the succession of subclone expansions. Comparative genomic hybridization, which can document the gains and losses of gene copy numbers across the cell genome, provides clear evidence of the loss of control of chromosome integrity in tumor progression.⁸ The development of next-generation DNA-sequencing technologies has led to the detection of widespread destabilization of gene copy numbers and nucleotide sequences.^{9,10} Defects in genome maintenance systems cannot detect and resolve these abnormal genome alterations¹. Therefore, favorable genotypes accumulate in subclones or premalignant cells. Accordingly, CIN is likely the primary cause and driving force of tumorigenesis and progression.¹¹

CHROMOSOME SEGREGATION AND CIN

The chromosomal changes that cause CIN commonly occur during tumor cell division.² Over 100 genes are involved in sister chromosome segregation in humans, and defects in some of the genes that control this segregation are found in cancer cells.^{6,12} The mitotic checkpoint plays a prominent role in chromosome segregation. This checkpoint is a control mechanism that ensures high-fidelity chromosome segregation. One of the key steps is regulated by the anaphase-promoting complex/cyclosome (APC/C). APC/C is a multi-subunit E3 ubiquitin ligase that controls mitotic progression and sister chromosome segregation. APC/C activation requires cell-division-cycle 20 (CDC20) as a co-activator to modulate the ubiquitination and degradation of mitotic substrates, including securin and cyclin B1.¹³ Degradation of securin releases the sequestering partner, separase. Active separase can cleave the cohesin links that hold the sister chromatins together. Degradation of cyclin B1 leads to the inactivation of cyclin-dependent kinase 1 and initiates mitotic exit. Numerous studies have determined the effect of the reduced expression of mitotic checkpoint genes, including *Bub1*, *Bub1b*, *Bub3*, *Mad1*, *Mad2*, *Cenp-E*, *Cmt2*, *Mps1*, *ZW10*, *ROD*, and *Zwilch*, and found that the haploinsufficiency of these genes resulted in chromosome mis-segregation (Table 1 and references within). Several key proteins,

including TOP2A, cooperate with APC/C^{cdc20} and regulate sister chromosome segregation (Figure 3).

ROLES OF TOP2A IN CIN

TOP2 is a group of highly conserved enzymes that catalyze the ATP-dependent transport of one intact DNA double helix through another.⁵¹ As a result, the intertwined parental strands of a replicating DNA ring can come apart, interlocked double-stranded DNA rings can become unlinked, and knots can be introduced or removed from DNA rings.⁵² Previous studies have revealed the important role of TOP2 activity in chromosome segregation through the use of inhibitors. Drugs that interfere with TOP2 have been reported to induce polyploidy and endoreduplication to different degrees, providing indirect evidence that this enzyme is required for the separation of sister chromatids.⁵³⁻⁵⁸

Human somatic cells replicate and segregate their genomes with remarkable precision. In the cell cycle, sister chromatids become entangled after DNA replication.⁵⁹ Normally, the decatenation checkpoint monitors the chromosome catenation status. Cells are arrested at the G₂ phase, and the onset of mitosis is delayed if sister chromatids are not fully separated.⁶⁰⁻⁶² Because complete chromatid decatenation is required for accurate chromatid segregation, it has been suggested that the attenuation of the decatenation checkpoint function contributes to the acquisition of CIN in cancer cells.⁶¹ TOP2A possess three distinct subunit dimerization interfaces (Figure 4A) and is maximally expressed in the G₂ and M phases of the cell cycle.⁶³⁻⁶⁶ It is specific for chromosome untangling and is essential for sister chromosome segregation before anaphase.⁶⁷⁻⁶⁹ One biochemical study performed in *Xenopus* egg extracts (XEEs) showed that centromeres were catenated while sister chromatids underwent bipolar attachment and that TOP2A activity was required after the onset of anaphase.⁶⁹ As an alternative approach, TOP2 catalytic inhibitors have been used to demonstrate the role of TOP2A in sister chromosome segregation. TOP2 catalytic inhibitors determine the relative importance of the enzyme in promoting chromosome segregation at the metaphase-anaphase transition. Inactivation of TOP2 by merbarone also resulted in polyploidy in male mouse meiotic cells.⁷⁰

TOP2 catalytic inhibitors inhibit the ATPase activity of TOP2A and stabilize this enzyme in a closed-clamp form, rather than stabilizing the TOP2A DNA-cleavable complex, which is the mechanism of action of TOP2 poisons (e.g. etoposide and teniposide).⁷¹ Therefore, in contrast to TOP2 poisons, TOP2 inhibitors do not induce extensive DNA breaks. Among the classes of catalytic TOP2 inhibitors, the bisdioxopiperazines (e.g., ICRF-154, ICRF-187, and ICRF-193) have been the most extensively studied.^{72,73} Andoh *et al* reported that ICRF-193, a catalytic, noncleavable-complex-forming-type TOP2 inhibitor, led to an absence of chromosome segregation at mitosis, with further accumulation of polyploid cells.⁷⁴ In addition, treating human leukemia cells with ICRF-187 led to endoreduplication, which resulted in large and highly polyploid cells.⁷⁵ However, these TOP2 inhibitor studies did not reveal whether a single isoform was responsible, and these phenotypes may have been complicated by side effects of the inhibitors. Gene targeting in mice showed that segregation was dependent on the alpha subunit of TOP2, not the beta subunit of TOP.^{76,77} When TOP2A's function was blocked after chromosome condensation, cells arrested at

metaphase, chromosomes failed to separate, and anaphase bridges formed,^{53,57,78,79} resulting in partial or complete chromosome gains or losses and polyploidy; this observation supports the theory that the enzyme is important in anaphase segregation.^{80,81}

As a whole, these reports support the theory that the catenation state of intertwined sister chromosomes is monitored in G₂ cells and that progression to mitosis is actively delayed when chromosomes are not sufficiently decatenated. The final step, decatenation of intertwined daughter molecules, can only be carried out by TOP2A.

TOP2A EXPRESSION REGULATION

TOP2A expression peaked in G₂/M phase cells and decreased when cells completed mitosis. Cell cycle-dependent TOP2A expression is essential, and TOP2A depletion in mammalian culture cells causes severe defects in chromosome segregation during anaphase.⁸² The expression level of human *TOP2A* is controlled by its promoter region. The *TOP2A* promoter does not contain a consensus TATA motif but contains two GC boxes and five CCAAT boxes that are located mostly in an inverted orientation (Figure 4B). The activity of the *TOP2A* promoter is regulated by various external stimuli, including the stages of the cell cycle, and by the TP53 tumor suppressor protein. Experimental studies using cell lines showed that TOP2A expression was negatively regulated by wild-type TP53 through its promoter region.^{83,84} In addition, Liu *et al* reported that *TOP2A* gene expression was regulated by *TP53* gene status and that several TP53 mutants exhibited reduced suppression of *TOP2A* gene expression.⁸⁵ This regulation was explained as a consequence of TP53 interfering with NF-Y binding to the regulatory sequences of the *TOP2A* promoter. NF-Y, a ubiquitous transcription factor, recognizes and binds to inverted CCAAT boxes (ICBs). The decrease in NF-Y activity is correlated with the decrease in *TOP2A* transcriptional activity, transcript level, and expression.⁸⁶ Furthermore, a chromatin immunoprecipitation analysis showed that reduced recruitment of NF-Y to *TOP2A* gene regulatory regions decreased its transcription.^{87,88}

GC boxes are common elements in promoters. In the *TOP2A* promoter, GC1 and GC2 flank ICB1 and ICB5, respectively. It has been reported that GC1 has a major role in the basal transcription of *TOP2A*, while GC2 functions in a more modulatory capacity.⁸⁹ Proteins bound to the GC2 element may act as repressors. The specificity proteins Sp1 and Sp3 have been implicated in the regulation of *TOP2A* transcription through binding to both GC1 and GC2. Sp1 is commonly known as a transcriptional activator and is able to up-regulate transcription in a variety of promoters, including *TOP2A*, while Sp3 is bifunctional. Sp3 is a transcriptional repressor of *TOP2A* and a common modulator of Sp1-dependent transcriptional activation.⁸⁹ Since Sp3 is functionally dominant over Sp1, relatively small increases in Sp3 levels or decreases in the Sp1/Sp3 ratio could result in reduced transcription of *TOP2A*. However, Mo *et al* found that Sp3 was a transcriptional activator.⁹⁰ It is possible that this discrepancy is cell type specific.

POST-TRANSLATIONAL MODIFICATIONS OF TOP2A

In mammalian cells, several different mechanisms regulate TOP2A's decatenation ability. Posttranslational modification, including phosphorylation, ubiquitination, and small

ubiquitin-like modifiers (SUMOylation), can alter its protein activity, stability, or localization (Figure 4C). The proteins involved in these modifications can regulate TOP2A's decatenation of intertwined daughter chromosomes. Mutations or alterations of the genes involving in TOP2A post-translational modification can lead to CIN and ultimately, cancer.

Phosphorylation

Phosphorylation is a key mechanism that regulates TOP2A's function. It has been proposed to affect the catalytic activity of TOP2A.⁹¹ Most of the phosphorylation sites in TOP2A protein are located within the C-terminal domain (Figure 4A).⁹²⁻⁹⁴ Some studies have reported that these phosphorylation sites are not important for enzymatic activity but are critical for the nuclear localization of TOP2A,⁹⁵ because deletion of this region or mutation of Ser-1376 or -1524 did not lead to inactivation of the enzyme.^{95,96} However, *Chikamori et al* demonstrated that Ser-1106 was a major phosphorylation site in the catalytic domain of TOP2A and that mutation of this Ser-1106 to alanine caused a decrease in enzymatic activity.⁹⁷ In addition, hypophosphorylation of Ser-1106 may be correlated with etoposide resistance. These findings establish that Ser-1106 phosphorylation plays a critical role in regulating TOP2A's decatenation function.

Several proteins are involved in the phosphorylation of TOP2A. Casein kinase II β , protein kinase C, and extracellular signal-related kinase 2 have been shown to phosphorylate TOP2A and enhance its activity.^{95,98,99} p38 γ is a member of the p38 mitogen-activated protein kinase family that can be activated by both stress and mitogenic signals. *Qi et al* reported that p38 γ phosphorylated the TOP2A Ser-1542 site and was important for TOP2A's stability and activity.¹⁰⁰ The polo-like kinase (Plk) family is also involved in the phosphorylation of TOP2A. Plk1 phosphorylates Ser1337 and Ser1524 of TOP2A, while Plk3 phosphorylates Thr1342.^{101,102} The maximum level of phosphorylation occurs in mitosis, and Plk1- and Plk2-associated phosphorylation can activate TOP2A. Although phosphorylation does not affect TOP2A's dynamic localization in chromosomes, it is required for its essential role in sister chromosome segregation, and the observed hyperphosphorylation could represent compensation for the reduced protein level.¹⁰³

Ubiquitination

As a tumor suppressor, BRCA1 plays a versatile role through its ability to participate in DNA damage response, checkpoint control, mitotic spindle assembly, centrosome duplication, and sister chromosome segregation¹⁰⁴. BRCA1 plays a critical role in the maintenance of chromosome stability, which participates in TOP2A -dependent DNA decatenation. Cells derived from conditional BRCA1-knockout mice have various chromosome abnormalities.^{105,106}

BRCA1 may regulate DNA decatenation through the ubiquitination of TOP2A. *Lou et al* reported that, in the absence of BRCA1, there was only one-third as much decatenation activity, and this contributed to a defect in chromosome segregation¹⁰⁷ that was also reported in BRCA1-defective embryonic mouse fibroblasts. BRCA1-deficient cells had many spontaneous chromosome abnormalities.^{105,106,108} *Shinagawa et al* found that BRCA1 is a regulator of TOP2A ubiquitination.¹⁰⁹ Other studies have supported this

mechanism and demonstrated that BRCA1 has a RING finger domain with documented E3 ubiquitin ligase activity.^{107,110} BRCA1 can form a complex that includes BRCA1 and pRb and then ubiquitinate TOP2A for its degradation. In this complex, pRb is another key factor required for the degradation and inactivation of TOP2A on exposure to oxidative stress.

