Targeting Genome Instability and DNA Repair

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68.1 Introduction

Genomic instability is a characteristic of most human cancers and plays critical roles in both cancer development and progression.

Genomic stability is dependent on faithful DNA repair and chromosome segregation during cell division [1].

To maintain genomic integrity, eukaryotes have evolved a system called the DNA damage response (DDR). DDR is a complex signal transduction pathway that allows cells to sense DNA damage and transduce this information to the cell to arrange the appropriate cellular responses to DNA damage [2, 3]. The failure to respond to DNA damage is a characteristic associated with genomic instability. This instability can manifest itself genetically on several different levels, ranging from simple DNA sequence changes to structural and numerical abnormalities at the chromosomal level. During S phase, the centrosome and genomic material are replicated concurrently, and replication errors are repaired prior to mitotic entry. During mitosis, equal segregation of chromosomes requires a bipolar mitotic spindle, telomeric preservation, and completion of the spindle assembly checkpoint. Ectopic amplification of centrosomes, telomerase dysfunction, and failure of the spindle assembly checkpoint may result in aborted mitosis. The majority of cancers exhibit chromosomal instability (CIN), which refers to the high rate by which chromosome structure and number changes over time in cancer cells compared with normal cells [4]. Although CIN is the major form of genomic instability in human cancers, other forms of genomic instability have also been described. These include accumulation of DNA base mutations and microsatellite instability (MIN), a form of genomic instability that is characterized by the expansion or contraction of the number of oligonucleotide repeats present in microsatellite sequences [4–6], and forms of genomic instability that are characterized by increased frequencies of base pair mutations [7].

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68.2 Hereditary Versus Sporadic Cancers

Familial breast cancer (BC) accounts for approximately 5–10% of BC cases. The most prevalent mutations leading to hereditary breast and ovarian cancer affect the homologous recombination (HR) genes BRCA1 and BRCA2. Heterozygous individuals carrying mutations of the BRCA1 or BRCA2 genes have a 40–80% risk of developing BC [8].

Patients (pts) with BRCA2 mutations have increased incidence of male breast, pancreas, and prostate cancer [3]. Tumors with BRCA1 or BRCA2 mutations are significantly associated with low level of 53BP1, indicating that 53BP1 mutation might confer a survival advantage in the absence of BRCA1 and BRCA2 [9]. Moreover, mutations in three additional HR genes, BACH1, PALB2, and RAD51C, have been identified in approximately 3% of familial BC pts and have been associated with a twofold increased risk of BC [10]. Mutations of CHK2, ATM, NBS1, and RAD50 have also been associated with a doubled risk of BC, indicating the importance of the ATM pathway, together with HR, in preventing BC formation. In hereditary cancers that are characterized by the presence of CIN, the genomic instability can also be attributed to mutations in DNA repair genes. The identification of mutations in DNA repair genes in hereditary cancers provides strong support for the *mutator hypothesis*, which states that genomic instability is present in precancerous lesions and drives tumor initiation by increasing the spontaneous mutation rate [4, 11, 12]. According to mutator hypothesis, the genomic instability in precancerous lesions results from mutations in caretaker genes, that is, genes that primarily function to maintain genomic stability [4, 11, 12]. Indeed, in inherited cancers, germline mutations targeting DNA repair genes are

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present in every cell of the patient's body. Thus, a single event—loss of the remaining wild-type allele—would lead to genomic instability and drive tumor development, as predicted by the mutator hypothesis. The classical caretaker genes are DNA repair genes and mitotic checkpoint genes [4]. Germline mutations in caretaker genes can explain the presence of genomic instability in inherited cancers. However, efforts to identify caretaker genes, the inactivation of which leads to genomic instability in sporadic (nonhereditary) cancers, have met with limited success [4, 13]. Thus, unlike hereditary cancers, the molecular basis of genomic instability in sporadic cancers remains unclear. A second hypothesis could explain the presence of CIN in sporadic cancers. That is *the oncogene-induced DNA replication stress model for cancer development* [14–18]. According to the second model, CIN in sporadic cancers results from the oncogene-induced collapse of DNA replication forks, which in turn leads to DNA double-strand breaks (DSBs) and genomic instability [4].

