Replication Stress: Cancer's Double-Edged Sword

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ABSTRACT: Cancer is fundamentally a disease of aberrant genetics. In contrast to nonmalignant cells, cancer cells are not only able to tolerate these genetic insults but are in fact enabled by them. While cancer treatment has progressed significantly in the last century, many current medical treatments rely on general cytotoxicity for rapidly dividing cells, rather than inhibiting the specific genetic lesions that enable malignant transformation. One promising area is the development of inhibitors targeted towards DNA replication. By targeting core pathways mutated in cancer, DNA replication inhibitor therapies offer new avenues for cancer treatment that spare patients the worst side effects of cytotoxic chemotherapy. One such target is RECQ1, a human helicase that shows great promise in cancer treatment when targeted as an adjuvant to other replication-based chemotherapies. In addition to summarizing the role replication stress plays in carcinogenesis, this paper discusses a triple combination of RECQ1, PARP1, and TOP1 inhibitors. While aggressive, this approach may increase the efficacy of these existing cancer therapies.

KEYWORDS: Biomedical and Health Sciences; Genetics and Molecular Biology of Disease; Cancer; Genomic Instability; DNA Repair; Replication Stress; Combined Inhibitors; RECQ1.

■ Introduction

Cancer is a broad grouping of diseases in which abnormal cells divide uncontrollably. Projected to kill over 600,000 people in the United States this year alone,[1] cancers are a subset of neoplasms – cells that grow without regulation and form a mass of tissue. However, in contrast to other neoplastic tissues, such as adenomas, cancer cells are malignant. They possess limitless replicative potential and may exhibit invasion into distant sites.

Despite their varying complexities and wide variety, many cancers may be characterized by certain key features, as laid out in 2000 by Hanahan and Weinberg's *The Hallmarks of Cancer* and updated in *Hallmarks of Cancer: The Next Generation*.[2,3] In the *Hallmarks*, Hanahan and Weinberg explicate six general characteristics of neoplastic disease — sustained growth signals, insensitivity to growth suppressors, avoidance of cell death, limitless replicative capabilities, induction of angiogenesis, and tissue invasion with the formation of metastases (Figure 1).[2] As cells progress towards a neoplastic state, they develop the abilities of each hallmark, capabilities that enable tumor growth and metastatic dissemination. In 2011, these hallmarks were updated to include reprogramming energy metabolism, avoiding immune damage, and acquisition of genomic instability, emphasizing the importance of continued investigation into this class of disease.[3]

While the *Hallmarks* deliver a descriptive analysis of cancer, inferential studies into the mechanisms underpinning the development of these hallmarks suggest an evolutionary model of carcinogenesis.[4,5] The dominant theory of carcinogenesis today entails a hypermutator phenotype that allows for stochastically generated gene mutations to be selected in a manner similar to that observed in Darwinian evolutionary theory (Figure 2).[5,6] It has been shown that many cancers achieve this hypermutator phenotype by developing genomic instability which allows cancer cells to sample a multitude of different phenotypes to best adapt to a microenvironmental niche.[7] Greater knowledge of the mechanisms that produce this genomic instability will not only improve our understanding of cancer as a disease but also enable us to develop new, targeted treatments.
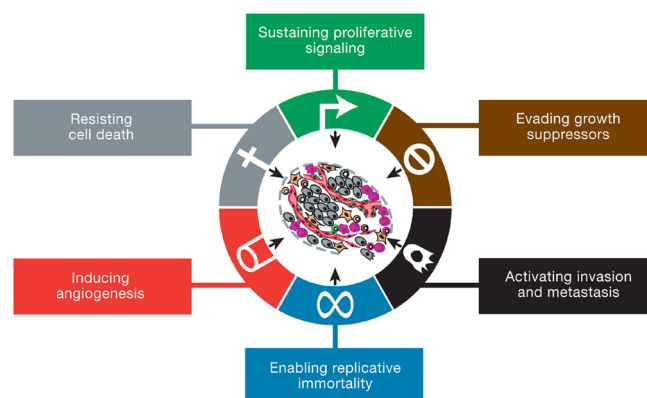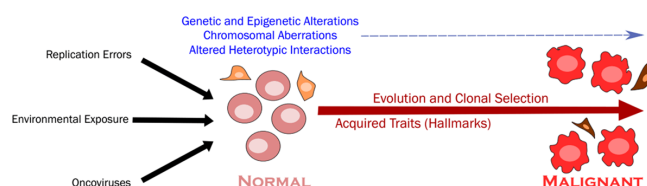