SUMOylation

SUMOs may be involved in diverse biological functions. The results of both genetic and biochemical studies indicate that the SUMO modification pathway plays an important role in proper cell cycle control, especially in the normal progression of mitosis. The results of studies in XEEs have implicated SUMOylation in TOP2A's decatenation ability.^{111,112} TOP2A is modified by SUMO-2/3 on mitotic chromosomes in the early stages of mitosis (Figure 4A).¹¹³ The inhibition of mitotic SUMOylation in the XEE assay system causes aberrant sister chromosome separation in anaphase and alters TOP2A's association with chromosomes.¹¹⁴ Similarly, PIAS γ , a member of the PIAS family, is unique in its capacity to bind mitotic chromosomes and is indispensable for accurate chromosome segregation in XEEs. PIAS γ with SUMO E3 ligase activity is a critical regulator of the mitotic SUMO-2 conjugation of TOP2A,¹¹⁵ and the activity of SUMO E3 ligase led to SUMO's modification of TOP2A in mitosis.¹¹⁶ In human cells, PIAS γ directs TOP2A to specific chromosome regions that require efficient removal of DNA catenations prior to anaphase. A lack of PIAS γ leads to a prolonged metaphase block, in which normal metaphase plates form and the spindle checkpoint is activated. Moreover, sister chromatids remain cohered, even if cohesin is removed by depleting hSgo1, because DNA catenations persist at centromeres.¹¹⁷

Recently, it was reported that the nuclear pole protein RanBP2 bound to TOP2A and regulated its SUMO modification, specifically in mitosis. RanBP2 catalyzed the SUMOylation of TOP2A, directing this protein to inner centromeres for accurate chromosome separation prior to anaphase onset. When RanBP2 was insufficient, this defect led to chromatin bridges in anaphase, which were linked to impaired TOP2A-mediated decatenation of sister chromatids.³⁷ The results of these studies demonstrate that SUMO modification leads TOP2A to accumulate at inner centromeres and is essential for proper sister chromosome separation in mitosis.

Deacetylation

Histone acetylases (HATs) and histone deacetylases (HDACs) modify nucleosomal histones. Tsai *et al* reported that two histone deacetylation enzymes, HDAC1 and HDAC2, were associated with TOP2 *in vivo* under normal physiological conditions.¹¹⁸ Their results indicate that TOP2A and TOP2B are substrates for HDAC1 and HDAC2 and that complexes containing HDAC1 or HDAC2 can increase TOP2 activity. HDAC1 and HDAC2 may facilitate chromatin modification by targeting a subunit of TOP2 to chromatin regions that are actively undergoing histone deacetylation. However, the mechanism by which TOP2 is modified by deacetylation *in vivo* requires further investigation. Although the balance between protein acetylation and deacetylation controls several physiological and pathological cellular processes, the role of HATs in acetylation of TOP2A is yet-to-be defined.

REGULATION OF TOP2A's DNA BINDING

Chromatin-Accessibility Complex

TOP2A is an ATP-dependent enzyme, and ATPase activity, which is important in TOP2A catalyzed hydrolysis, is stimulated by free DNA. The chromatin-accessibility complex uses energy to increase the general accessibility of DNA in chromatin. When its ATPase activity is stimulated by nucleosomal DNA, it facilitates TOP2A's hydrolysis ability. The ATPase subunit of the chromatin-accessibility complex, ISWI, and TOP2A are located in the same complex. Nucleosome remodeling by ISWI may help TOP2A bind to chromatin, which will enhance TOP2A's hydrolysis.¹¹⁹

SWI/SNF Complex

A similar mechanism is found in another ATP-dependent chromatin remodeling complex, SWI/SNF. The mammalian SWI/SNF complex is composed of two distinct groups, the Brm/Brg1-associated factor (BAF) and polybromo-associated BAF (PBAF) complexes. The BAF complex is composed of one of two mutually exclusive catalytic ATPase subunits, BRG1 or BRM, whereas the PBAF complex uses only BRG1 as the catalytic subunit. The deletion of BRG1 or the expression of the tumor-associated G1232D mutant BRG1 reduced TOP2A's decatenation ability and caused anaphase bridges.³⁹ The loss of another subunit of the BAF complex, BAF250a, also resulted in TOP2A decatenation defects. In the BAF complex, TOP2A associated with BRG1 through a direct interaction with BAF250a. Moreover, TOP2A binding to DNA was dependent on BAF's function. The BRG1 mutants reduced the association between TOP2A and chromatin. Consequently, reduced binding of TOP2A to chromatin reduced TOP2A's function and weakened its ability to associate with substrate DNA during decatenation.

DNA Damage Checkpoint 1 Complex

A number of gene products are involved in TOP2A's decatenation function (Table 2). Mediator of DNA damage checkpoint 1 (MDC1) is an important multi-functional checkpoint protein that can interact with TOP2A.⁹⁶ Luo *et al* found within the 71 amino acids of the TOP2A C-terminal region (residues 1461-1531), Ser 1524 was required for MDC1 binding to TOP2A (Figure 4A); mutation of the Ser 1524 site abolished this interaction. In addition, phosphorylation of Ser 1524 occurred preferentially when cells were in G2/M transition.⁹⁶ These findings indicate that MDC1-TOP2A interaction and TOP2A Ser 1524 phosphorylation of are involved in the decatenation checkpoint. Luo *et al* further demonstrated that when phosphorylated, Ser 1524 of TOP2A acted as a binding site for MDC1 and the following MDC1-TOP2A interaction recruited this complex to chromatin. Although the MDC1-TOP2A interaction is not required for checkpoint activation induced by DNA damage, it is required for activation of the decatenation checkpoint. Mutation of Ser 1524 results in a defective decatenation checkpoint. These results reveal an important role of MDC1-TOP2A interaction in checkpoint activation and the maintenance of chromosome stability.

ALTERATIONS OF TOP2A AND RELATED GENES IN CANCERS TOP2A

TOP2A is considered a specific marker for cell proliferation, and it plays an important role in malignant tumors. As indicated by the findings discussed above, several interacting partners of TOP2A and mitotic checkpoint proteins regulate accurate chromosome segregation. The abnormal alteration of TOP2A and related genes consistently contributes to the human cancer genome (Table 3). The expression, genetic alteration, and enzyme activity of TOP2A have been studied in several types of malignancies.¹²⁰⁻¹²⁵ In primary invasive ovarian carcinoma, both protein and mRNA levels of TOP2A expression were increased in high-grade and advanced-stage tumors; there was also a correlation between high expression and poor survival.¹²¹ In Depowski *et al's* study, TOP2A was a candidate marker of increased cell proliferation and poor prognosis in breast cancer.¹²² These authors also reported that it was preferentially expressed in a more aggressive subset of breast tumors (HER-2/neu overexpressed), and HER-2/neu was overexpressed in tumors with increased TOP2A expression. Since TOP2A is located close to HER-2/neu on chromosome 17, the levels of TOP2A gene copy number were assessed in samples with HER-2/neu amplification. Bhargava *et al'* reported that TOP2A amplification was found in 39% (25/64) of HER-2-amplified tumors and was not detected in the absence of HER-2 amplification in their study.¹⁵⁴ TOP2A deletion was also seen in 7 (11%) of 64 tumors. However, whether TOP2A amplification leads to higher TOP2A protein levels is controversial. Mueller *et al* reported that TOP2A gene amplification in breast tumors was not predictive of high TOP2A protein expression.¹²³

In addition to human breast and ovarian cancer, elevated expression of TOP2A has been identified in oral cancer, nasopharyngeal carcinoma, esophageal cancer, lung cancer, gallbladder carcinoma, hepatocellular cancer, and colorectal cancer.¹²⁵ TOP2A overexpression in these cancers is associated with an aggressive tumor phenotype, advanced disease stage, tumor recurrence, and decreased overall survival.

BRCA1

As one of the representative interacting partners of TOP2A, BRCA1 is located on chromosome 17q21.31 and encodes a critical tumor suppressor. Low expression of BRCA1 was previously shown to increase the growth rate of benign and malignant breast tissue.¹⁴¹ In another study, loss of nuclear BRCA1 expression was significantly associated with high histological grade.¹⁴² Interestingly, low BRCA1 expression is associated with the development of distant metastasis and a poor prognosis in sporadic breast cancer.¹⁴³ Dorairaj *et al* reported that the rs8176318 G > T 3'UTR variant of BRCA1 is associated with decreased BRCA1 expression, both in vitro and in vivo. Moreover, they showed that this variant was predictive of aggressive and stage IV disease.¹⁴⁴

Importantly, BRCA1 mutations account for the majority of hereditary breast and ovarian cancers. Compared to BRCA2-mutation carriers, BRCA1-mutation carriers have a higher risk of developing both breast and ovarian cancer. The risk of breast cancer in individuals with the BRCA1 mutation range from 50% to 80%.¹⁴⁵ Meanwhile, over 10% of human ovarian cancers contain BRCA1 mutations in the coding region, which result in genetic instability.¹⁰³

In addition to human breast and ovarian cancer, *BRCA1* mutation carriers were found to have an increased risk of pancreatic cancer and prostate carcinoma.¹⁴⁰

BRG1

BRG1 is a critical interacting partner of TOP2A and is mutated in various malignancies, including prostate, lung, breast, colon, pancreas, ovary, and colon carcinomas. Allelotype analyses have often shown loss of heterozygosity at the 19p13 region, where *Brg1* is localized. In addition, a number of anti-cancer proteins, such as BRCA1, p53, and Rb, have been functionally linked to BRG1, further suggesting that *Brg1* loss can hasten cancer development. The results of these studies suggest that BRG1 has a tumor-suppressive role in a wide range of human cancers.¹⁴⁶

Medina *et al* reported a high incidence of inactivating mutations of *Brg1* in lung cancer cell lines.¹⁴⁷ BRG1 was inactive in a quarter of the 59 lines. Such alterations were more common in non-small cell lung cancer (NSCLC) cell lines. Thirty-five percent of the NSCLC cell lines carried *Brg1*-inactivating mutations, compared with only approximately 5% of SCLC cell lines. An earlier study performed an immunohistochemical analysis to evaluate the levels of BRG1 in primary NSCLCs and found that 30% of tumors had no detectable protein, suggesting BRG1 inactivation.¹⁴⁸ Furthermore, loss of nuclear expression of *Brg1* was associated with a low survival rate in NSCLC patients. Glaros *et al* found that heterozygous loss of *Brg1* could serve as an initiating event in lung cancer development, whereas complete loss of *Brg1* could promote tumor progression rather than tumor initiation.¹⁴⁹

In pancreatic tumors, *Brg1* also acts as a tumor suppressor. Mutations in *Brg1* and other members of the SWI/SNF complex were observed in over 30% of human pancreatic ductal adenocarcinoma cases.¹⁵⁰ Furthermore, decreased *Brg1* expression was associated with the IPMN precursor lesion that pancreatic ductal adenocarcinoma is thought to arise from.¹⁵¹ As in lung cancer, *Brg1*-inactivating mutations and deletions were found in pancreatic cancer cell lines. Taken as together, loss of BRG1 affects cancer development.

