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Synthetic Lethality and Cancer: Cohesin and PARP at the Replication Fork

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Abstract

Cohesins are mutated in a significant number of tumors of various types making them an attractive target for chemotherapeutic intervention. However, cohesins have a spectrum of cellular roles including sister chromatid cohesion, transcription, replication, and repair. Which of these roles are central to cancer biology and which roles can be exploited for therapeutic intervention? Genetic interaction networks in yeast have identified synthetic lethal interactions between mutations in cohesin and replication fork mediators. These interactions are conserved in worms and in human cells suggesting that inhibition of replication fork stability mediators such as poly (ADP-ribose) polymerase (PARP) could result in the specific killing of tumors with cohesin mutations. These findings also highlight the utility of genetic interaction networks in model organisms for the identification of clinically relevant interactions. Here we review this type of approach, emphasizing the power of synthetic lethal interactions to reveal new avenues for development of cancer therapeutics.

Keywords

synthetic lethality; cohesion; replication fork; PARP; cancer; genetic networks

Leveraging synthetic lethal interactions to treat cancer

Tumor cells are genetically distinct from the surrounding, noncancerous tissue. The specific genetic differences that distinguish tumor cells from normal cells can be exploited to yield selective killing of cancers¹. These genetic differences often make the cancer cell dependent on the activity of a particular gene or pathway for viability that is not essential for normal cell growth. Thus, a cancer cell harboring an oncogenic mutation may be susceptible to loss of another gene that is not otherwise essential. This is known as synthetic lethality: when mutations in either of two genes are independently viable, but when present together the combination results in inviability (Figure 1A). The two most common models presented to explain synthetic lethality are that (i) the two genes function in parallel pathways, with each contributing to some process essential to viability, or (ii) the two genes encode proteins that form part of an essential complex that is partially functional in the absence of either one of the genetic interacting partners, but whose function is lost by the absence of both. A

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clinically relevant synthetic lethal interaction has been observed between mutations in the breast cancer susceptibility genes *BRCA1* or *BRCA2* and inhibition of poly(ADP-ribose) polymerases (PARPs). *BRCA1*- and *BRCA2*-defective cells are sensitive to siRNA-mediated knockdown or chemical inhibition of PARP^{2,3}. These synthetic lethal interactions have led to the clinical testing of PARP inhibitors as potential anti-cancer drugs^{4,5}.

This finding suggested a new approach to developing cancer therapeutics. However, one of the major challenges of the synthetic lethal chemotherapeutic paradigm is finding synthetic lethal partners for mutations commonly found in tumors. The number of possible gene-pair or gene-drug combinations that need to be tested can be prohibitive in cell culture. Because many of the pathways underlying genomic stability and proliferation control are evolutionarily conserved, genome-wide genetic interaction screens in model organisms, such as yeasts or *Caenorhabditis elegans*, can identify genes or processes that when inhibited lead to synthetic lethality with tumor-associated mutations (Figure 1B). Synthetic lethal interaction mapping has been done most extensively in yeast, such as *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, due to the amenability of yeast to large-scale genetic analysis^{6,7}. Synthetic lethal interactions identified in model organisms can be brought forward for further development in tissue culture and xenograft models as monotherapies or combination therapies for the treatment of human tumors.

Here we discuss recent data from large-scale genetic interaction screens and more focused investigations that demonstrate that cohesins, which are frequently found mutated in a range of tumors, have a critical role in replication fork maintenance. Furthermore, we discuss how synthetic lethal interactions uncovered in yeast suggest that disruption of replication fork mediators, in particular PARPs, could be an effective synthetic lethal strategy to specifically kill tumor cells with cohesin dysfunction.

What is cohesin?

Genetic screens in yeast identified cohesin as critical for sister chromatid cohesion (SCC) and accurate chromosome segregation^{8,9}. Since that initial discovery it has been shown that the structure and function of cohesin is well conserved across organisms¹⁰. The cohesin core complex is comprised of four proteins: Smc1 and Smc3, which form a heterodimer, the kleisin Mcd1 (also known as Scc1 or Rad21) and Scc3. In humans the role of Scc3 is filled by two mitotic Scc3 paralogs; STAG1 (Stromal Antigen 1/SA-1) and STAG2 (Stromal Antigen 2/SA-2) and a meiotic Scc3 paralog STAG3 (Stromal Antigen 3/SA-3). The four core proteins form a ring-shaped complex that can tether chromatin strands and facilitate sister chromatid cohesion until the appropriate time for chromosome segregation (reviewed in¹¹). There are cohesin accessory proteins that are essential for efficient sister chromatid cohesion, such as Pds5, the cohesin loader complex Scc2-Scc4, and the acetyl transferase Eco1, and there are non-essential cohesin-associated proteins that have been shown to contribute to the efficacy of SCC and other cohesin functions (Figure 2A).