Figure 1: The six original hallmarks of cancer.[3]

Figure 2: The transformation process is an evolutionary mechanism.[4]

■ Discussion

At the core of genomic instability is deoxyribonucleic acid (DNA). In order for life to propagate, DNA must be replicated, thereby passing information from one generation to the next. In eukaryotic cells, this replication occurs once

during the cell cycle in the S-phase. This must be done with high precision,[8] and there are multiple mechanisms that ensure this high-fidelity replication.

DNA Structure:

DNA is a double helix made of two strands of nucleotides linked by a sugar-phosphate backbone and annealed to one another by hydrogen bonding between complementary base pairs (Figure 3). The two strands run in opposite directions and are said to be antiparallel meaning one strand runs in the 5'→3' direction, while the other runs 3'→5'. The molecular structures of the nitrogenous bases comprising DNA allow complementary hydrogen bonds between specific bases — Adenine (A) with Thymine (T) and Guanine (G) with Cytosine (C).
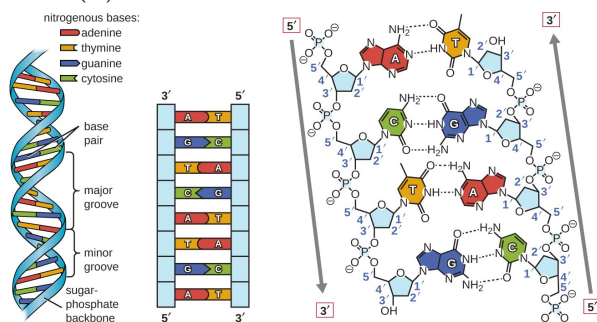

Figure 3: Structure of DNA.[9]

The two antiparallel strands separate during DNA replication and each of the original strands serves as a template for a new strand. The new strands form when nucleotides are added by DNA polymerase (DNA pol) based on complementarity with the original strand. This results in semi-conservative DNA replication, in which two new DNA double helices, each consisting of one original strand and one daughter strand, are formed (Figure 4). This guarantees that each resulting daughter cell will have a full complement of double-stranded DNA.
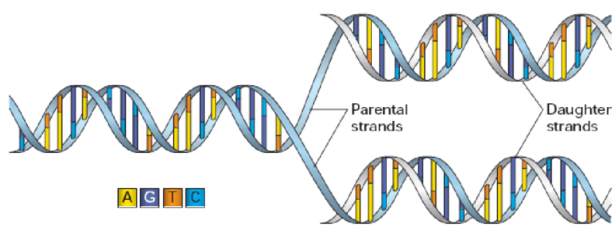

Figure 4: DNA Replication.[10]

DNA Replication:

While the principles of DNA replication are in many ways intuitive, the molecular process by which replication occurs involves a complex apparatus of proteins and nucleic acids that ensures proper regulation.

Before DNA replication can occur, the two original DNA strands must be separated to allow polymerases and other proteins to access the template strand. This separation is mediated by a group of enzymes called helicases, which couple their unwinding action to adenosine triphosphate (ATP) hydrolysis. As base-pair hydrogen bonds are broken and the DNA strands separated, ATP hydrolysis provides

the energy for helicase translocation along the DNA double helix, typically in the 5'→3' direction.[11]

This unwinding action produces a torsional strain on the DNA strands, which is rectified by a class of enzymes called the topoisomerases. This torsional strain results from DNA strands being twisted into tighter helices than their structures allow, termed positive supercoiling.[12] This torsional strain can cause structural distortions and DNA damage. DNA gyrase, which is a member of the topoisomerase family, relaxes these twists via negative supercoiling — twisting *against* the helical conformation, unwinding, and straightening the structure of the helix.[13] Single-stranded binding (SSB) proteins then bind to the single-stranded DNA (ssDNA) strands after they have been separated to prevent the strands from reannealing while also protecting the ssDNA from nucleolytic cleavage.[14]