THE POTENTIAL ROLE OF TOP2A IN PERSONALIZED CANCER THERAPY

The homeostasis and balance of the topoisomerase network are critical. Too much or too few of the key enzymes will lead to topological stress on the chromosomes and cell death. Thus, TOP2 and TOP1 are often highly expressed to cope with stress. This may lead to a “topoisomerase addiction” phenotype in CIN. Targeting this addiction may kill these cells.¹⁵⁵ In addition, the level of TOP2A increases 2-3-fold during G₂/M phase,¹⁵⁶ and the expression level is much higher in rapidly proliferating cells than in quiescent cell populations.¹⁵⁷ Targeting both TOP2A-mediated DNA cleavage and cell proliferation has been an attractive approach in cancer therapy. Another isoform of TOP2, TOP2B, does not change significantly during the cell cycle. It has been suggested that targeting TOP2B leads to several undesirable consequences, with little clear benefit.¹⁵⁸

TOP2-targeting Agents in Clinic

Several TOP2 inhibitors/poisons have been approved by the US Food and Drug Administration, including etoposide, teniposide, doxorubicin, idarubicin, epirubicin, and mitoxantrone (Figure 5). Both etoposide and teniposide damage DNA by interacting with TOP2 to form cleavable complexes that prevent the relegation of DNA; this leads to double-strand DNA breaks. Etoposide is used to treat a wide variety of malignancies, including SCLC, testicular cancer, neuroblastoma, leukemias, and lymphomas, while teniposide is used in pediatric patients with poor-prognosis acute lymphocytic leukemia.¹⁵⁷ Like etoposide and teniposide, anthracyclines intercalate into DNA and are commonly used to treat breast cancer, leukemias, lymphomas, and sarcomas. The anthracyclines that are currently approved for use in the United States are doxorubicin, daunorubicin, epirubicin, and idarubicin. Doxorubicin is the most commonly used anthracycline for the treatment of solid tumors. Mitoxantrone is an anthracenedione that targets TOP2 and is the only agent of its class approved for clinical use. In the past, these drugs were used without considering whether the cancer had a G₂/M checkpoint defect and CIN. Now, an important goal of clinical research is to maximize the therapeutic efficacy of TOP2-targeting drugs while minimizing the risk of toxicity. Thus, it is critical to better understand the role of TOP2A and the genes that regulate TOP2A in personalized chemotherapy.

Potential Roles of TOP2A in Personalized Cancer Therapy

The TOP2A level is believed to be a major determinant of cellular sensitivity to targeting this enzyme. Thus, genetic and molecular defects that lead to increased TOP2A levels may serve as predictors of a better response to TOP2A inhibitors/poisons. Because *TP53* deficiency is one of the most common genetic aberrations in human cancers and *TP53* negatively regulates *TOP2A* expression, cancer cells with the *TP53* mutation have elevated TOP2A levels and are more sensitive to TOP2A inhibitors/poisons.⁸⁵ Similarly, it has been shown that the level of TOP2A partly depends on the ratio of Sp1:Sp3 in the cells, which also affects the sensitivity of cancer cells to TOP2A inhibitors.⁸⁹ Wang *et al* reported that alterations in NF-Y, a ubiquitous transcription factor that interacts with the *TOP2A* promoter, had a significant effect on *TOP2A* expression and has important consequences in TOP2A-targeting agents.⁸⁶ Identifying the key factors that regulate TOP2A will help us develop an accurate model for predicting patients' response to TOP2A-targeted chemotherapy.

Because TOP2A is an enzyme, its activity may more closely determine cellular sensitivity to TOP2A-directed chemotherapy. Cancer cells with a defect in TOP2A decatenation activity, which is caused by TOP2A modifications or other factors, are resistant to topoisomerase inhibitors/poisons. Since phosphorylation of TOP2A regulates its decatenation activity, researchers have studied the role of altered TOP2A phosphorylation in drug sensitivity. Chikamori *et al* found that cancer cells with the TOP2A S1106A site mutation were resistant to etoposide and amsacrine,⁹⁷ demonstrating that site-specific phosphorylation regulates sensitivity to TOP2-targeting drugs. Thus, hypophosphorylation of TOP2A may be responsible for patients' lack of response to treatment. Chen *et al* reported that hypophosphorylation of TOP2A in teniposide-resistant cells was two times higher than in parental cells.¹⁵⁹ A subsequent study by Ritke *et al* in etoposide-resistant K562 human

leukemia cells revealed that hypophosphorylation of TOP2A in these cells was due to decreased levels of Casein kinase II β .¹⁶⁰ In addition, as it is a kinase, ERK2 has been shown to phosphorylate TOP2A and enhance its activity.⁹⁹ *Kolb et al* reported that inhibition of ERK1 and ERK2 activation using specific inhibitors markedly attenuated the G₂/M arrest induced by etoposide.¹⁶¹ Similarly, p38 γ , which is important to TOP2A's stability and activity, actively regulates the drug-TOP2A signal transduction; this can be exploited to increase the therapeutic activity of TOP2 drugs. In addition to phosphorylation, other posttranslational modifications of TOP2A, including ubiquitination, can regulate the decatenation and localization of this enzyme. Unlike phosphorylation, which can enhance TOP2A activity, BRCA1 ubiquitination reduces TOP2A activity.¹⁰² The absence of functional BRCA1 enhances cellular sensitivity to etoposide.¹⁶²

A major challenge to the clinical use of TOP2 inhibitors/poisons is that they are associated with the development of secondary cancers. Etoposide is widely used for the treatment of many cancer types; however, it is associated with an increased risk of secondary leukemia, particularly acute myelogenous leukemia. TOP2 inhibitors/poisons, including etoposide, often cause rearrangements that involve the mixed lineage leukemia gene on chromosome 11q23, which is associated with this secondary cancer. The risk of secondary acute myelogenous leukemia appears to be dependent on the drug dose. Pedersen-Bjergaard reported that the risk of developing secondary leukemia was 336 times higher with etoposide doses of > 2.0 g/m² than with doses of \leq 2.0 g/m².¹⁶³ On the basis of these data, CIN status may be an important factor that should be considered when the dose of TOP2 inhibitors/poisons are chosen because cancer cells with different TOP2A decatenation activity levels have different sensitivity to topoisomerase inhibitors and low-dose regimens induce a lower risk of leukemogenesis.

Another challenge in clinically using TOP2-targeting agents is drug resistance. In resistant cells, TOP2A alterations have been found at both expression level and catalytic activity of the enzyme. Point mutations in the *TOP2A* gene have also been found in resistant cells. Cancer cells with the TOP2A decatenation defect are resistant to TOP2 inhibitors, indicating that cancer cells' sensitivity to these drugs can be affected by genes that are involved in TOP2A-mediated CIN. Mutations or alterations in these genes may result in different prognoses after treatment with topoisomerase inhibitors. Thus, further studies of TOP2 drug sensitivity and CIN genes may open a new avenue for the development of personalized cancer therapy.

CONCLUDING REMARKS

A comprehensive characterization of cancer genome landscapes unequivocally established that genomic instability is a hallmark of tumorigenesis. Genomic instability manifests in two major genotypes: genetic mutations and CIN. Genetic mutations are believed to be initiated by mutations of a group of DNA repair genes. However, the initiating events for CIN are still elusive. The picture is further muddled because chromosomal copy number alterations are also observed in normal cells; thus, the initiating events for CIN may regularly occur in subclones of normal cells, but only a small percentage evolve into neoplasms.¹⁶⁴ Therefore, although CIN is fully manifested as a tumor phenomenon, it is unlikely to be tumor specific.

Rather, it is mostly likely a pre-cancer phenotype that is intimately tied to cell division, a highly regulated and fundamental process with multiple checkpoints to monitor for errors and remove strayed cells. In particular to the CIN phenotype, correct chromosome segregation requires extensive choreography with key “conductors,” such as TOP2A to cut and paste, spin, and package. The more cell divisions that occur, the higher the chance of errors and the higher the chances of cells surviving the monitoring system and becoming neoplastic. Cancer cells either have CIN or the high mutation phenotype, illustrating that not only do these two genetic aberrations have distinct mechanisms but also that they have an equilibrium and “stability” that is likely supported by a new set of machinery. In other words, these CIN cells are “addicted” to something, which if characterized, would qualify as the Achilles heel.

The results of our literature review and analysis support the hypothesis that TOP2A is an Achilles heel in cancer therapy. However, the reported responses to TOP2 inhibitors/poisons have been limited. This is not surprising given that not all cancer cells are “addicted” to TOP2. TOP2’s activities are subject to multiple levels of regulation—copy number, gene expression control, post-translational modifications, partner protein interactions, and feedback loops—that lead to other changes in checkpoint control, such as the compensatory regulation of TOP1. Therefore, to fully explore TOP2 as an effective target for therapy, more in-depth investigations of the TOP2 regulatory network are needed. Careful interrogation of the clinical annotations that are tied to genomic characterization will help us identify prognostic factors that can be used to stratify patients by responsive or resistant disease; only those with responsive would receive TOP2 inhibitors/poisons.

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REFERENCES

1. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011; 144:646–674. [PubMed: 21376230]
2. Lengauer C, Kinzler KW, Vogelstein B. Genetic instabilities in human cancers. *Nature*. 1998; 396:643–649. [PubMed: 9872311]
3. Geigl JB, Obenaus AC, Schwarzbraun T, Speicher MR. Defining ‘chromosomal instability’. *Trends Genet*. 2008; 24:64–69. [PubMed: 18192061]
4. Michor F, Iwasa Y, Vogelstein B, Lengauer C, Nowak MA. Can chromosomal instability initiate tumorigenesis? *Semin Cancer Biol*. 2005; 15:43–49. [PubMed: 15613287]
5. Chen J, Fu L, Zhang LY, Kwong DL, Yan L, Guan XY. Tumor suppressor genes on frequently deleted chromosome 3p in nasopharyngeal carcinoma. *Chin J Cancer*. 2012; 31(5):215–222. [PubMed: 22360856]
6. Kops GJ, Weaver BA, Cleveland DW. On the road to cancer: aneuploidy and the mitotic checkpoint. *Nat Rev Cancer*. 2005; 5:773–785. [PubMed: 16195750]
7. Duijff PH, Benezra R. The cancer biology of whole-chromosome instability. *Oncogene*. 2013; 32:4727–4736. [PubMed: 23318433]