68.3 Cellular Mechanisms that Prevent or Promote Genomic Instability

68.3.1 Telomere Damage

Telomeres, which are located at the ends of each chromosome, consist of approximately 5–10 kbp of specialized, tandem repeat, noncoding DNA complexed with a variety of telomere-associated proteins [1, 19, 20]. These elements create a protective cap that prevents the recognition of the chromosomal termini as DSBs and their consequent aberrant repair via nonhomologous end joining (NHEJ) or HR [1, 21–24]. Due to the inability of DNA polymerase to fully replicate the ends of linear DNA molecules, in the absence of compensatory mechanisms, telomeric DNA is lost at the rate of approximately 100 base pairs (bp) per telomere per cell division [1, 25–27]. In normal somatic cells, this telomere erosion is used by the cell to monitor its division history, with moderate telomere shortening triggering either irreversible cell-cycle arrest, termed replicative senescence, or apoptosis [1]. This block to continued proliferation is thought to have evolved to prevent the development of cancer in long-lived organisms by restricting the uncontrolled outgrowth of transformed cell clones and also by preventing further telomere erosion which would accompany such abnormal growth and eventually destabilize the telomeres leading to CIN [1, 25, 28].

68.3.2 Centrosomes

Centrosome amplification, the presence of greater than two centrosomes during mitosis, is a common characteristic of most solid and hematological tumors that may induce multipolar mitoses, chromosome missegregation, and subsequent genetic imbalances that promote tumorigenesis [1, 29].

The centrosome is the primary microtubule organizing center in dividing mammalian cells [1]. The centrosome is duplicated in a semiconservative fashion with one daughter centriole formed next to a preexisting mother centriole, and this process only occurs once in every cell cycle [1, 30, 31].

Centrosome amplification arises from many different mechanisms, including centrosome over duplication [1, 31, 32], de novo assembly [1, 33], and mitotic failure downstream from mono- [34] or multipolar division [35]. Given that centrosome clustering may be advantageous for cancer cell survival, this process may be an attractive and specific therapeutic target [36–38]. Bipolar chromosome attachment during mitosis is ensured by a quality control mechanism known as the spindle assembly checkpoint [1]. The assembly checkpoint relies upon kinase signaling to delay cell-cycle progression and correct attachment errors. Aurora kinase B, for example, detects misattached chromosomes [1], and overexpression of the kinase is sufficient to disrupt the checkpoint and promote tetraploidy [1]. Moreover, mutations or expression changes in other checkpoint gene products may compromise the checkpoint and favor tumorigenesis [39].

68.3.3 DNA Methylation and Chromatin Remodeling

A vast array of epigenetic mechanisms contribute to the genomic instability in cancer cells [40]. One of them is the DNA methylation, which consists of the addition of a methyl group at the carbon 5 position of the cytosine pyrimidine ring or the number 6 nitrogen of the adenine purine ring [41]. Most cytosine methylation occurs in the context of cytosine-phosphate-guanine (CpG) dinucleotides and occurs via a group of DNA methyltransferase enzymes resulting in silencing of gene transcription [1]. A prominent example is the aberrant methylation of CpG islands in the promoter regions of DNA mismatch repair (MMR) genes that result in cancer cells with a "mutator phenotype" [1, 42]. In addition to DNA methylation, histone molecules that form the primary protein component of chromatin also regulate genome stability as well as gene transcription [43]. A number of posttranslational modifications such as acetylation, deacetylation, methylation, phosphorylation, and ubiquitination have been identified that alter the function of histones [1]. Various combinations of these posttranslational histone modifications have been hypothesized to form a "histone code" that dictate distinct chromatin structures that can affect genome stability pathways and transcription [1]. Therefore, in most cases, histone acetylation enhances transcription while histone deacetylation represses transcription.

In addition, histone acetylation can affect DNA repair. Similarly, histone ubiquitination can also modify DNA repair capacity [1, 44]. Finally, histone phosphorylation is an early event following DNA damage and required for efficient DNA repair [1].