Although the best-characterized role of cohesin is the regulation of SCC, the cohesin complex has additional roles. Cohesin establishment is tightly linked to replication, it has a role in the activation of replication origins¹², and, along with cohesin-associated proteins, it is needed for replication stress tolerance and the restart of stalled replication forks^{13–15}. In addition, cohesin has a critical role in homologous recombination (HR)-mediated repair of double strand DNA breaks during G2¹⁶. It has also been shown that cohesin can affect gene expression through its effects on chromatin organization by defining regions of active and inactive chromatin^{17–21}.

Cohesin is mutated or misexpressed in different tumor types

Many tumors exhibit genomic instability, and, given the function of cohesin in chromosome segregation and DNA repair, it is not surprising that cohesin dysfunction has been observed in a number of different tumor types. The cohesin and cohesin-associated genes RAD21, SMC3, PTTG1, PDS5A, ESP1, WAPL and ESCO2 have been found to be overexpressed in certain tumors (reviewed in ²²). The overexpression of cohesin can correlate with poor prognosis in breast cancers ^{23, 24}. Reduction of cohesin overexpression can decrease cell growth and increase the cytotoxicity of etoposide and bleomycin, ²⁵ suggesting that cohesin overexpression may contribute directly to tumor proliferation and resistance to DNA damaging agents. Conversely, underexpression of cohesin has also been associated with tumors. RAD21 is underexpressed in some oral squamous cell carcinomas ²⁶, and down-regulation of the cohesin-associated gene SGO1 has been linked to chromosomal instability in colorectal cancer ²⁷ supporting the notion that cohesin levels are critical to prevent tumorigenesis.

In addition to cohesin expression changes, mutations affecting cohesin have been found in a number of different tumor types. The first link between cohesin mutations and tumors was made in a study that sequenced 102 human homologues of yeast genes that are required to prevent chromosomal instability ²⁸. From an initial screen of 36 colorectal tumors, somatic mutations were found in five genes, of which four were cohesin or cohesin-accessory genes. Further sequencing of these four cohesin genes in 95 additional tumors identified five more mutations for a total of ten mutations in cohesin or cohesin-associated genes: four in the core cohesin SMC1A, four in the cohesin-loader NIPBL/Sccl, one in SMC3, and one in the meiotic Sccl paralog STAG3/SA-3. Although each of the cohesin genes was found mutated at a low frequency (0.7 – 3.0 %), collectively, mutations affecting cohesin or cohesin-associated genes were identified in ~7.5% of all colorectal tumors tested. In this study the frequency of cohesin mutations in colorectal tumors is likely to be an underestimate, as only four cohesin genes were sequenced in the larger panel of tumors. Cohesin mutations are not limited to colorectal tumors. More recently, sequencing has identified recurrent cohesin mutations in leukemias ^{29–31}, ovarian tumors ^{32, 33}, Ewing's sarcomas, melanomas, and glioblastomas ³⁴.

Based on currently available data, the most frequently mutated cohesin in tumors is STAG2. A detailed analysis of STAG2 in tumors used Affymetrix 250K single-nucleotide polymorphism (SNP) arrays, DNA sequencing, and Western blotting to determine copy number alterations, mutations, and loss of expression of STAG2 in human cancer cell lines from several tumor types ³⁴. This analysis found that STAG2 was mutated or not expressed in 21% of Ewing's sarcomas and in 19% of both melanoma and glioblastoma tumors. Another study looking at STAG2 expression in colorectal carcinomas (CRC), gastric carcinomas (GC), non-small cell lung cancers (NSCLC), and prostate carcinomas (PCA) found loss of STAG2 expression in 27% of GC, 23% of CRC, and 30% of PCA ³⁵. Interestingly, the authors could not find mutations in the STAG2 gene in the tumors lacking STAG2 expression. Similarly, two tumors lacking STAG2 expression in the first study ³⁴ were not correlated with mutations in the STAG2 gene suggesting that STAG2 expression may be silenced in some tumors.