While the above enzymes are imperative for successful eukaryotic DNA replication, the key enzyme remains DNA polymerase, which catalyzes the addition of deoxyribonucleotide 5′-triphosphates (dNTPs) to the elongating DNA polymer, and which also possesses endonucleolytic activity.[15] Free nucleotides align opposite their complementary base partners, and DNA polymerase moves along the two strands in a 5'→3' direction, covalently joining these free nucleotides.[16]
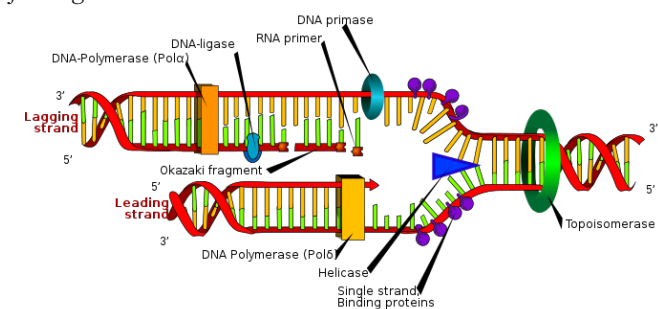

Figure 5: Replication Machinery.[17]

Helicase's unwinding of the double-stranded DNA by breaking hydrogen bonds between base pairs occurs at specific regions called origins of replication. This action creates a replication fork, with the two-parent DNA strands running in antiparallel directions. While each DNA strand is replicated at a given replication fork, DNA polymerase can only synthesize in a 5'→3' direction, meaning one of the parent strands will be replicated continuously, and one will be replicated discontinuously. Consequently, the replication fork proceeds in a single direction by generating a continuous leading strand and a lagging strand consisting of discontinuous Okazaki fragments (Figure 5). Because DNA polymerase can only join nucleotides to the 3'-hydroxyl group of ribonucleotides, replication is initiated by de novo synthesis of an RNA primer by primase, thus providing DNA polymerase with a free 3'-hydroxyl to which a nucleotide may be joined.[18] On the lagging strand, DNA polymerase elongates away from the replication fork, eventually meeting the RNA primer for the previously synthesized Okazaki fragment, prompting termination of its synthetic function and initiation of its endonucleolytic function. This results in degradation of the RNA primer of the previously synthesized

Okazaki fragment and replacement of these ribonucleotides with deoxyribonucleotides by DNA polymerase. The Okazaki fragments are then joined by DNA ligase to form a continuous strand by covalently joining the sugar-phosphate backbones together with a phosphodiester bond.

DNA replication must be highly accurate to maintain genetic integrity. High-fidelity DNA replication relies on three key aspects — specific nucleotide selection by replicative polymerases, exonucleolytic proofreading, and post-replication DNA mismatch repair (MMR).[19]

As aforementioned, the eukaryotic genome is replicated by DNA polymerases, consisting of pol α, pol δ, and pol ε. Polymerase α synthesizes approximately 20–30 nucleotides during Okazaki fragment origination, and polymerase δ then extends this lagging strand.[20] DNA pol α lacks its proofreading; however, any errors it introduces are later removed by polymerase δ and its exonucleolytic proofreading activity.[21,22] Errors that persist despite this proofreading are then corrected by MMR.[23] More complex errors must be corrected by the DNA damage response (DDR), discussed below.

DNA Repair:

Cells have multiple repair pathways in place to recognize and correct DNA damage, known collectively as the DNA damage response (DDR) system. There are five significant pathways — base excision repair (BER), homologous recombination (HR), non-homologous end joining (NHEJ), nucleotide excision repair (NER), and mismatch repair (MMR).[24] A brief description of BER, HR, and NHEJ follows, as these three are of significance to therapies discussed below.