8. Watanabe T, Kobunai T, Yamamoto Y, Matsuda K, Ishihara S, Nozawa K, et al. Chromosomal instability (CIN) phenotype, CIN high or CIN low, predicts survival for colorectal cancer. *J Clin Oncol.* 2012; 30:2256–2264. [PubMed: 22547595]
9. Wang X. Discovery of molecular associations among aging, stem cells, and cancer based on gene expression profiling. *Chin J Cancer.* 2013; 32:155–161. [PubMed: 23298462]
10. Parker BC, Zhang W. Fusion genes in solid tumors: an emerging target for cancer diagnosis and treatment. *Chin J Cancer.* 2013; 32:594–603. [PubMed: 24206917]
11. Duesberg P, Li R. Multistep carcinogenesis: a chain reaction of aneuploidizations. *Cell Cycle.* 2003; 2:202–210. [PubMed: 12734426]
12. Barber TD, McManus K, Yuen KW, Reis M, Parmigiani G, Shen D, et al. Chromatid cohesion defects may underlie chromosome instability in human colorectal cancers. *Proc Natl Acad Sci USA.* 2008; 105:3443–3448. [PubMed: 18299561]
13. Peters JM. The anaphase-promoting complex: proteolysis in mitosis and beyond. *Mol Cell.* 2002; 9:931–943. [PubMed: 12049731]
14. Tang Z, Shu H, Oncel D, Chen S, Yu H. Phosphorylation of Cdc20 by Bub1 provides a catalytic mechanism for APC/C inhibition by the spindle checkpoint. *Mol Cell.* 2004; 16:387–397. [PubMed: 15525512]
15. Jeganathan K, Malureanu L, Baker DJ, Abraham SC, van Deursen JM. Bub1 mediates cell death in response to chromosome mis-segregation and acts to suppress spontaneous tumorigenesis. *J Cell Biol.* 2007; 179:255–267. [PubMed: 17938250]
16. Baker DJ, Jeganathan KB, Cameron JD, Thompson M, Juneja S, Kopecka A, et al. BubR1 insufficiency causes early onset of aging-associated phenotypes and infertility in mice. *Nat Genet.* 2004; 36:744–749. [PubMed: 15208629]
17. Taylor SS, Ha E, McKeon F. The human homologue of Bub3 is required for kinetochore localization of Bub1 and a Mad3/Bub1-related protein kinase. *J Cell Biol.* 1998; 142:1–11. [PubMed: 9660858]
18. Chen RH, Shevchenko A, Mann M, Murray AW. Spindle checkpoint protein Xmad1 recruits Xmad2 to unattached kinetochores. *J Cell Biol.* 1998; 143:283–295. [PubMed: 9786942]
19. Fang G, Yu H, Kirschner MW. The checkpoint protein MAD2 and the mitotic regulator CDC20 form a ternary complex with the anaphase-promoting complex to control anaphase initiation. *Genes Dev.* 1998; 12:1871–1883. [PubMed: 9637688]
20. Luo X, Fang G, Coldiron M, Lin Y, Yu H, Kirschner MW, et al. Structure of the Mad2 spindle assembly checkpoint protein and its interaction with Cdc20. *Nature Struct Biol.* 2000; 7:224–229. [PubMed: 10700282]
21. Weaver BA, Bonday ZQ, Putkey FR, Kops GJ, Silk AD, Cleveland DW. Centromere-associated protein-E is essential for the mammalian mitotic checkpoint to prevent aneuploidy due to single chromosome loss. *J Cell Biol.* 2003; 162:551–563. [PubMed: 12925705]
22. Habu T, Kim SH, Weinstein J, Matsumoto T. Identification of a MAD2-binding protein, CMT2, and its role in mitosis. *EMBO J.* 2002; 21:6419–6428. [PubMed: 12456649]
23. Xia G, Luo X, Habu T, Rizo J, Matsumoto T, Yu H. Conformation-specific binding of p31(comet) antagonizes the function of Mad2 in the spindle checkpoint. *EMBO J.* 2004; 23:3133–3143. [PubMed: 15257285]
24. London N, Biggins S. Mad1 kinetochore recruitment by Mps1-mediated phosphorylation of Bub1 signals the spindle checkpoint. *Genes Dev.* 2014; 28:140–152. [PubMed: 24402315]
25. Tipton AR, Ji W, Sturt-Gillespie B, Bekier ME 2nd, Wang K, Taylor WR, et al. Monopolar spindle 1 (MPS1) kinase promotes production of closed MAD2 (C-MAD2) conformer and assembly of the mitotic checkpoint complex. *J Biol Chem.* 2013; 288:35149–35158. [PubMed: 24151075]
26. Kops GJ, Kim Y, Weaver BA, Mao Y, McLeod I, Yates JR 3rd, et al. ZW10 links mitotic checkpoint signaling to the structural kinetochore. *J Cell Biol.* 2005; 169:49–60. [PubMed: 15824131]
27. Lin YT, Chen Y, Wu G, Lee WH. Hec1 sequentially recruits Zwint-1 and ZW10 to kinetochores for faithful chromosome segregation and spindle checkpoint control. *Oncogene.* 2006; 25:6901–6914. [PubMed: 16732327]

28. Buffin E, Lefebvre C, Huang J, Gagou ME, Karess RE. Recruitment of Mad2 to the kinetochore requires the Rod/Zw10 complex. *Curr Biol.* 2005; 15:856–861. [PubMed: 15886105]
29. Williams BC, Li Z, Liu S, Williams EV, Leung G, Yen TJ, et al. Zwilch, a new component of the ZW10/ROD complex required for kinetochore functions. *Mol Biol Cell.* 2003; 14:1379–1391. [PubMed: 12686595]
30. Jallepalli PV, Waizenegger IC, Bunz F, Langer S, Speicher MR, Peters JM, et al. Securin is required for chromosomal stability in human cells. *Cell.* 2001; 105:445–457. [PubMed: 11371342]
31. Wirth KG, Wutz G, Kudo NR, Desdouets C, Zetterberg A, Taghybeeglu S, et al. Separase: a universal trigger for sister chromatid disjunction but not chromosome cycle progression. *J Cell Biol.* 2006; 172:847–860. [PubMed: 16533945]
32. Ricke RM, van Ree JH, van Deursen JM. Whole chromosome instability and cancer: a complex relationship. *Trends Genet.* 2008; 24:457–466. [PubMed: 18675487]
33. Sudakin V, Chan GK, Yen TJ. Checkpoint inhibition of the APC/C in HeLa cells is mediated by a complex of BUBR1, BUB3, CDC20, and MAD2. *J Cell Biol.* 2001; 154:925–936. [PubMed: 11535616]
34. Nilsson J, Yekezare M, Minshull J, Pines J. The APC/C maintains the spindle assembly checkpoint by targeting Cdc20 for destruction. *Nat Cell Biol.* 2008; 10:1411–1420. [PubMed: 18997788]
35. Jeganathan KB, Baker DJ, van Deursen JM. Securin associates with APC (Cdh1) in prometaphase but its destruction is delayed by Rae1 and Nup98 until the metaphase/anaphase transition. *Cell Cycle.* 2006; 5:366–370. [PubMed: 16479161]
36. García-Higuera I, Machado E, Dubus P, Cañamero M, Méndez J, Moreno S, et al. Genomic stability and tumour suppression by the APC/C cofactor Cdh1. *Nat Cell Biol.* 2008; 10:802–811. [PubMed: 18552834]
37. Dawlaty MM, Malureanu L, Jeganathan KB, Kao E, Sustmann C, Tahk S, et al. Resolution of sister centromeres requires RanBP2-mediated SUMOylation of topoisomerase II α . *Cell.* 2008; 133:103–115. [PubMed: 18394993]
38. Coelho PA, Queiroz-Machado J, Carmo AM, Moutinho-Pereira S, Maiato H, Sunkel CE. Dual role of topoisomerase II in centromere resolution and aurora B activity. *PLoS Biol.* 2008; 6:e207. [PubMed: 18752348]
39. Dykhuizen EC, Hargreaves DC, Miller EL, Cui K, Korshunov A, Kool M, et al. BAF complexes facilitate decatenation of DNA by topoisomerase II α . *Nature.* 2013; 497:624–627. [PubMed: 23698369]
40. Privette LM, Weier JF, Nguyen HN, Yu X, Petty EM. Loss of CHFR in human mammary epithelial cells causes genomic instability by disrupting the mitotic spindle assembly checkpoint. *Neoplasia.* 2008; 10:643–652. [PubMed: 18592005]
41. Giménez-Abián JF, Sumara I, Hirota T, Hauf S, Gerlich D, de la Torre C, et al. Regulation of sister chromatid cohesion between chromosome arms. *Curr Biol.* 2004; 14:1187–1193. [PubMed: 15242616]
42. Huang B, Shang ZF, Li B, Wang Y, Liu XD, Zhang SM, et al. The Catalytic Subunit of DNA-Dependent Protein Kinase Associates with PLK1 and is Involved in Proper Chromosome Segregation and Regulation of the Cytokinesis. *J Cell Biochem.* 2014; 115:1077–88. [PubMed: 24166892]
43. Liu H, Jia L, Yu H. Phospho-H2A and cohesin specify distinct tension-regulated Sgo1 pools at kinetochores and inner centromeres. *Curr Biol.* 2013; 23:1927–1933. [PubMed: 24055156]
44. Kaitna S, Mendoza M, Jantsch-Plunger V, Glotzer M. Incenp and an aurora-like kinase form a complex essential for chromosome segregation and efficient completion of cytokinesis. *Curr Biol.* 2000; 10:1172–1181. [PubMed: 11050385]
45. Adams RR, Maiato H, Earnshaw WC, Carmena M. Essential roles of Drosophila inner centromere protein (INCENP) and aurora B in histone H3 phosphorylation, metaphase chromosome alignment, kinetochore disjunction, and chromosome segregation. *J Cell Biol.* 2001; 153:865–880. [PubMed: 11352945]
46. Lee BH, Amon A. Role of Polo-like kinase CDC5 in programming meiosis I chromosome segregation. *Science.* 2003; 300:482–486. [PubMed: 12663816]

47. Kallio MJ, Nieminen M, Eriksson JE. Human inhibitor of apoptosis protein (IAP) survivin participates in regulation of chromosome segregation and mitotic exit. *FASEB J.* 2001; 15:2721–2723. [PubMed: 11687505]
48. Maney T, Hunter AW, Wagenbach M, Wordeman L. Mitotic centromere-associated kinesin is important for anaphase chromosome segregation. *J Cell Biol.* 1998; 142:787–801. [PubMed: 9700166]
49. Prasanth SG, Prasanth KV, Stillman B. Orc6 involved in DNA replication, chromosome segregation, and cytokinesis. *Science.* 2002; 297:1026–1031. [PubMed: 12169736]
50. Ji P, Smith SM, Wang Y, Jiang R, Song SW, Li B, et al. Inhibition of gliomagenesis and attenuation of mitotic transition by MIIP. *Oncogene.* 2010; 29:3501–3508. [PubMed: 20418911]
51. Champoux JJ. DNA topoisomerases: structure, function, and mechanism. *Annu Rev Biochem.* 2001; 70:369–413. [PubMed: 11395412]
52. Wang JC. Cellular roles of DNA topoisomerases: a molecular perspective. *Nat Rev Mol Cell Biol.* 2002; 3:430–440. [PubMed: 12042765]
53. Sumner AT. Inhibitors of topoisomerase II delay progress through mitosis and induce doubling of the DNA content in CHO cells. *Exp Cell Res.* 1995; 217:440–447. [PubMed: 7698244]
54. Chen M, Beck WT. Teniposide-resistant CEM cells, which express mutant DNA topoisomerase II, when treated with non-complex-stabilizing inhibitors of the enzyme, display no cross-resistance and reveal aberrant functions of the mutant enzyme. *Cancer Res.* 1993; 53:5946–5953. [PubMed: 8261408]
55. Cummings J, Sumner AT, Slavotinek A, Meikle I, Macpherson JS, Smyth JF. Cytogenetic evaluation of the mechanism of cell death induced by the novel anthracenylamino acid topoisomerase II catalytic inhibitor NU/ICRF 500. *Mutat Res.* 1995; 344:55–62. [PubMed: 7565893]
56. Sumner AT. Induction of diplochromosomes in mammalian cells by inhibitors of topoisomerase II. *Chromosoma.* 1998; 107:486–490. [PubMed: 9914381]
57. Ferguson LR, Whiteside G, Holdaway KM, Baguley BC. Application of fluorescence in situ hybridization to study the relationship between cytotoxicity, chromosome aberration, and changes in chromosome number after treatment with the topoisomerase II inhibitor amsacrine. *Environ Mol Mutagen.* 1998; 27:255–262. [PubMed: 8665870]
58. Kallio M, Lahdetie J. Effects of DNA topoisomerase inhibitor merbarone in male mouse meiotic divisions in vivo: cell cycle arrest and induction of aneuploidy. *Environ Mol Mutagen.* 1997; 29:16–27. [PubMed: 9020303]
59. Holm C. Coming undone: how to untangle a chromosome. *Cell.* 1994; 77:955–957. [PubMed: 8020101]
60. Deming PB, Cistulli CA, Zhao H, Graves PR, Piwnicka-Worms H, Paules RS, et al. The human decatenation checkpoint. *Proc Natl Acad Sci USA.* 2001; 98:12044–12049. [PubMed: 11593014]
61. Downes CS, Clarke DJ, Mullinger AM, Giménez-Abián JF, Creighton AM, Johnson RT. A topoisomerase II-dependent G2 cycle checkpoint in mammalian cells. *Nature.* 1994; 372:467–470. [PubMed: 7984241]
62. Paulovich AG, Toczyski DP, Hartwell LH. When checkpoints fail. *Cell.* 1997; 88:315–321. [PubMed: 9039258]
63. Schmidt BH, Burgin AB, Deweese JE, Osheroff N, Berger JM. A novel and unified two-metal mechanism for DNA cleavage by type II and IA topoisomerases. *Nature.* 2010; 465:641–644. [PubMed: 20485342]
64. Schmidt BH, Osheroff N, Berger JM. Structure of a topoisomerase II-DNA-nucleotide complex reveals a new control mechanism for ATPase activity. *Nat Struct Mol Biol.* 2012; 19:1147–54. [PubMed: 23022727]
65. Wendorff TJ, Schmidt BH, Heslop P, Austin CA, Berger JM. The structure of DNA-bound human topoisomerase II alpha: conformational mechanisms for coordinating inter-subunit interactions with DNA cleavage. *J Mol Biol.* 2012; 424:109–124. [PubMed: 22841979]
66. Villman K, Stahl E, Liljegren G, Tidefelt U, Karlsson MG. Topoisomerase II-alpha expression in different cell cycle phases in fresh human breast carcinomas. *Mod Pathol.* 2002; 15:486–491. [PubMed: 12011253]