One explanation for the high frequency of STAG2 loss is that STAG2 is on the X chromosome so there is only one copy in males and one active copy in females. Therefore, a single somatic mutation or silencing event is sufficient to result in loss of STAG2. Tumor cells that have lost STAG2 expression are viable, which is surprising given the importance of cohesin for chromosome segregation. The viability of STAG2-deficient cells is possibly due to the fact that STAG2 has a paralog, STAG1, and that cohesin complexes utilize either

STAG1 or STAG2 depending on chromosomal context. STAG1 is the predominant form in telomeric regions, and loss of STAG1 results in cell death due to defective telomere replication^{36,37}. By contrast, STAG2 is associated with cohesin complexes at the centromere, and its loss results in defective centromeric cohesion³⁶. Interestingly, frequent loss of STAG2 but not STAG1 has been observed in tumors. Similarly, DNA sequencing of AML tumors identified mutations in STAG2 but not STAG1 or STAG3³⁰ suggesting that certain cohesin genes are more mutable in tumors than others. A similar phenomenon is observed in randomly generated cohesin mutations in yeast with some cohesins much more mutable to a chromosome instability phenotype³⁸.

The role of cohesin in tumors

Although much is known about the function of cohesin in regulating SCC and DNA damage repair, it is not as yet clear which aspects of cohesin biology might contribute to tumor progression (reviewed in²²). Because cohesin is essential for cell viability it is not surprising that most cohesin mutations identified in tumors exist as heterozygous missense mutations, with the exception of STAG2 (discussed above). The fact that cohesin mutations in tumors are frequently missense mutations and most often heterozygous suggests that cohesin function in these tumors is reduced and not completely absent. The relative disruption of function by these mutations could have important implications for which cohesin functions are affected by the mutations in tumors. In yeast, limiting levels of cohesin subunits have been shown to result in a differential effect on the different roles of cohesin. Systematic reduction of cohesin subunit levels demonstrated that chromosome condensation, DNA stability in repetitive regions, and DNA repair are affected when cohesin levels are reduced by 70%, whereas SCC and chromosome segregation remained unaffected even when cohesin was reduced by 87%³⁹. Consistent with this observation from yeast, heterozygous mutations in the human cohesin loader component NIPBL/Sccl result in developmental abnormalities associated with Cornelia de Lange syndrome but do not affect SCC^{40,41}. This suggests that the heterozygous mutations in tumors, if they cause reduced function, are more likely to affect DNA repair, repetitive DNA stability, and chromosome condensation, and not have a major effect on chromosome segregation. This hypothesis is supported by the observation that of 19 AML cases with cohesin mutations, 12 had normal cytogenetics, six had fewer than four cytogenetic abnormalities, and only one exhibited complex cytogenetics³⁰. Similarly, STAG2 deficient tumor-derived cell lines and matched lines that had STAG2 restored demonstrated that loss of STAG2 results in mild chromosome cohesion defects and only a modest increase in aneuploidy³⁴. The nature of the cohesin mutations observed in tumors suggest that processes other than SCC are disrupted.

Using genetic networks to understand cohesin dysfunction

One approach to understand the biological effects of cohesin mutations is to screen for genetic interactions with other mutations⁴². Identifying negative genetic interactions such as synthetic lethality/growth defects or positive genetic interactions such as phenotypic suppression can deconvolve the functions associated with mutations in multifunctional proteins such as cohesins. In a cross-species analysis, the model organisms, *S. cerevisiae* and *C. elegans* were used to construct a network of evolutionarily conserved negative genetic interactions with cohesin mutations with the aim of better understanding the biology of cohesin dysfunction and identifying potential therapeutic targets for tumors with cohesin mutations⁴³. Synthetic genetic array (SGA) (Box 1) analysis of temperature sensitive (ts) missense mutants of two core cohesins and one cohesin loader against a collection of non-essential gene deletions and conditional alleles of essential genes that encompassed approximately 95% of all genes in *S. cerevisiae* identified 1695 statistically significant negative genetic interactions, which were filtered based on magnitude (EC < 0.3) to yield