68.3.4 Mitochondrial DNA Alteration in Human Cancers

Mitochondria are the key component of the oxidative phosphorylation system to generate cellular adenosine triphosphate. Mitochondrial genetic reprogramming and energy balance within cancer cells play a pivotal role in tumorigenesis [1]. Most human cells contain hundreds of nearly identical copies of mt-DNA, which are maternally inherited. A substantial number of studies identified somatic mt-DNA mutations involving coding and noncoding mt-DNA regions in various cancers [1].

Table 68.1 DNA lesions generated by endogenous and exogenous DNA damage [3]

Exogenous DNA damage	Dose exposure (mSV)	DNA lesions generated
Peak hr. sunlight	–	Pyrimidine dimers (6-4) photoproducts
Cigarette smoke	–	DSBs
Chest-X-ray	0.02	DSBs
Mammography	0.4	DSBs
Body CT scan	7	DSBs
Tumor PET scan	10	DSBs
Airline travel	0.005/h	DSBs
Endogenous DNA damage	*Dose lesions generated*	*Number lesions/cell/day*
Depurination	AP site	10,000
Cytosine deamination	Base transition	100–500 s
SAM-induced methylation	3 meA	600
	7 meA	4000
	O^6 meG	10–30
Oxidation	8oxoG	400–1500

68.4 DNA Repair Pathways

Repeated exposure to both exogenous and endogenous insults challenges the integrity of cellular genomic material. To maintain genomic integrity, DNA must be protected from damage induced by environmental agents or generated spontaneously during DNA metabolism.

Environmental DNA damage can be produced by physical or chemical sources. For example, the ultraviolet (UV) component of sunlight can cause up to 1 × 105 DNA lesions per cell per day, many of which are pyrimidine dimers. If left unrepaired, dimers that contain cytosine residues are prone to deamination, which can ultimately result in cytosine being replaced with thymine in the DNA sequence. Likewise, ionizing radiation (e.g., from sunlight or cosmic radiation) can cause single-strand breaks (SSBs) and DSBs in the DNA double helix backbone. If misrepaired—for example, the inaccurate rejoining of broken DNA ends at DSBs—these breaks can induce mutations and lead to widespread structural rearrangement of the genome [45]. Table 68.1 [46, 47] showed environmental agents that cause DNA damage and mutations.

Spontaneous DNA alterations can be due to dNTP misincorporation during DNA replication, interconversion between DNA bases caused by deamination, loss of DNA bases following DNA depurination, and modification of DNA bases by alkylation. Additionally, DNA breaks and oxidized DNA bases can be generated by reactive oxygen species (ROS) derived from normal cellular metabolism.

Organisms respond to chromosomal insults by activating a complex damage response pathway. This pathway regulates known responses such as cell-cycle arrest and apoptosis (programmed cell death) and has been shown to control additional processes including direct activation of DNA repair mechanisms. Most of the subtle changes to DNA, such as oxidative lesions, alkylation products, and SSBs, are repaired through a series of mechanisms that is termed base excision repair (BER). In BER, damaged bases are first removed from the double helix, and the "injured" section of the DNA backbone is then excised and replaced with newly synthesized DNA [48]. Key to this process are members of the poly(ADP-ribose) polymerase (PARP) family. The PARP family has 16 members, but only PARP1 and PARP2 have been implicated in the DDR [49]. PARP1 and PARP2 are activated by SSBs and DSBs and catalyze the addition of poly (ADP-ribose) chains on proteins to recruit DDR factors to chromatin at breaks [3]. Mispaired DNA bases are replaced with correct bases by MMR [50]. In addition to BER, the pool of deoxynucleotides (deoxyadenosine triphosphate (dATP), deoxythymidine triphosphate (dTTP), deoxyguanosine triphosphate (dGTP), and deoxycytidine triphosphate (dCTP)) that provide the building blocks of DNA can be chemically modified before they are incorporated into the double helix. The nucleotide pool is, therefore, continually "sanitized" by enzymes such as nudix-type motif 5 (NUDT5). Whereas small base adducts are repaired by BER, some of the bulkier single-strand lesions that distort the DNA helical structure, such as those caused by ultraviolet light, are processed by nucleotide excision repair (NER) through the removal of an oligonucleotide of approximately 30 bp containing the damaged bases. NER is often subclassified into transcription-coupled NER, which occurs where the lesion blocks and is detected by elongating RNA polymerase, and global-genome NER, in which the lesion is detected not as part of a blocked transcription process but because it disrupts