67. Cortes F, Pastor N, Mateos S, Dominguez I. Roles of DNA topoisomerases in chromosome segregation and mitosis. *Mutat Res*. 2003; 543:59–66. [PubMed: 12510017]
68. Yanagida M. Basic mechanism of eukaryotic chromosome segregation. *Philos Trans R Soc Lond B Biol Sci*. 2005; 360:609–621. [PubMed: 15897183]
69. Wang LH, Schwarzbraun T, Speicher MR, Nigg EA. Persistence of DNA threads in human anaphase cells suggests late completion of sister chromatid decatenation. *Chromosoma*. 2008; 117:123–135. [PubMed: 17989990]
70. Kallio M, Lahdetie J. Effects of DNA topoisomerase inhibitor merbarone in male mouse meiotic divisions in vivo: cell cycle arrest and induction of aneuploidy. *Environ Mol Mutagen*. 1997; 29:16–27. [PubMed: 9020303]
71. Roca J, Ishida R, Berger JM, Andoh T, Wang JC. Antitumor bisdioxopiperazines inhibit yeast DNA topoisomerase II by trapping the enzyme in the form of a closed protein clamp. *Proc Natl Acad Sci USA*. 1994; 91:1781–1785. [PubMed: 8127881]
72. Andoh T, Sato M, Narita T, Ishida R. Role of DNA topoisomerase II in chromosome dynamics in mammalian cells. *Biotechnol Appl Biochem*. 1993; 18:165–174. [PubMed: 8251115]
73. Ishida R, Sato M, Narita T, Utsumi KR, Nishimoto T, Morita T, et al. Inhibition of DNA topoisomerase II by ICRF-193 induces polyploidization by uncoupling chromosome dynamics from other cell cycle events. *J Cell Biol*. 1994; 126:1341–1351. [PubMed: 8089169]
74. Ishida R, Miki T, Narita T, Yui R, Sato M, Utsumi KR, et al. Inhibition of intracellular topoisomerase II by antitumor bis(2,6-dioxopiperazine) derivatives: mode of cell growth inhibition distinct from that of cleavable complex-forming type inhibitors. *Cancer Res*. 1991; 51:4909–4916. [PubMed: 1654205]
75. Hasinoff BB, Abram ME, Barnabé N, Khélifa T, Allan WP, Yalowich JC. The catalytic DNA topoisomerase II inhibitor dexrazoxane (ICRF-187) induces differentiation and apoptosis in human leukemia K562 cells. *Mol Pharmacol*. 2001; 59:453–461. [PubMed: 11179439]
76. Akimitsu N, Adachi N, Hirai H, Hossain MS, Hamamoto H, Kobayashi M, et al. Enforced cytokinesis without complete nuclear division in embryonic cells depleting the activity of DNA topoisomerase II α . *Genes Cells*. 2003; 8:393–402. [PubMed: 12653966]
77. Akimitsu N, Adachi N, Hirai H, Hossain MS, Hamamoto H, Kobayashi M, et al. DNA topoisomerase II β and neural development. *Science*. 2000; 287:131–134. [PubMed: 10615047]
78. Uemura T, Ohkura H, Adachi Y, Morino K, Shiozaki K, Yanagida M. DNA topoisomerase II is required for condensation and separation of mitotic chromosomes in *S. Pombe*. *Cell*. 1987; 50:817–925.
79. Clarke DJ, Johnson RT, Downes CS. Topoisomerase II inhibition prevents anaphase chromatid segregation in mammalian cells independently of the generation of DNA strand breaks. *J Cell Sci*. 1993; 105:563–568. [PubMed: 8408285]
80. Carpenter AJ, Porter AC. Construction, characterization, and complementation of a conditional-lethal DNA topoisomerase II α mutant human cell line. *Mol Biol Cell*. 2004; 15:5700–5711. [PubMed: 15456904]
81. Johnson M, Phua HH, Bennett SC, Spence JM, Farr CJ. Studying vertebrate topoisomerase 2 function using a conditional knockdown system in DT40 cells. *Nucleic Acids Res*. 2009; 37:e98. [PubMed: 19494182]
82. Chang CJ, Goulding S, Earnshaw WC, Carmena M. RNAi analysis reveals an unexpected role for topoisomerase II in chromosome arm congression to a metaphase plate. *J Cell Sci*. 2003; 116:4715–26. [PubMed: 14600258]
83. Sandri MI, Isaacs RJ, Ongkeko WM, Harris AL, Hickson ID, Broggin M, et al. p53 regulates the minimal promoter of the human topoisomerase II α gene. *Nucleic Acids Res*. 1996; 24:4464–4470. [PubMed: 8948636]
84. Wang Q, Zambetti GP, Suttle DP. Inhibition of DNA topoisomerase II α gene expression by the p53 tumor suppressor. *Mol Cell Biol*. 1997; 17:389–397. [PubMed: 8972219]
85. Liu D, Huang CL, Kameyama K, Hayashi E, Yamauchi A, Sumitomo S, et al. Topoisomerase II α gene expression is regulated by the p53 tumor suppressor gene in nonsmall cell lung carcinoma patients. *Cancer*. 2002; 94:2239–47. [PubMed: 12001123]

86. Wang H, Jiang Zg, Wong YW, Dalton WS, Futscher BW, Chan VT. Decreased CP-1 (NF-Y) activity results in transcriptional down-regulation of topoisomerase IIalpha in a doxorubicin-resistant variant of human multiple myeloma RPMI 8226. *Biochem Biophys Res Commun.* 1997; 237:217–224. [PubMed: 9268689]
87. Belluti S, Basile V, Benatti P, Ferrari E, Marverti G, Imbriano C. Concurrent inhibition of enzymatic activity and NF-Y-mediated transcription of Topoisomerase-II α by bis-DemethoxyCurcumin in cancer cells. *Cell Death Dis.* 2013; 4:e756. [PubMed: 23928695]
88. Stros M, Polanská E, Struncová S, Pospíšilová S. HMGB1 and HMGB2 proteins up-regulate cellular expression of human topoisomerase IIalpha. *Nucleic Acids Res.* 2009; 37:2070–2086. [PubMed: 19223331]
89. Williams AO, Isaacs RJ, Stowell KM. Down-regulation of human topoisomerase IIalpha expression correlates with relative amounts of specificity factors Sp1 and Sp3 bound at proximal and distal promoter regions. *BMC Mol Biol.* 2007; 8:36. [PubMed: 17511886]
90. Mo YY, Wang Q, Beck WT. Down-regulation of topoisomerase IIalpha in CEM cells selected for merbarone resistance is associated with reduced expression of Sp3. *Cancer Res.* 1997; 57:5004–8. [PubMed: 9371492]
91. Watt PM, Hickson ID. Structure and function of type II DNA topoisomerases. *Biochem J.* 1994; 303:681–695. [PubMed: 7980433]
92. Ishida R, Iwai M, Marsh KL, Austin CA, Yano T, Shibata M, et al. Threonine 1342 in human topoisomerase IIalpha is phosphorylated throughout the cell cycle. *J Biol Chem.* 1996; 271:30077–30082. [PubMed: 8939955]
93. Daum JR, Gorbisky GJ. Casein kinase II catalyzes a mitotic phosphorylation on threonine 1342 of human DNA topoisomerase IIalpha, which is recognized by the 3F3/2 phosphoepitope antibody. *J Biol Chem.* 1998; 273:30622–30629. [PubMed: 9804834]
94. Escargueil AE, Plisov SY, Filhol O, Cochet C, Larsen AK. Mitotic phosphorylation of DNA topoisomerase II alpha by protein kinase CK2 creates the MPM-2 phosphoepitope on Ser-1469. *J Biol Chem.* 2000; 275:34710–34718. [PubMed: 10942766]
95. Redwood C, Davies SL, Wells NJ, Fry AM, Hickson ID. Casein kinase II stabilizes the activity of human topoisomerase II α in a phosphorylation-independent manner. *J Biol Chem.* 1998; 273:3635–3642. [PubMed: 9452492]
96. Luo K, Yuan J, Chen J, Lou Z. Topoisomerase IIalpha controls the decatenation checkpoint. *Nat Cell Biol.* 2009; 11:204–210. [PubMed: 19098900]
97. Chikamori K, Grabowski DR, Kinter M, Willard BB, Yadav S, Aebersold RH, et al. Phosphorylation of serine 1106 in the catalytic domain of topoisomerase II alpha regulates enzymatic activity and drug sensitivity. *J Biol Chem.* 2003; 278:12696–12702. [PubMed: 12569090]
98. Sahyoun N, Wolf M, Besterman J, Hsieh T, Sander M, LeVine H 3rd, et al. Protein kinase C phosphorylates topoisomerase II: topoisomerase activation and its possible role in phorbol ester-induced differentiation of HL-60 cells. *Proc. Natl. Acad. Sci. USA.* 1986; 83:1603–1607. [PubMed: 3006058]
99. Shapiro PS, Whalen AM, Tolwinski NS, Wilsbacher J, Froelich-Ammon SJ, Garcia M, et al. Extracellular signal-regulated kinase activates topoisomerase IIalpha through a mechanism independent of phosphorylation. *Mol Cell Biol.* 1999; 19:3551–3560. [PubMed: 10207078]
100. Qi X, Hou S, Lepp A, Li R, Basir Z, Lou Z, Chen G. Phosphorylation and stabilization of topoisomerase I I α protein by p38 γ mitogen-activated protein kinase sensitize breast cancer cells to its poisons. *J Biol Chem.* 2011; 286:35883–35890. [PubMed: 21878638]
101. Li H, Wang Y, Liu X. Plk1-dependent phosphorylation regulates functions of DNA topoisomerase IIalpha in cell cycle progression. *J Biol Chem.* 2008; 283:6209–6221. [PubMed: 18171681]
102. Iida M, Matsuda M, Komatani H. Plk3 phosphorylates topoisomerase I Ialpha at Thr(1342), a site that is not recognized by Plk1. *Biochem J.* 2008; 411:27–32. [PubMed: 18062778]
103. Ganapathi RN, Ganapathi MK. Mechanisms regulating resistance to inhibitors of topoisomerase II. *Front Pharmacol.* 2013; 4:89. [PubMed: 23914174]