The BER pathway is recruited when a molecular insult is made to a single nucleobase through exposure to radiation or genotoxic molecules.[25] This single-nucleobase damage is recognized by DNA glycosylase, which removes the damaged base from the strand by cleavage of the N-glycosidic bond via an activated water.[25] AP endonuclease then recognizes the abasic site (AP-site) and creates a nick in the phosphodiester backbone.[25] DNA polymerase then inserts a base at the AP-site, after which DNA ligase seals the nick.[25]

Homologous recombination (HR) and non-homologous end-joining (NHEJ) both repair double-strand breaks in DNA.[26] Such breaks can form from exposure to reactive oxygen species (ROS) generated by normal respiratory metabolism inside a cell, external agents such as ionizing radiation, and certain chemotherapeutic drugs.[26] Additionally, during meiosis, HR facilitates recombination between homologous maternal and paternal chromosomes to produce gametes.[26] The primary mechanism by which HR occurs has been elucidated. Specialized helicases unwind the damaged DNA into its two constituent strands, allowing HR proteins to facilitate pairing between the damaged DNA and the same segment on the undamaged homologous chromosome.[26] DNA polymerases are then recruited to repair the break through replication, using the homologous strand as a template.[27] This process is relatively slow and requires extensive homology between regions of the two chromosomes, but results in the high-fidelity repair of damaged DNA (Figure 6).[27]
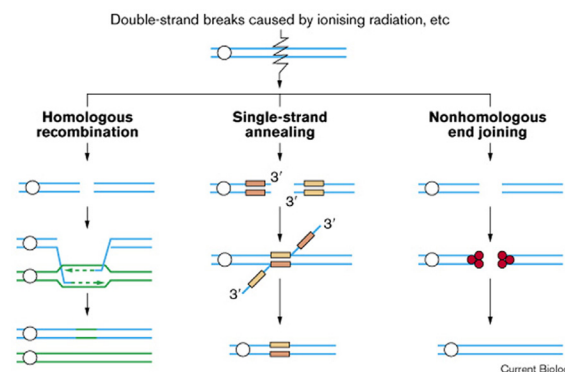

Figure 6: DNA repair pathways for double-strand breaks.[26]

In contrast, NHEJ requires no homology with another DNA duplex, instead of removing the damaged DNA and ligating the two break ends.[28] NHEJ is directed by microhomologies (short homologous DNA sequences) found on the ends of the double-strand breaks, as only when these sequences are compatible does NHEJ repair accurately.[28]

DNA Replication Stress & Cancer Therapy:

DNA replication faces several obstacles, both intracellular and extracellular, which can cause replication stress wherein DNA synthesis is stalled or slowed, resulting in DNA damage.[29] In eukaryotic cells, replication origins form bidirectional replication forks.[30] Once DNA replication begins, the cells need to maintain accuracy, speed, and proper resource allocation to maintain polymerase processivity.[30] Thus, replication origins are fired in a regulated fashion, thereby preventing the potentially catastrophic consequences of replication stalling — cytotoxicity, deleterious mutation, and malignant transformation.[30]

The primary mechanism by which fork stalling can cause DNA damage is through the formation of extended stretches of ssDNA, which occurs when replicative helicases continue to unwind the template DNA despite polymerase stalling.[31] These stretches of ssDNA alongside the newly replicated, yet stalled, dsDNA activate a primer-template junction replication stress response.[32] This results in the recruitment of replication stress response proteins, most notably the protein kinase ATM and Rad3-related (ATR).[33-36] ATR is a major replication stress response kinase that phosphorylates substrates in order to help the cell continue DNA replication despite stress.[33-36] ATR's activation inhibits cell cycle progression, gives more time for repair, and stabilizes the stalled fork.

ATR senses replication stress through interaction with protective ssDNA-binding proteins, such as ribonucleoprotein A (RPA), which bind to the extended ssDNA segments to prevent DNA damage.[37] Through this protein-protein interaction, ATR becomes active, setting off a chain of molecular signaling events that culminate in the phosphorylation of Chk1 kinase.[38] Additionally, ATR activation triggers the cell cycle S-M checkpoint, thereby preventing entry into mitosis when unreplicated DNA is present.[39] In the absence of ATR or Chk1, cells are unable to respond efficiently to stalled replication forks, resulting in the inappropriate firing of new replication forks, causing RPA depletion and DNA damage.[40] Furthermore, as the burden of ssDNA increases due to further

origin firing, there is a greater probability of replication fork collapse, resulting in DNA double-strand breaks (DSBs) and unreplicated DNA.[41] If these cells are allowed to enter the M phase, their unreplicated chromosomes will activate cell death through mitotic catastrophe.[40] Indeed, while the genomic instability conferred by increased replication stress can be advantageous to cancer, excessive replication stress can result in cell death, a phenomenon that holds great therapeutic potential.[42,43]