437 interactions with 365 array genes (Figure 2B). One of the goals of the SGA analyses was to identify therapeutic targets that could be effective with a number of different cohesin mutations (Box 2). To this end, interactions were limited to the 33 interaction partners that showed a negative genetic interaction with at least two of the three cohesin mutations and had an obvious human homolog. The 33 interactors were enriched for genes involved in replication, microtubule biology, and mRNA processing. Surprisingly, none of the interactors were explicitly DNA repair genes, which would have been expected if the cohesin mutations were leading to DNA breaks. To identify the most promising potential therapeutic targets, the network was further limited to those interactions that resulted in complete synthetic lethality with at least two of the query genes. This resulted in 19 interactions between three cohesin mutations and just seven interactors: *ctf18*, *dcc1*, *csn3*, *chl1*, *ctf4*, *rad61*, and *mdm20* (Table 1). The seven interactors were remarkably similar; all the interactors except *mdm20*, which encodes a subunit of the NatB N-terminal acetyltransferase, have been linked to the replication fork (Figure 2A). In addition, all seven interactors, including *mdm20*, are sensitive to sublethal doses of the replication stressors hydroxyurea, camptothecin, or methyl methanesulfonate ⁴⁴, suggesting that the interactors have a role in responding to or tolerating replication stress. All the interactors share a role mediating replication fork stability in response to replication stress and several are components of the Fork Protection Complex (FPC) and are required for activation of the DNA Replication Checkpoint (DRC). The synthetic lethality of cohesin mutations and components of the FPC and DRC suggests that cells require additional replication fork stability to maintain viability when cohesin is mutated.

The synthetic lethality of cohesin mutations with a number of different replication fork mediators in yeast makes replication fork mediators attractive targets for therapeutic intervention. However, none of the replication fork mediators identified in the SGA screens have known inhibitors. Based on the fact that many of the general mechanisms that maintain replication fork stability are conserved between yeast and humans, the synthetic lethality observed between cohesin mutations and replication fork mediators would be expected to extend to replication fork mediators found in human cells that are not conserved in yeast.

Cohesin at the Replication Fork

The specific synthetic lethality of cohesin mutations and replication fork mediator mutations strongly argue for a role for cohesin in maintaining replication fork stability. A detailed analysis of cohesin and replication in yeast demonstrated that cohesin has a direct role in the recovery of stalled replication forks ¹⁴. ChIP-chip analysis revealed that cohesin is transiently enriched at active early replication origins when replication is perturbed by treatment with hydroxyurea or methyl methanesulfonate and at naturally occurring replication pause sites ¹⁴. Furthermore, the localization of cohesin to sites of replication stress was dependent on the Mre11-Rad50-Xrs2 complex and one of either of the checkpoint kinases Mec1 or Tel1 but not γ -H2AX. Consistent with these observations, a thermosensitive *SCC1* mutant, *scc1-73*, was shown to be exquisitely sensitive to sublethal doses of hydroxyurea or methyl methanesulfonate. Following methyl methanesulfonate treatment, replication slowed in *scc1-73* mutants, and DNA fiber combing experiments detected a four to five-fold increase in unreplicated DNA compared to wild type. These replication progression defects were also associated with a decrease in branched X-spike DNA molecules, which are intermediates of the HR-related template-switching mechanism of replication fork restart suggesting that cohesin accumulation at stalled forks is needed for efficient template switching. From this study it is clear that cohesin has a critical role in maintaining replication fork integrity that is dependent on the MRX complex but is independent of DSB formation.

In light of these studies a model emerges for the synthetic lethality of cohesin and replication fork mediators. Mutations in cohesin result in sensitivity to replication stress and reliance on replication fork mediators to efficiently replicate the genome, probably through replication fork restart mechanisms such as HR, fork regression and reversal, and template switching. When replication fork mediators are mutated or inhibited in cells with cohesin mutations, endogenous replication stress is sufficient to block the restart of stalled replication forks and completion of replication resulting in inviability.