104. Yang D, Khan S, Sun Y, Hess K, Shmulevich I, Sood AK, et al. Association of BRCA1 and BRCA2 mutations with survival, chemotherapy sensitivity, and gene mutator phenotype in patients with ovarian cancer. *JAMA*. 2011; 306:1557–1565. [PubMed: 21990299]
105. Xu X, Qiao W, Linke SP, Cao L, Li WM, Furth PA, et al. Genetic interactions between tumor suppressors Brca1 and p53 in apoptosis, cell cycle and tumorigenesis. *Nat Genet*. 2001; 28:266–271. [PubMed: 11431698]
106. Xu X, Wagner KU, Larson D, Weaver Z, Li C, Ried T, et al. Conditional mutation of Brca1 in mammary epithelial cells results in blunted ductal morphogenesis and tumour formation. *Nat Genet*. 1999; 22:37–43. [PubMed: 10319859]
107. Lou Z, Minter-Dykhouse K, Chen J. BRCA1 participates in DNA decatenation. *Nat Struct Mol Biol*. 2005; 12:589–593. [PubMed: 15965487]
108. Wang RH, Yu H, Deng CX. A requirement for breast-cancer-associated gene 1 (BRCA1) in the spindle checkpoint. *Proc Natl Acad Sci USA*. 2004; 101:17108–17113. [PubMed: 15563594]
109. Shinagawa H, Miki Y, Yoshida K. BRCA1-mediated ubiquitination inhibits topoisomerase II alpha activity in response to oxidative stress. *Antioxid Redox Signal*. 2008; 10:939–949. [PubMed: 18162055]
110. Gilmore PM, Quinn JE, Mullan PB, Andrews HN, McCabe N, Carty M, et al. Role played by BRCA1 in regulating the cellular response to stress. *Biochem Soc Trans*. 2003; 31:257–262. [PubMed: 12546697]
111. Azuma Y, Arnaoutov A, Dasso M. SUMO-2/3 regulates topoisomerase II in mitosis. *J Cell Biol*. 2003; 163:477–487. [PubMed: 14597774]
112. Diaz-Martinez LA, Gimenez-Abian JF, Azuma Y, Guacci V, Gimenez-Martin G, Lanier LM, et al. PIASgamma is required for faithful chromosome segregation in human cells. *PloS One*. 2006; 1:e53. [PubMed: 17183683]
113. Obado SO, Bot C, Echeverry MC, Bayona JC, Alvarez VE, Taylor MC, et al. Centromere-associated topoisomerase activity in bloodstream form *Trypanosoma brucei*. *Nucleic Acids Res*. 2011; 39:1023–1033. [PubMed: 20864447]
114. Azuma Y. Analysis of SUMOylation of topoisomerase IIalpha with *Xenopus* egg extracts. *Methods Mol Biol*. 2009; 582:221–231. [PubMed: 19763953]
115. Azuma Y, Arnaoutov A, Anan T, Dasso M. PIASy mediates SUMO-2 conjugation of Topoisomerase-II on mitotic chromosomes. *EMBO J*. 2005; 24:2172–2182. [PubMed: 15933717]
116. Takahashi Y, Yong-Gonzalez V, Kikuchi Y, Strunnikov A. SIZ1/SIZ2 control of chromosome transmission fidelity is mediated by the sumoylation of topoisomerase II. *Genetics*. 2006; 172:783–794. [PubMed: 16204216]
117. Díaz-Martínez LA, Giménez-Abián JF, Azuma Y, Guacci V, Giménez-Martín G, Lanier LM, et al. PIASgamma is required for faithful chromosome segregation in human cells. *PloS One*. 2006; 1:e53. [PubMed: 17183683]
118. Tsai SC, Valkov N, Yang WM, Gump J, Sullivan D, Seto E. Histone deacetylase interacts directly with DNA topoisomerase II. *Nat Genet*. 2000; 26:349–353. [PubMed: 11062478]
119. Varga-Weisz PD, Wilm M, Bonte E, Dumas K, Mann M, Becker PB. Chromatin-remodelling factor CHRAC contains the ATPases ISWI and topoisomerase II. *Nature*. 1997; 388:598–602. [PubMed: 9252192]
120. Meng H, Chen R, Li W, Xu L, Xu L. Correlations of TOP2A gene aberrations and expression of topoisomerase II α protein and TOP2A mRNA expression in primary breast cancer: a retrospective study of 86 cases using fluorescence in situ hybridization and immunohistochemistry. *Pathol Int*. 2012; 62:391–399. [PubMed: 22612507]
121. Faggad A, Darb-Esfahani S, Wirtz R, Sinn B, Sehoul J, Könsen D, et al. Topoisomerase IIalpha mRNA and protein expression in ovarian carcinoma: correlation with clinicopathological factors and prognosis. *Mod Pathol*. 2009; 22:579–588. [PubMed: 19270648]
122. Depowski PL, Rosenthal SI, Brien TP, Stylos S, Johnson RL, Ross JS. Topoisomerase IIalpha expression in breast cancer: correlation with outcome variables. *Mod Pathol*. 2000; 13:542–547. [PubMed: 10824926]

123. Mueller RE, Parkes RK, Andrusis I, O'Malley FP. Amplification of the TOP2A gene does not predict high levels of topoisomerase II alpha protein in human breast tumor samples. *Genes Chromosomes Cancer*. 2004; 39:288–297. [PubMed: 14978790]
124. Washiro M, Ohtsuka M, Kimura F, Shimizu H, Yoshidome H, Sugimoto T, et al. Upregulation of topoisomerase IIalpha expression in advanced gallbladder carcinoma: a potential chemotherapeutic target. *J Cancer Res Clin Oncol*. 2008; 134:793–801. [PubMed: 18204862]
125. Lan, J.; Huang, HY.; Lee, SW.; Chen, TJ.; Tai, HC.; Hsu, HP., et al.
126. Sherr CJ, McCormick F. The RB and p53 pathways in cancer. *Cancer Cell*. 2002; 2:103–112. [PubMed: 12204530]
127. Yamasaki L. Role of the RB tumor suppressor in cancer. *Cancer Treat Res*. 2003; 115:209–239. [PubMed: 12613199]
128. Muller PA, Vousden KH. p53 mutations in cancer. *Nat Cell Biol*. 2013; 15:2–8. [PubMed: 23263379]
129. Li L, Davie JR. The role of Sp1 and Sp3 in normal and cancer cell biology. *Ann Anat*. 2010; 192:275–283. [PubMed: 20810260]
130. Lin WC, Yan MD, Yu PN, Li HJ, Kuo CC, Hsu CL, et al. The role of Sp1 and EZH2 in the regulation of LMX1A in cervical cancer cells. *Biochim Biophys Acta*. 2013; 1833:3206–3217. [PubMed: 24018208]
131. Kang R, Zhang Q, Zeh HJ 3rd, Lotze MT, Tang D. HMGB1 in cancer: good, bad, or both? *Clin Cancer Res*. Aug 1. 2013; 19:4046–57. [PubMed: 23723299]
132. Chen J, Xi B, Zhao Y, Yu Y, Zhang J, Wang C. High-mobility group protein B1 (HMGB1) is a novel biomarker for human ovarian cancer. *Gynecol Oncol*. 2012; 126:109–117. [PubMed: 22484401]
133. Yang GL, Zhang LH, Bo JJ, Huo XJ, Chen HG, Cao M, et al. Increased expression of HMGB1 is associated with poor prognosis in human bladder cancer. *J Surg Oncol*. 2012; 106:57–61. [PubMed: 22237993]
134. Liu Y, Xie C, Zhang X, Huang D, Zhou X, Tan P, et al. Elevated expression of HMGB1 in squamous-cell carcinoma of the head and neck and its clinical significance. *Eur J Cancer*. 2010; 46:3007–3015. [PubMed: 20724142]
135. Wu D, Ding Y, Wang S, Zhang Q, Liu L. Increased expression of high mobility group box 1 (HMGB1) is associated with progression and poor prognosis in human nasopharyngeal carcinoma. *J Pathol*. 2008; 216:167–175. [PubMed: 18680137]
136. Eckerdt F, Yuan J, Strebhardt K. Polo-like kinases and oncogenesis. *Oncogene*. 2005; 24:267–276. [PubMed: 15640842]
137. Koivunen J, Aaltonen V, Peltonen J. Protein kinase C (PKC) family in cancer progression. *Cancer Lett*. 2006; 235:1–10. [PubMed: 15907369]
138. Qi X, Tang J, Loesch M, Pohl N, Alkan S, Chen G. p38gamma mitogen-activated protein kinase integrates signaling crosstalk between Ras and estrogen receptor to increase breast cancer invasion. *Cancer Res*. 2006; 66:7540–7547. [PubMed: 16885352]
139. Bordeleau L, Panchal S, Goodwin P. Prognosis of BRCA-associated breast cancer: a summary of evidence. *Breast Cancer Res Treat*. 2010; 119:13–24. [PubMed: 19789974]
140. Lynch HT, Deters CA, Snyder CL, Lynch JF, Villeneuve P, Silberstein J, et al. BRCA1 and pancreatic cancer: pedigree findings and their causal relationships. *Cancer Genet Cytogenet*. 2005; 158:119–125. [PubMed: 15796958]
141. Thompson ME, Jensen RA, Obermiller PS, Page DL, Holt JT. Decreased expression of BRCA1 accelerates growth and is often present during sporadic breast cancer progression. *Nat Genet*. 1995; 9:444–450. [PubMed: 7795653]
142. Lee WY, Jin YT, Chang TW, Lin PW, Su IJ. Immunolocalization of BRCA1 protein in normal breast tissue and sporadic invasive ductal carcinomas: a correlation with other biological parameters. *Histopathology*. 1999; 34:106–112. [PubMed: 10064388]
143. Seery LT, Knowlden JM, Gee JM, Robertson JF, Kenny FS, Ellis IO, Nicholson RI. BRCA1 expression levels predict distant metastasis of sporadic breast cancers. *Int J Cancer*. 1999; 84:258–262. [PubMed: 10371343]