While ATR responds to stalled replication forks specifically, there are several molecular perturbations may cause further stalling to occur. Nicks, gaps, and stretches of ssDNA, when encountered by DNA replication machinery, can be converted into DSBs, leading to catastrophic DNA damage.[29] Moreover, DNA lesions — physical barriers to replication fork progression — generated by endogenous or exogenous DNA-damaging agents, such as UV light and chemical mutagens, can cause replication stress if left unrepaired.[44] Furthermore, despite the high fidelity of DNA polymerases, both polymerase δ, and polymerase ε may fail to distinguish dNTPs from rNTPs. These mistakes should ideally be rectified by RNase H2-mediated ribonucleotide excision repair. However, these errors have been found recently to be made at an atypically high rate,[45,46] producing yet another source of replication stress through the erroneous incorporation of the wrong nucleotide species. Additional sources of replication stress include collisions between replicative and transcriptional complexes, inherently unstable DNA regions, and drug-mediated nucleotide depletion.[47-50]

As aforementioned, cancer is able to develop in many cases because of its genomic instability, which confers the potential for the rapid adaptation necessary for its unrestrained growth. Tumor cells achieve this instability through persistent replication stress, which is included as an "enabling characteristic" of cancer in the revisited *Hallmarks* review.[3,29,51] However, this enabling characteristic has been exploited by therapeutics that target repair pathways needed to ameliorate this replication stress.[52] This allows for the selective killing of cancer cells while sparing nonmalignant ones.[52] Such therapeutics are based on the principle of synthetic lethality whereby one can kill cancer cells that have become "addicted" to a specific cellular pathway due to the absence of another, through its inhibition.[3] Replication stress is essentially increased within these tumor cells,[43] an exploitation of their predilection for it. The most notable example of this strategy is the use of poly (ADP-ribose) polymerase (PARP) inhibitors in breast cancer-associated (BRCA1/2)-deficient cancers, discussed below.

Current Methods for Cancer Therapy Targeting DNA Replication Stress:

Camptothecin (CPT) – Topo I inhibitor. While topoisomerases can broadly be described as enzymes that cleave and repair phosphodiester bonds during normal DNA replication,[53] there are actually two distinct subtypes: Topo I, which creates a single-stranded DNA nick, and Topo II, which creates a double-stranded DNA break.[54] In addition to endonuclease activity, both Topo I and Topo II possess ligase activity, allowing them to repair the DNA breaks once the threat of damage from helicase-induced supercoiling is resolved.[54] Because fail-

ure to repair these DNA breaks is lethal, this ligase activity can be targeted as a form of chemotherapy.

Camptothecin (CPT) is a cytotoxic alkaloid that binds to Topo I, forming Topo I-cc, a stable covalent ternary complex with DNA. CPT exerts its cytotoxic effects in the S phase, during which it converts ssDNA breaks into 1-ended dsDNA breaks as the replication fork collides with Topo I-cc.[55] Unlike 2-ended dsDNA breaks, which are caused by ionizing radiation, 1-ended dsDNA breaks cannot be repaired by NHEJ.[56] As a result of Topo I-cc, Ataxia telangiectasia mutated (ATM) and ATR, both members of the phosphatidylinositol 3-kinase (PI 3-kinase) like family of protein kinases (PIKKs) involved in cellular responses to DNA damage, are activated, leading to recruitment of HR repair pathways.[54] CPT additionally prompts hyper-phosphorylation of FANCJ, a DNA helicase important in the DNA replication stress response,[57] and suppression of microsatellite instability in an ATR-dependent manner.[58,59] Failure to activate any part of this stress response pathway in the presence of CPT results in DNA damage and apoptosis. Thus, CPT arrests cancer growth.