Replication fork stress response and PARP

The response to replication stress in both yeast and vertebrate cells is remarkably similar even though some of the proteins mediating the response are not conserved. In response to camptothecin, which traps topoisomerase I (Top1) on the nicked DNA intermediate during replication, replication forks in human cells and yeast cells slow progression and can regress to form a four-armed reversed fork⁴⁵, a structure that has been proposed to promote replication fork restart^{46, 47}. Yeast and vertebrate cells also accumulate components of the Mre11-Rad50-Xrs2/Mre11-Rad50-Nbs1 complex in response to replication stress^{14, 48–50}. In human cells both of these responses are at least partially dependent on PARP1^{45, 49, 51}. The PARP family of proteins catalyze post-translational modifications of target proteins through poly(ADP-ribosyl)ation, which has been implicated in a number of biological processes including DNA repair, replication, transcription, mitochondrial function, and cell division⁵². PARP1 has been shown to mediate various aspects of DNA repair including the repair of single strand breaks (SSBs)⁵³, and loss or inhibition of PARP1 results in synthetic lethality with cells lacking BRCA1 or BRCA2^{3, 54}. This synthetic lethal interaction has led to the development of small molecule PARP inhibitors as potential chemotherapeutic agents. The initial models for PARP1-BRCA1 and PARP1-BRCA2 synthetic lethality proposed that loss of PARP1 resulted in an increase of SSBs that became double stranded breaks (DSBs) when encountered by the replication fork and that these replication-derived DSBs required BRCA1- and BRCA2-mediated HR for repair. More recently, this model has been challenged by the observations that SSBs do not accumulate in PARP mutants⁵⁵, and SSB repair mutants are not synthetic lethal with BRCA1 or BRCA2⁵⁶. Furthermore, Ewing Sarcoma cells containing the *EWS-FLI1* translocation are very sensitive to PARP inhibition even though the cells appear to be proficient for DNA damage repair⁵⁷. Although the mechanism of PARP BRCA1/2 synthetic lethality remains unclear, it is apparent that PARP plays a role in non-HR resolution of replication fork intermediates. PARP inhibitors sensitize cells to chemicals that cause replication fork stress such as hydroxyurea, topoisomerase poisons, and alkylating agents^{49, 51, 58}. These studies demonstrate that PARP1 is a mediator of replication fork stability.

Consistent with the role of PARP in maintaining replication fork stability, mutations in PARP genes in *C. elegans* result in somatic cell proliferation defects in combination with a hypomorphic mutation in *him-1*, the *C. elegans* *SMC1* ortholog. These proliferation defects are similar to those observed for *him-1* mutants that have had replication fork mediators depleted by RNA interference. Furthermore, treatment with PARP inhibitors reduced the proliferation of cultured human colorectal tumor-derived cells depleted for different cohesin components demonstrating that the SL interaction is conserved in human cells and is not specific to a single cohesin component⁴³. More recently, the PARP-cohesin interactions have been recapitulated using glioblastoma-derived STAG2 deficient cell lines and paired STAG2 complemented cell lines³⁴, demonstrating that loss of the cohesin STAG2 correlates with hypersensitivity to PARP inhibition (M. Bailey, N. O'Neil, and P. Hieter, unpublished results). As cohesin mutations are recurrent in tumors of various types, the SL interaction between cohesin mutations and PARP inhibitors could represent a new therapeutic approach for the treatment of these tumors. Further work testing the efficacy of

PARP inhibitors on cohesin-mutated tumor growth in xenograft models could lead to clinical trials of PARP inhibitors in the treatment of tumors with cohesin mutations.

Concluding remarks

Construction of genetic interaction networks in yeast has revealed highly connected synthetic lethal interactions between mutations in cohesin and replication fork mediators. When cohesin is dysfunctional, cells rely on replication fork stress response proteins to complete replication. From recent data, a model is emerging in which replication forks experiencing replication stress are remodeled to promote non-DSB dependent restart. In vertebrate cells these processes are catalyzed by PARP (Figure 3). It remains to be discovered how these responses are initiated in yeast, which lack PARP. The fact that the synthetic lethal interactions between cohesin and replication fork mediators extends to PARP suggests inhibition of other recently discovered replication fork protecting proteins such as SMARCAL1 or ZRANB3 (Reviewed in ^{59, 60}) could be an effective treatment for tumors with cohesin or cohesin-associated mutations as well. Based on these initial successes, we expect that genetic networks in yeast and other model organisms will be useful tools for predicting more clinically relevant genetic and chemical-genetic interactions.

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Highlights

Synthetic lethal networks in yeast identify clinically relevant genetic interactions.

Cohesins are recurrently mutated in a variety of tumor types.

Cohesin mutations are synthetic lethal with mutations in replication fork mediators.

PARP inhibitors can selectively kill tumors with cohesin mutations.