144. Dorairaj JJ, Salzman DW, Wall D, Rounds T, Preskill C, Sullivan CA, et al. A germline mutation in the BRCA1 3'UTR predicts Stage IV breast cancer. *BMC Cancer*. Jun 10. 2014; 14:421. [PubMed: 24915755]
145. Antoniou A, Pharoah PD, Narod S, Risch HA, Eyfjord JE, Hopper JL, et al. Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case Series unselected for family history: a combined analysis of 22 studies. *Am J Hum Genet*. 2003; 72:1117–1130. [PubMed: 12677558]
146. Gunduz E, Gunduz M, Nagatsuka H, Beder L, Demircan K, Tamamura R, et al. Epigenetic alterations of BRG1 leads to cancer development through its nuclear-cytoplasmic shuttling abnormalities. *Med Hypotheses*. 2006; 67:1313–1316. [PubMed: 16824695]
147. Medina PP, Romero OA, Kohno T, Montuenga LM, Pio R, Yokota J, et al. Frequent BRG1/SMARCA4-inactivating mutations in human lung cancer cell lines. *Hum Mutat*. 2008; 29:617–622. [PubMed: 18386774]
148. Reisman DN, Sciarrotta J, Wang W, Funkhouser WK, Weissman BE. Loss of BRG1/BRM in human lung cancer cell lines and primary lung cancers: correlation with poor prognosis. *Cancer Res*. 2003; 63:560–566. [PubMed: 12566296]
149. Glaros S, Cirrincione GM, Palanca A, Metzger D, Reisman D. Targeted knockout of BRG1 potentiates lung cancer development. *Cancer Res*. 2008; 68:3689–3696. [PubMed: 18483251]
150. Shain AH, Giacomini CP, Matsukuma K, Karikari CA, Bashyam MD, Hidalgo M, et al. Convergent structural alterations define SWI/SNF chromatin remodeler as a central tumor suppressive complex in pancreatic cancer. *Proc Natl Acad Sci USA*. 2012; 109:E252–259. [PubMed: 22233809]
151. Dal Molin M, Hong SM, Hebbar S, Sharma R, Scrimieri F, de Wilde RF, et al. Loss of expression of the SWI/SNF chromatin remodeling subunit BRG1/SMARCA4 is frequently observed in intraductal papillary mucinous neoplasms of the pancreas. *Hum Pathol*. 2012; 43:585–591. [PubMed: 21940037]
152. Patel AN, Goyal S, Wu H, Schiff D, Moran MS, Haffty BG. Mediator of DNA damage checkpoint protein 1 (MDC1) expression as a prognostic marker for nodal recurrence in early-stage breast cancer patients treated with breast-conserving surgery and radiation therapy. *Breast Cancer Res Treat*. 2011; 126:601–607. [PubMed: 20521098]
153. Yuan C, Bu Y, Wang C, Yi F, Yang Z, Huang X, et al. NFBDB1/MDC1 is a protein of oncogenic potential in human cervical cancer. *Mol Cell Biochem*. 2012; 359:333–346. [PubMed: 21853275]
154. Bhargava R, Lal P, Chen B. HER-2/neu and topoisomerase IIa gene amplification and protein expression in invasive breast carcinomas: chromogenic in situ hybridization and immunohistochemical analyses. *Am J Clin Pathol*. 2005; 123:889–895. [PubMed: 15899781]
155. Li Y, Xiong H, Yang DQ. Functional switching of ATM: sensor of DNA damage in proliferating cells and mediator of Akt survival signal in post-mitotic human neuron-like cells. *Chin J Cancer*. 2012; 31:364–372. [PubMed: 22739265]
156. Bower JJ, Karaca GF, Zhou Y, Simpson DA, Cordeiro-Stone M, Kaufmann WK. Topoisomerase IIalpha maintains genomic stability through decatenation G(2) checkpoint signaling. *Oncogene*. 2010; 29:4787–4799. [PubMed: 20562910]
157. Hande KR. Topoisomerase II inhibitors. *Update on Cancer Therapeutics*. 2008; 3(1):13–26.
158. Nitiss JL. Targeting DNA topoisomerase II in cancer chemotherapy. *Nat Rev Cancer*. 2009; 9:338–350. [PubMed: 19377506]
159. Chen M, Beck WT. DNA topoisomerase II expression, stability, and phosphorylation in two VM-26-resistant human leukemic CEM sublines. *Oncol Res*. 1995; 7:103–111. [PubMed: 7579726]
160. Ritke MK, Murray NR, Allan WP, Fields AP, Yalowich JC. Hypophosphorylation of topoisomerase II in etoposide (VP-16)-resistant human leukemia K562 cells associated with reduced levels of beta II protein kinase C. *Mol Pharmacol*. 1995; 48:798–805. [PubMed: 7476909]
161. Kolb RH, Greer PM, Cao PT, Cowan KH, Yan Y. ERK1/2 signaling plays an important role in topoisomerase II poison-induced G2/M checkpoint activation. *PLoS One*. 2012; 7:e50281. [PubMed: 23166842]

162. Kennedy RD, Gorski JJ, Quinn JE, Stewart GE, James CR, Moore S, et al. BRCA1 and c-Myc associate to transcriptionally repress psoriasin, a DNA damage-inducible gene. *Cancer Res.* 2005; 65:10265–10272. [PubMed: 16288014]
163. Pedersen-Bjergaard J, Daugaard G, Hansen SW, Philip P, Larsen SO, Rorth M. Increased risk of myelodysplasia and leukaemia after etoposide, cisplatin, and bleomycin for germ-cell tumours. *Lancet.* 1991; 338:359–363. [PubMed: 1713639]
164. de Voer RM, Geurts van Kessel A, Weren RD, Ligtenberg MJ, Smeets D, Fu L, et al. Germline mutations in the spindle assembly checkpoint genes BUB1 and BUB3 are risk factors for colorectal cancer. *Gastroenterology.* 2013; 145:544–547. [PubMed: 23747338]

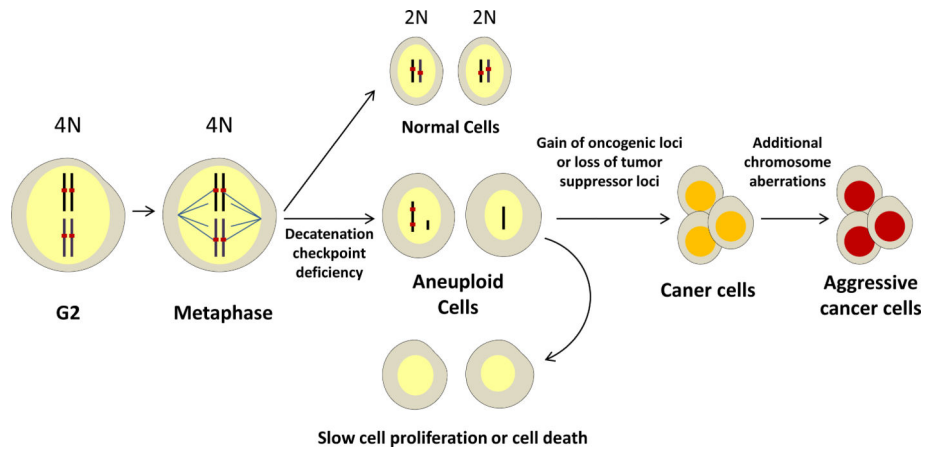


Figure 1. Cancer biology of CIN

Normally, a 4N cell in G2 enters mitosis, aligns its chromosomes in the metaphase plate, and equally distributes the DNA over two nuclei and subsequently, two daughter cells.³⁰ The decatenation checkpoint controls sister chromosome segregation and delays entry into mitosis until the knotted chromosomes have been decatenated by TOP2A. When the decatenation checkpoint is defective, chromosomal mis-segregation is observed. This causes aneuploidy and CIN. CIN can provide these evolving cell subclones with a mechanism that fosters unremitting genomic and mutational plasticity; this leads to increased cell survival, increased cell proliferation, and carcinogenesis. Furthermore, the decatenation checkpoint deficiency can result in additional chromosome imbalances of cancer cells, increasing tumor malignancy. CIN can slow the proliferation rate and cause cell death in cells with chromosomal alternations but no unremitting genomic and mutational plasticity.

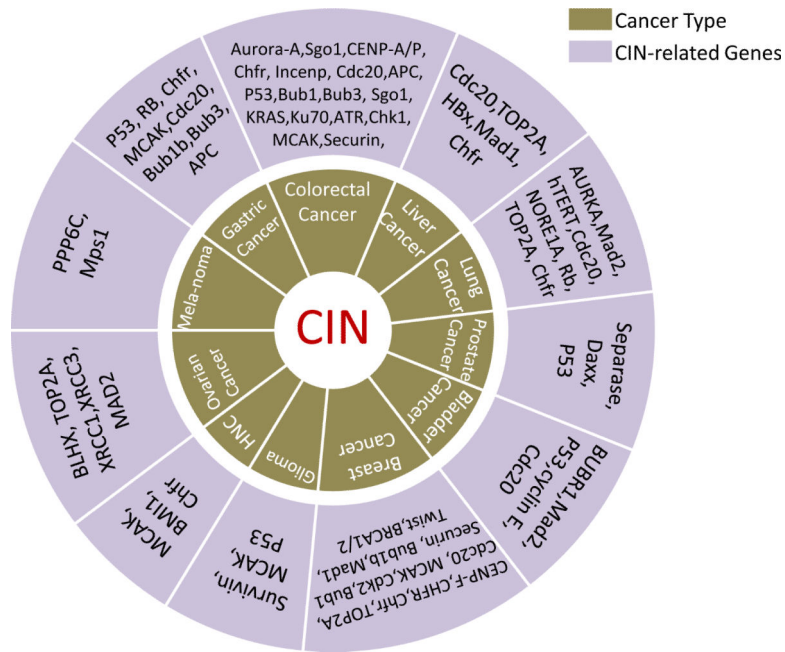


Figure 2. CIN-related gene aberrations in solid tumors
 CIN was recognized as a distinct feature of most solid tumors that have numerous CIN-related gene aberrations. The inner circle shows various cancer types. The outer circle shows related genes that cause CIN.

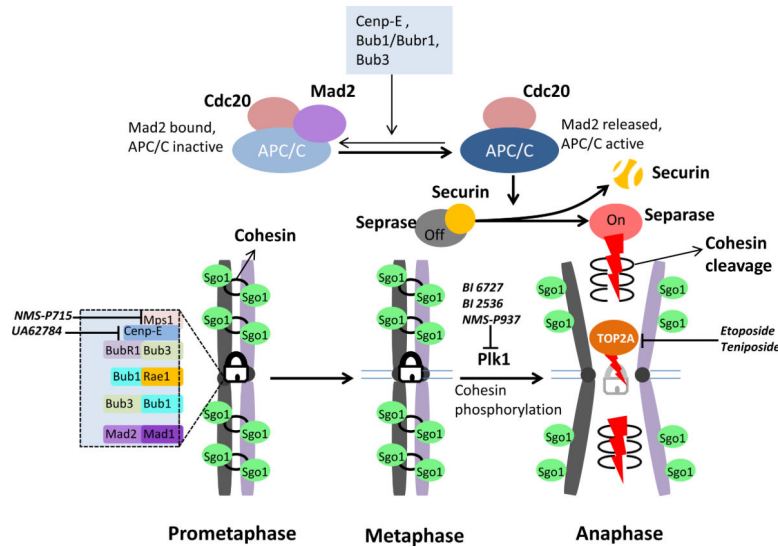


Figure 3. Regulation of sister chromatid separation

In pro-metaphase, various mitotic checkpoint proteins, including Mps1, Rae1, Bub1, Bub3, BubR1, Mad1, and Mad2, bind kinetochores that lack attachment or tension. Unattached chromosomes apparently generate a signal that delays progress to anaphase until all sister chromatids are attached to the spindle apparatus. This signal is transduced by the spindle-checkpoint complex that includes CENPE and the Mad/Bub proteins, resulting in inhibition of APC/C^{Cdc20}. After attachment of the last kinetochore to the mitotic spindle, the “wait anaphase” signal is extinguished. This allows APC/C^{Cdc20} to become active, resulting in the ubiquitin-dependent degradation of cohesion. The separase is then activated, and the protease catalyzes the cleavage of cohesion complexes that contain Rec8, which bridges the aligned sister chromatids. The kinase Plk1 marks Rec8 at chromatid arms by phosphorylation so that Rec8 will be degraded by separase, while a complex of Sgo1 is recruited by Bub1 to centromeres. Sgo1 can bind and protect Rec8 from phosphorylation by Plk1. Sister chromatid separation is also dependent on TOP2A, which targets inner centromeres and decatenates centromeric DNA. The newly separated chromatids can then migrate poleward along the spindle axis during anaphase. Once the chromosomes become bi-oriented, Sgo1 is dephosphorylated at Thr-346 and Sgo1 no longer binds to cohesion. NMS-P715, NMS-P937, BI6727, BI2536, UA62784, etoposide, and teniposide target the mitotic checkpoint for cancer therapy.