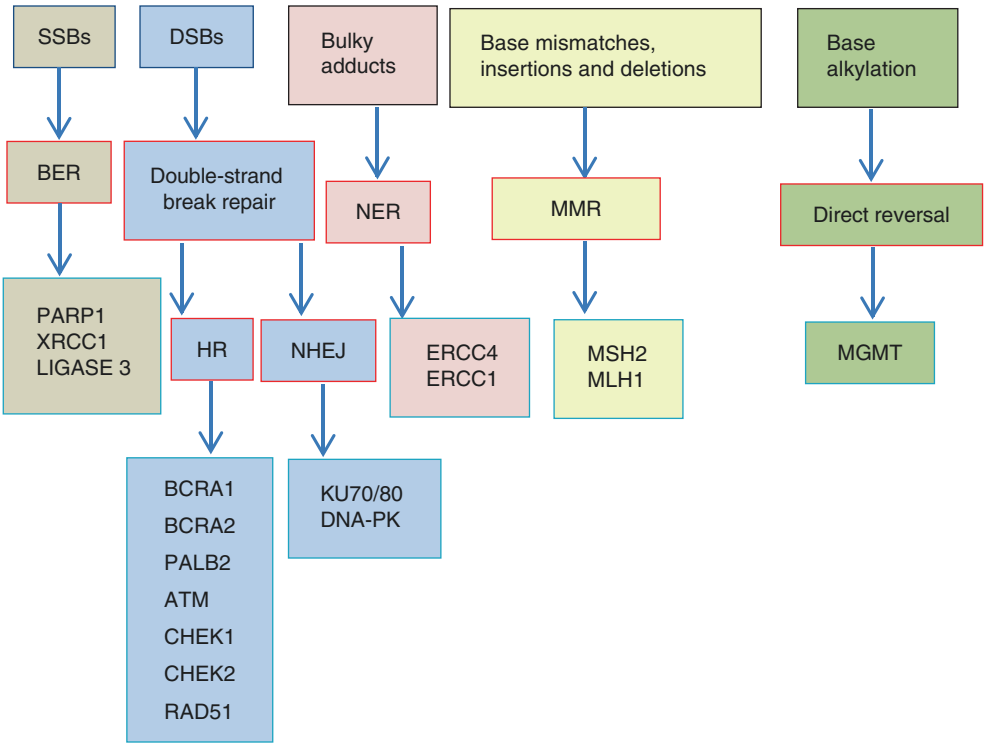

Fig. 68.1 DNA Repair Mechanisms maintain genomic stability. *SSBS* single-strand break, *DSBs* double-strand break, *HR* homologous recombination, *NHEJ* nonhomologous and joining, *MMR* mismatch repair

base pairing and distorts the DNA helix. Although these processes detect lesions using different mechanisms, they repair them in a similar way: DNA surrounding the lesion is excised and then replaced using the normal DNA replication machinery. Excision repair cross-complementing protein 1 (ERCC1) is key to this excision step. The major mechanisms that cope with DSBs are HR [51] and NHEJ [52]. HR acts mainly in the S and G2 phases of the cell cycle and is a conservative process in that it tends to restore the original DNA sequence to the site of damage. Part of the DNA sequence around the DSB is removed (known as resection), and the DNA sequence on a homologous sister chromatid is used as a template for the synthesis of new DNA at the DSB site. Crucial proteins involved in mediating HR include those encoded by the BRCA1, BRCA2, RAD51, and PALB2 genes. In contrast to HR, NHEJ occurs throughout the cell cycle. Rather than using a homologous DNA sequence to guide DNA repair, NHEJ mediates repair by directly ligating the ends of a DSB together. Sometimes this process can cause the deletion or mutation of DNA sequences at or around the DSB site. Therefore, compared with HR, NHEJ, although mechanistically simpler, can often be mutagenic.