Box 1: Synthetic Genetic Array Analysis

Synthetic genetic array (SGA) is a technology used to identify genetic interactions in yeast on a genome-wide scale. In SGA, a haploid yeast strain bearing a “query” mutation in a gene of interest is mated to an arrayed collection of yeast strains bearing knockouts of nonessential genes, or temperature-sensitive or hypomorphic alleles of essential genes. Diploids are selected using nutritional or drug-resistance markers contributed by each of the parents, and then these diploids are induced to undergo meiosis and sporulation.

The query strain carries with it two crucial kinds of genetic constructs: the first is a recessive drug-resistance allele. The gene most commonly used is *CAN1*, as *can1* mutants are resistant to the arginine analog canavanine. The recessive drug resistance marker is used to counterselect against diploids that do not undergo meiosis. The query strain also carries a nutritional gene driven by a mating type-specific promoter. For example, *STE2pr-SpHis5⁺* confers histidine prototrophy only to MAT α yeast. This prevents the growth of MAT α yeast, and thus prevents unwanted mating during spore germination, and further selects against unsporulated diploids.

Following sporulation, the yeast are grown on medium selecting against diploids, for a single mating type, and for each of the mutant genes or alleles. In parallel, the yeast are grown on medium selective only for the array mutation. The plates are then imaged and colony size, which is an indirect measure of growth rate, is digitally analyzed. Statistical analyses are used to normalize colony size to the entire population and then compare colony sizes between experimental and control samples. Negative or aggravating genetic interactions, such as synthetic lethality, are found when colony size is significantly less than the cumulative growth defect predicted between the query and array mutations. A positive genetic interaction, sometimes known as an alleviating interaction, occurs when the colony size of the double mutant is equal to or greater than that of the single mutant, depending on the model used.

Box 2: Synthetic lethal screening for clinically relevant interactions

Many of the pathways involved in chromosome stability are evolutionarily conserved. Therefore, information from experiments with model organisms can have relevance to the biology of human tumors. Yeast provides an experimental platform from which to investigate the function and biological relevance of mutations and combinations of mutations identified in human cancer tumors. Synthetic lethal genetic interactions with disease-associated mutations can identify potential therapeutic targets and provide biological context in an unbiased manner and uncover unexpected genetic interactions.

The genetic amenability of yeast and the development of high throughput genetic techniques such as synthetic genetic array (SGA) analysis make it possible to screen a mutation of interest (query gene) against mutations in every other gene in the genome (array gene).

To identify relevant gene interactions the “noise” of false positive interactions must be reduced. Although some of the false positives can be minimized by technical considerations such as technical and biological replicates, experimental design considerations can boost the signal to noise ratio. For example, comparing SGA data sets derived from mutations in different genes of a complex or biological pathway can eliminate gene- or allele-specific interactions and identify genetic interaction partners that are common to a biological process. In this way the interactors identified have a broader synthetic lethal profile and would be expected to interact with a number of different related mutations.

Similarly, the confidence in genetic interactions can be further strengthened if there are interactions between the query gene and multiple array genes that form complexes or pathways. For example, in one study cohesin mutations were synthetic lethal with both *dcc1* and *ctf8*, which are both part of the Ctf18-RFC complex.

Prior knowledge, such as biological function and phenotype, can also be used to organize large genetic interaction networks to identify relevant function-related groups of interactions or “hubs”. If multiple interactors share functions, such as tolerance of specific types of DNA damage or cell cycle control, it is possible that the interaction will extend to other genes within that pathway or broader process. This can even extrapolate to genes that are not present in yeast allowing for an expansion of the genetic network to include human specific genes.

Figure 1. Synthetic Lethality in tumors and yeast. A. Synthetic lethal interactions can be exploited to specifically kill tumor cells. In the example, tumor cells with mutations in a tumor-associated CIN (Chromosome Instability) gene require the activity of the non-essential gene A for viability. If gene A is inhibited, tumor cells with mutations in the CIN gene are inviable, whereas non-tumor cells are viable. In this way, genetic differences in tumor cells can be leveraged to affect tumor specific killing with minor effects on the surrounding non-tumorous cells. B. Synthetic lethal screening with cohesin mutations in yeast. Haploid mutants carrying sub-lethal mutations in cohesin are mated to haploid mutants carrying viable mutations of every other gene in the yeast genome using SGA technology (See Box 1). Cells carrying hypomorphic mutations in cohesin require the activity of certain other genes for viability. When these second site genes are mutated, cohesin mutants are no longer viable and colonies do not grow. Growth defects can be measured by digital imaging and comparison to single mutant growth.

A

B

Figure 2.