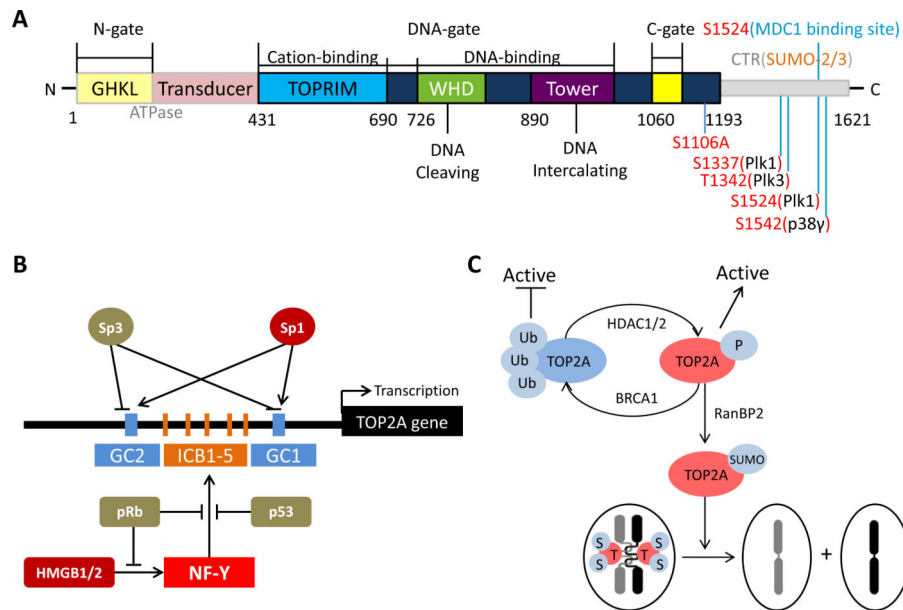


Figure 4. Regulation of TOP2A

A) Domain arrangement of TOP2A. TOP2A possesses 3 distinct subunit dimerization interfaces, termed the N-gate, DNA-gate, and C-gate. Functional regions and post-modification sites are colored and labeled. GHKL, GHKL (gyrase, Hsp90, histidine kinase, MutL) family ATPase; TOPRIM, Mg²⁺ ion-binding Topoisomerase/Primase fold; WHD, winged helix domain; CTR, C-terminal domain. **B)** Transcriptional regulation of *TOP2A* expression. The expression of human *TOP2A* is controlled by its promoter region that contains two GC boxes and five CCAAT boxes. NF-Y recognizes and binds to the ICBs. This binding of NF-Y to the *TOP2A* promoter can be promoted by HMGB1/2 and inhibited by pRb. In the *TOP2A* promoter, GC1 and GC2 flank ICB1 and ICB5, respectively. Two specificity proteins, Sp1 and Sp3, regulate *TOP2A* transcription by binding to both GC1 and GC2. Sp1 is a transcriptional activator and can up-regulate *TOP2A* transcription, while Sp3 is a transcriptional repressor of TOP2A and a common modulator of Sp1-dependent transcriptional activation. **C)** Post-translational modifications of TOP2A. TOP2A is activated by phosphorylation and enhanced by HDAC1 and HDAC2, but it is inhibited by the E3 ubiquitin ligase activity of BRCA1. SUMO modification, which is catalyzed by RanBP2, leads TOP2A to accumulate at inner centromeres and is essential for proper sister chromosome separation in mitosis. P, phosphorylation; S, SUMOylation; T, TOP2A.

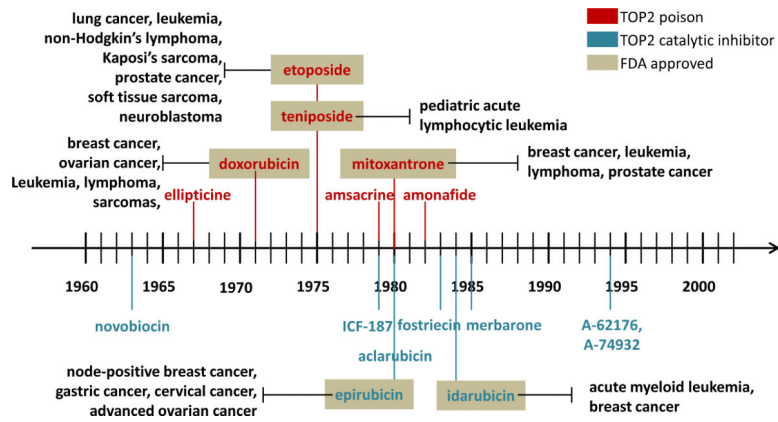


Figure 5. TOP2-targeting agents in chemotherapy

Drugs that target TOP2 fall into one of two categories: poisons (red) or catalytic inhibitors (blue). US Food and Drug Administration-approved TOP2-targeted inhibitors (grey) include etoposide, teniposide, doxorubicin, idarubicin, epirubicin, and mitoxantrone.

Table 1

Proteins involved in chromosome segregation

Protein	Description	Function	Ref.
Bub1	Budding uninhibited by benzimidazole 1	Inhibit CDC20 by phosphorylation	14,15
Bub1b	Bub1p	Encoding Bub receptor 1 protein	16
Bub3	Budding uninhibited by benzimidazole 3	Localize Bub1 and BubR1 to kinetochores	17
Mad1	Mitotic arrest deficient 1	Recruit Mad2 to unattached kinetochores	18
Mad2	Mitotic arrest deficient 2	Bind to CDC20 and inhibit APC/C activity	19,20
Cenp-E	Centromere protein E	Activate Bubr1 at the unattached kinetochore	21
CMT2	Charcot-Marie-Tooth gene 2	Inhibit mitotic checkpoint signaling by antagonizing MAD2	22,23
MPS1	Monopolar spindle 1	Phosphorylate Bub1	24,25
ZW10	Zeste white 10 protein	Recruit the MAD1–MAD2 heterodimer to unattached kinetochores	26,27
ROD	Rough deal protein	Recruit the MAD1–MAD2 heterodimer to unattached kinetochores	26,28
Zwilch	N/A	Recruit the MAD1/2 heterodimer to unattached kinetochores	26,29
Securin	N/A	Activate separase	30
Separase	N/A	Cleave the cohesion links	31,32
CDC20	Cell-division cycle protein 20	Cofactor of APC/C	33,34
Cdh1	Cadherin-1	Cofactor of APC/C	35,36
TOP2A	Topoisomerase IIalpha	Decatenation checkpoint, separate knotted and intertwined DNA molecules, decatenate intertwined daughter DNA duplexes	37-39
Chfr	Checkpoint with Forkhead and RING finger domains	E3 ubiquitin ligase, inhibit cyclin B nuclear import, regulate Mad2 and BubR1 functions	40
Nup98	Nucleoporin 98	Prevent cohesion degradation	35
Rae1	Bub3-related protein RNA export factor 1	Prevent cohesion degradation	35
RanBP2	Ran binding protein 2	Promote disentanglement of sister chromatids	37
Plk1	Polo-like kinase I	Phosphorylate and remove cohesion complex proteins	41,42
Sgo1	Shugoshin 1, inner centromere protein	Counteract phosphorylation of cohesion; may directly inhibit separase	43
Incenp	Inner centromere protein	Localize along chromosome arms in anaphase	44,45
Cdc5	Polo-like kinase	Phosphorylate and remove meiotic cohesin	46
Survivin	Inhibitor of apoptosis protein	Unclear; may play multiple roles in regulating apoptosis and cell division	47
MCAK	Mitotic centromere-associated kinesin	Coordinate onset of sister centromere separation	48
Orc6	Origin recognition complex 6	Coordinate chromosome replication and segregation	49
MIIP	Migration and invasion inhibitor protein	Interact with CDC20 and inhibit APC/C activity	50

Table 2

Factors involved in regulation of TOP2A's activity

Factor	Description	Mechanism	Function	Ref.
CKII β	Casein kinase I β	Phosphorylation	Enhance TOP2A activity	94
PKC	Protein kinase C	Phosphorylation	Enhance TOP2A activity	97
ERK2	Extracellular signal-related kinase 2	Phosphorylation	Enhance TOP2A activity	98
p38 γ	p38 MAPK family	Phosphorylation of Ser1542 of TOP2A	TOP2A stability and activity	99
Plk1	Polo-like kinase 1	Phosphorylation of Ser1337 and Ser1524 of TOP2A	Activate TOP2A	100
Plk3	Polo-like kinase 3	Phosphorylation of Thr1342 of TOP2A	Activate TOP2A	101
BRCA1	E3 ubiquitin ligase activity	Ubiquitination	Reduce TOP2A activity	105
Rb	Retinoblastoma protein	Unknown	Inhibit TOP2A activity	111
SUMO2/3	SUMO isoforms	SUMOylation	Centromeric localization of TOP2A	114
PIAS γ	SUMO E3 ligase	SUMOylation	Centromeric localization of TOP2A	114,115
RanBP2	Nuclear pore complex protein with SUMO E3 ligase activity	SUMOylation	Direct TOP2A to inner centromeres, accumulate TOP2A in metaphase	37
HDAC1/2	Histone deacetylase 1 and 2	Deacetylation	Enhance TOP2A activity	116
ISWI	Nucleosome remodeling factor	Interaction of TOP2A with chromatin	Facilitate the hydrolysis ability of TOP2A	119
BRG1	Transcription activator, member of the SWI/SNF family	Interaction of TOP2A with chromatin	Enhance TOP2A activity	39
MDC1	Multi-functional checkpoint protein	Unknown	Activate the decatenation checkpoint controlled by TOP2A	95

Table 3

Genetic aberrations of TOP2A and related genes in human cancers

Protein	Gene alteration event	Cancer type	Ref.
TOP2A	Amplified or deleted (depending on cancer types)	Breast cancer, esophageal cancer, ovarian cancer, gallbladder cancer, oral cancer, hepatocellular cancer, prostate cancer	120-125
P53	Mutation, deletion, epigenetic silencing	Lung cancer, breast cancer, gastric cancer, colorectal cancer, bladder cancer, ovarian cancer, hepatocellular cancer, head and neck cancer, pancreatic cancer, nasopharyngeal carcinoma, lymphoma	126-128
Rb	Mutation, deletion, epigenetic silencing	Lung cancer, breast cancer, gastric cancer, colorectal cancer, bladder cancer, ovarian cancer, hepatocellular cancer, head and neck cancer, pancreatic cancer, nasopharyngeal carcinoma, lymphoma	126-128
Sp1	Overexpression	Breast carcinomas, thyroid cancer, hepatocellular carcinomas, pancreatic cancer, colorectal cancer, gastric cancer, lung cancer, cervical cancer	129,130
HMGB1	Overexpression	Gastric colorectal cancer, prostate cancer, skin cancer, pancreatic tumor, NSCLC, hepatocellular cancer, head and neck cancer, bladder cancer, nasopharyngeal carcinoma	131-135
Plk1	Mutation, overexpression	Melanoma, breast cancer, ovarian cancer, thyroid cancer, colorectal cancer, prostate cancer, pancreatic cancer, head and neck cancer, NSCLC, non-Hodgkin lymphoma	136
PKC	Mutation, up- or down-regulation (depending on cancer type)	Gastric cancer, esophageal cancer, colorectal cancer, bladder cancer, breast cancer, ovarian cancer, hepatocellular cancer, basal cell cancer, prostate cancer, endometrial cancer, pancreatic cancer, renal cancer, B-cell lymphoma	137
p38 γ	Overexpression	Breast cancer	138
BRCA1	Downregulation, mutation	Breast cancer, ovarian cancer, prostate cancer, pancreatic cancer, lung cancer	99, 139-145
BRG1	Mutation	Breast cancer, ovarian cancer, prostate cancer, lung cancer, pancreatic cancer, colorectal cancer	146-151
MDC1	Overexpression	Cervical cancer, breast cancer	152,153