PARP1 Inhibitors. The poly (ADP-ribose) polymerases (PARP) are a family of DNA repair proteins that are important for BER (Figure 7).[60] PARP1, a specific enzyme, detects sites of ssDNA and synthesizes poly (ADP) ribose (PAR), which then recruits other repair proteins to the damaged DNA site, leading to repair of the DNA damage through BER.[61,62] When PARP, and therefore BER, is inhibited, SSBs accumulate and become DSBs.[61] Accumulation of DSBs drives cellular dependence on repair via HR and NHEJ. Dependence on pathways such as HR, in which BRCA1 and BRCA2 proteins play a major role, has allowed inhibition of PARP for therapeutic benefit. Indeed, because cells with BRCA deficiencies are unable to repair DSBs, PARP inhibition has proven to be a potent method of chemotherapy in BRCA-deficient cancers.[63] PARP inhibitors additionally enhance various other chemotherapies, such as irinotecan and carboplatin, by inhibiting the repair of SSBs induced by these agents. PARP inhibitors have been tested clinically, and there are currently several PARP inhibitors in development testing the two concepts — synthetic lethality in patients with deficient HR and removal of the cell's ability to repair DNA damage caused by certain chemotherapies.[61]
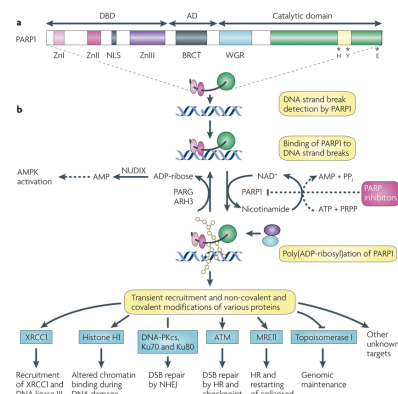

Figure 7: Structural & Functional Characteristics of PARP1.[61]

RECQ1 as an Emerging Anticancer Target:

Helicases are essential in maintaining genomic stability.[11] They play many roles in nucleic acid metabolism, including DNA replication, repair, transcription, and chromosome segregation.[64] Specifically, the RecQ family of helicases has proven indispensable for the maintenance of genomic integrity. These helicases serve as regulators of genetic recombination at stalled replication forks and help suppress elevated sister chromatid exchange.[65] RecQ Helicases are also involved in DSB repair, MMR, BER, and telomere maintenance.[65]

One human RecQ helicase, RECQ1, has been recognized in recent years to play a particularly significant role. RECQ1 (also known as RECQL or RECQL1) is involved in multiple cellular processes, primarily implicated in the maintenance of chromosomal stability, and its loss has been linked to tumorigenesis.[65,66] Studies of primary fibroblasts from RECQ1-knockout mice and human cells depleted of RECQ1 by RNA interference exhibit marked chromosomal instability.[67,68] Cancer cells have marked genomic instability, and having to rely on fewer DNA repair pathways due to mutations in other DNA repair factors leaves them vulnerable to detrimental effects from disrupting a specific DNA repair pathway that plays a major role, such as RECQ1. Due to the functions in DDR and repair pathways that DNA helicases perform, targeted inhibition of RECQ1 has thus arisen as a novel approach that is informative for developing anticancer approaches harnessing DNA replication.[69] RECQ1 silencing offers a powerful emerging avenue for cancer treatment, as it offers a targeted therapy that exploits the replication stress inherent to cancer.

Structure & Functions of RECQ1:

The *RECQ1* gene is located on chromosome 12p12 and it encodes a 649 amino acid protein weighing 73 kDa.[70] Like replicative helicases, RECQ1 catalyzes ATP-dependent unwinding of DNA and can unwind a plethora of DNA structures, including stalled replication forks.[71] It is the most abundant RecQ homolog present in humans,[72] and contains four domains: N-terminus, a core helicase, the RecQ-specific C-terminal (RQC) domain, and C-terminus (Figure 8). In addition to helicase activity, RECQ1 also activates the annealing of complementary ssDNA in an ATP-independent manner, facilitating HR DNA repair.[73]
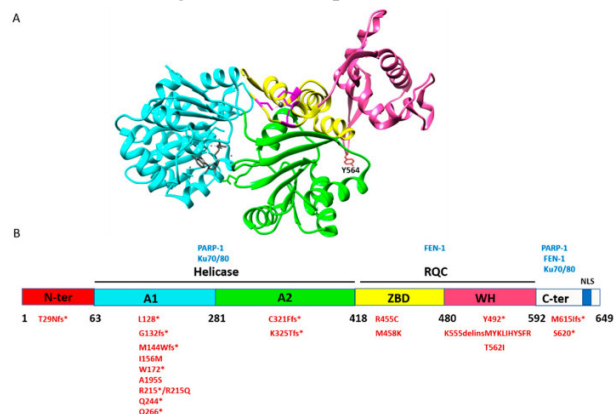