A. Model of the cohesins Smc1, Smc3, Scc1, and Scc3 and cohesin-associated proteins Scc2-Scc4, Pds5, and Rad61 at an active replication fork. Pds5 and Rad61 are bound tightly to cohesin until the replication fork passes. Cohesion is established after passage of the replication fork and coincides with the disassociation of Pds5 and Rad61. Synthetic lethal interactors with cohesin mutations are colored orange ⁴³. (B) Three different cohesin mutations affecting *smc1*, *scc1*, and *scc2* were screened using synthetic genetic arrays (SGA) to identify synthetic lethal interactions, which may have clinical relevance for the treatment of tumors containing cohesin mutations. i. SGA data filtered to eliminate interactions that lack statistical significance and with minor effects on growth. ii. Data further filtered to only include interactors with human homologs and that interact with at least two of the query genes to eliminate gene- or allele-specific interactions. iii. Data further filtered to only include synthetic lethal interactions (no growth) to identify the strongest genetic interactions. Orange denotes proteins known to function at the replication

fork. It is of note that all hits are non-essential genes and when mutated exhibit sensitivity to the chemical replication fork stresses hydroxyurea, methyl methanesulfonate, and camptothecin.

Figure 3.

A speculative model of the synthetic lethal interaction of cohesin mutations and PARP inhibition. A. In non-tumor cells with wild type cohesin, replication proceeds normally and PARP is not activated. B. In non-tumor cells with wild type cohesin, inhibition of PARP does not affect DNA replication, as PARP is not required. C. In tumor cells with cohesin mutations or dysfunction resulting in either a quantitative or qualitative change in cohesin function, replication forks can stall. Stalled replication forks activate PARP1, which in turn parylates itself and other substrates (poly-ADP ribose (PAR) chains are shown in green). PAR chains localize PAR-binding proteins to the stalled fork. These proteins include the chromatin remodelers MTA1 and CHD4⁷¹, and DNA repair proteins such as MRE11^{45, 49, 51}. PARP activity promotes the regression of the stalled fork to form a four-armed reversed fork that could facilitate DSB-independent replication fork restart. D. When PARP is inhibited in tumor cells with cohesin mutations and stalled replication forks, the

necessary proteins are not recruited to the stalled fork, the replication fork does not regress, and the replication fork breaks resulting in a DSB.

Table 1

Replication fork stress response genes

<i>S. cerevisiae</i>	Role in Replication	<i>C. elegans</i>	Human
Csm3	Csm3, together with Tof1, Mrc1, and Ctf4, is part of the intra-S phase fork protection complex (FPC) ⁶¹ and is required for the DRC after genotoxic stress.	F23C8.9	TIPIN
Ctf4	Ctf4 is a component of the FPC and interacts with a number of replication and cohesion proteins linking DNA synthesis to sister chromatid cohesion ⁶²⁻⁶⁴ .	F17C11.10	WDHD1
Dcc1	Ctf8 and Dcc1 are components of the Ctf18 Replication Factor C-like complex. Ctf18-RFC controls replication fork velocity, spacing, and the ability to restart forks stalled by genotoxic stress ^{13, 15} . Ctf18-RFC also activates the Mrc1-dependent DNA replication checkpoint (DRC) to stabilize stalled forks and prevent the firing of late replication origins in response to genotoxic stress ^{15, 65} .	K09H9.2	DCC1
Ctf8		T22C1.4	CTF8
Chl1	Less is known about Chl1, a probable helicase; however, it is known that it interacts with Ctf18 and Eco1 ⁶⁶ .	<i>chl-1</i>	DDX11
Rad61	Rad61/Wapl inhibits cohesion establishment outside of S phase and maintains the proper cycling of cohesion establishment during replication and repair ⁶⁷⁻⁶⁹ . In human cells the association of Rad61/Wapl and cohesin slows the replication fork and loss of Rad61/Wapl allows forks to progress rapidly even in the absence of the Alt-RFC ¹³ .	<i>wapl-1</i>	WAPAL
None	PARP is required to form regressed replication forks in response to replication stress and blocks MRX resection at stalled replication forks ^{45, 70} .	<i>pme-1</i>	PARP1
None		<i>pme-2</i>	PARP2
None	SMARCAL1 protects against replication stress ⁵⁹ .	C16A3.1	SMARCAL1
None	ZRANB3 protects against replication stress ⁶⁰ .	None	ZRANB3