Figure 8: Human RECQ1 Protein; A – Crystal Structure, B – Domain Structure.[74]

RECQ1 & Cancer Expression:

Overexpression of RECQ1 has been observed in multiple malignancies,[75] including glioblastoma, multiple myeloma, ovarian cancers, hematological cancers, hepatocellular carcinoma, and head and neck cancers.[76-81] Silencing RECQ1 results in cancer cell death, suggesting its activity is cancer-protective.[82] Furthermore, depletion of RECQ1 sensitizes cancer cells to several genotoxic chemotherapeutics, including camptothecin, hydroxyurea, and temozolomide.[83] This finding indicates that RECQ1 may be necessary for cancer resistance to these agents. Moreover, RECQ1 expression is increased in response to DNA damage, enabling some malignant cells to persist despite drug-mediated replication fork stalling.[74] This enables some cancer cells to survive the cytotoxic effect of chemotherapeutic drugs, and therefore, additional inhibition of RECQ1 could be effective.

RECQ1 Silencing in Breast & Brain Cancer:

Evidence for such efficacy has been shown with mutations in RECQ1 and subsequent implications in breast cancer pathogenesis. A clear connection between RECQ1 and estrogen receptor alpha (ERα) has been established.[84] ERα is a master regulatory transcription factor in breast cancer, and as reported by Lu *et al*., the expression of *ESR1*, the gene encoding ERα, is directly activated by RECQ1.[84] They report that >35% of RECQ1 binding sites were also bound by ERα genome-wide, suggesting that RECQ1 may act as a novel cofactor for ER-alpha. These findings demonstrate a new mechanism by which RECQ1 regulates gene expression in ER-positive breast cancer cells, thereby driving disease and serving as a critical elaboration on several previous reports suggesting RECQ1 as a breast cancer susceptibility gene.[74]

Immunohistochemical and Western blot analyses comparing malignant and nonmalignant cells have also implicated RECQ1 as a driver of glioblastoma.[76] Mendoza-Maldonado *et al*. demonstrate that RECQ1 is overexpressed in tumors, dramatically so in human brain glioblastoma relative to control brain tissues.[76] They demonstrate higher levels of DNA damage in RECQ1-depleted glioblastoma cells, indicating that RECQ1 plays a protective role in glioblastoma survival, as well as a significant decrease in T98G proliferation when RECQ1 is depleted.[76] Furthermore, this work also shows that silencing RECQ1 expression sensitizes glioblastoma to hydroxyurea and temozolomide, two agents widely used to treat brain tumors — as RECQ1 is essential to genomic stability, DNA damage was increased and thus T98G and U87 cells were more susceptible to treatment.[76]

Moreover, using xenotransplantation of human glioblastoma cells into zebrafish embryos, Vittori *et al*. were able to elaborate on the findings of Mendoza-Maldonado *et al*.[85] They confirmed the growth-inhibiting effects of RECQ1 silencing in U87 glioblastoma cells *in vivo* in a zebrafish embryo model.[85] Using this approach, they were able to corroborate the *in vitro* effects of RECQ1 silencing observed by Mendoza-Maldonado *et al*. in an *in vivo* setting.[85] The results, by looking at fluorescently-labeled cells and observing tumor-associated angiogenesis, effects of drugs, and involvement of signaling pathways in tumor progression

demonstrated that RECQ1 silencing reduces the rate of increase in the number of U87 tumor cells through the result of cell cycle perturbation by RECQ1 knockout.[86-89]

Looking Forward:

With their work in breast and brain cancer, Lu *et al.*, Vittori *et al.*, and Mendoza-Maldonado *et al.* solidify the efficacy and role of RECQ1 inhibitors in cancer therapy.[76,84,85] However, while sole RECQ1 inhibition has potential, when combined with the aforementioned TOP1 and PARP1 inhibitors, it may have a greater impact. In research recently published in *Nature Structural & Molecular Biology* by Berti *et al.*, it was demonstrated how cancer cells respond to the damage caused by topoisomerase I inhibitors.[90] When TOP1 inhibition causes replication stress via the formation of nicks, the replication machinery pauses and reverses its course instead of colliding with the TOP1-induced DNA lesion. They find that two important cellular proteins, PARP and RECQ1, regulate this fork reversal mechanism.[90] They demonstrate an interplay between these two proteins whereby PARP activity at sites of CPT-induced DNA damage inhibits RECQ1-mediated premature replication fork restart.[90] This interplay suggests a potential therapeutic benefit in targeting RECQ1 and PARP as adjuvants to TOP1 inhibition, thus preventing repair of CPT-induced DNA damage by PARP. With TOP1 and PARP1 inhibition, any disruption of RECQ1 activity would prevent cells from restarting reversed forks. This proposed triple combination treatment highlights how RECQ1 inhibitors with PARP1 inhibitors like Olaparib and TOP1 inhibitors like Camptothecin, could cause excessive replication stress in malignant cells, resulting in targeted cell death. Cancer cells relying on DNA repair pathways like TOP1 and PARP1 would not even be able to turn to RECQ1 if these were inhibited. Evidence for such treatment has arisen in work surrounding multiple myeloma cells (MMCs) — Viziteu *et al.* demonstrated RECQ1 depletion sensitized MMCs to PARP inhibitors.[77] RECQ1 inhibitors involved like so, as adjuvants to others harnessing replication stress, should ideally be the new target in battling cancer as they could increase the efficacy of combinational cancer therapies.

However, RECQ1-based therapies in cancer are still mainly at a preclinical stage due to challenges in applying helicase inhibitors. In general, it is difficult to develop highly specific small molecule inhibitors. A significant barrier is bioavailability — the extent and rate at which the drug can access the site of action by entering the systemic circulation. Brosh & Datta recognize that among other cellular bioavailability parameters, inhibitor aqueous solubility, nonspecific binding to the cell membrane and extracellular matrix, cellular uptake, and intracellular metabolic stability must be considered for successful exploitation.[91] With helicases specifically, another challenge lies in ensuring helicase-interacting compounds reach their desired subcellular location — one potential method for nuclear-targeted delivery is nanoparticles.[91] With nuclear localization sequences (NLS), they should help deliver the small molecule inhibitors to target tumor sites. This specific delivery heightens the therapeutic effects and lessens negative consequences if achieved with sufficient concentration. With issues on the potency of helicase inhibitors, they need to be able to have their intended biological effects at minimal concentrations. Since the field of helicase inhibitors is relatively new, there are concerns with drug resistance mechanisms as well. While the basic principle is to compromise a helicase-dependent pathway of repair leading to synthetic lethality, other repair pathways might get involved that interfere with the helicase inhibitor.[91] This requires further research and prioritized attention. Lastly, there is a challenge with the structural configuration of inhibiting DNA repair proteins. The inhibitor compound needs to approach its target effectively; however, the proteins' flexibility and intrinsic conformational states cannot ensure a good fit.[91] Brosh & Datta report that machine learning algorithms and artificial intelligence can help such treatments with advanced structure-based drug design.[91] These obstacles need to be addressed if targets for therapy from cancer predisposition syndromes associated with mutations in DNA repair genes, like helicase inhibitors, can be built.

■ Conclusion

In summary, although replication fork instability can be a boon for cancer adaptation, we can leverage our knowledge to turn this stress into an advantage, exploiting its vulnerability. While RECQ1 is one such promising avenue, the complexity of replication makes it rich with therapeutic potential. Just as recognition of immune evasion as a hallmark of cancer has enabled the development of novel therapies in the form of checkpoint inhibitors and CAR T-cells, so too can the recognition of genomic instability as a central feature of cancer development lead to groundbreaking targeted cancer treatments.[2,3]

■ Acknowledgements

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