SSBs repaired by single-strand break repair (SSBR), whereas DSBs are processed either by NHEJ or HR [3]. DNA repair is carried out by a plethora of enzymatic activities that chemically modify DNA to repair DNA damage, including nucleases, helicases, polymerases, topoisomerases, recombinases, ligases, glycosylases, demethylases, kinases, and phosphatases.

In summary DDR can be divided into a series of distinct, but functionally interwoven, pathways, which are defined largely by the type of DNA lesion they process (Fig. 68.1). DDR pathways encompass a similar set of tightly coordinated processes: namely, the detection of DNA damage, the accumulation of DNA repair factors at the site of damage, and finally the physical repair of the lesion.

MMR [50] is crucial to the DDR. Key to the process of MMR are proteins encoded by the mutS and mutL homologue genes, such as MSH2 and MLH1.

Finally, translesion synthesis and template switching allow DNA to continue to replicate in the presence of DNA lesions that would otherwise halt the process. Translesion synthesis and template switching are therefore usually considered to be part of the DDR. In translesion synthesis, relatively high-fidelity DNA replication polymerases are transiently replaced with low-fidelity "translesion" polymerases that are able to synthesize DNA using a template strand encompassing a DNA lesion. Once the replication fork passes the site of the lesion, the low-fidelity DNA polymerases are normally replaced with the usual high-fidelity enzyme, which allows DNA synthesis to continue as normal. In template switching, the DNA lesion is bypassed at the replication fork by simply leaving a gap in DNA synthesis opposite the lesion. After the lesion has passed the replication

fork, the single-strand gap is repaired using template DNA on a sister chromatid, similar to the process used during HR.

Although sometimes considered distinct from the DDR, the mechanisms that control the integrity of telomeric DNA at the end of each human chromosome also act as a barrier against genomic instability and mutation [53].

The core DDR machinery does not work alone but is coordinated with a set of complementary mechanisms that are also crucial to maintaining the integrity of the genome. For example, chromatin-remodeling proteins allow the DNA repair apparatus to gain access to the damaged DNA [54]. DDR core components interact with the cell-cycle checkpoint and chromosome segregation machinery. These interactions allow DNA repair to occur before mitosis takes place and ensure that the correct complement of genetic material is passed on to daughter cells [55].

68.5 Therapeutic Targeting of Genomic Instability in BC

When as CIN, and as changes to the structure of DNA, such as nucleotide substitutions, insertions, and deletions, they occur in crucial "driver" genes (of which there are probably fewer than ten per tumor), these mutations can alter cell behavior, confer a selective advantage, and drive the development of the disease. Importantly, these mutations can also influence how the tumor will respond to therapy. Alongside key driver mutations, emerging data from cancer genome sequencing suggests that a typical tumor may contain many thousands of other genetic changes. These "passenger" mutations do not contribute directly to the disease but are probably collateral damage from exposure to various environmental factors or defects in the molecular mechanisms that maintain the integrity of the genome. DNA damage causes cell-cycle arrest and cell death either directly or following DNA replication during the S phase of the cell cycle. Cellular attempts to replicate damaged DNA can cause increased cell killing, thus making DNA-damaging treatments more toxic to replicating cells than to non-replicating cells. However, the toxicity of DNA-damaging drugs can be reduced by the activities of several DNA repair pathways that remove lesions before they become toxic. The efficacy of DNA damage-based cancer therapy can thus be modulated by DNA repair pathways. In addition, some of these pathways are inactivated in some cancer types. These two features make DNA repair mechanisms a promising target for novel cancer treatments. Increasing knowledge of DNA repair permits rational combination of cytotoxic agents and inhibitors of DNA repair to enhance tumor cell killing. Thus, DNA repair inhibitors can be used in combination with a DNA-damaging anticancer agent. This will increase the efficiency of the cancer treatment by inhibiting DNA repair-mediated removal of toxic DNA lesions.

Moreover DNA repair inhibitors can be used as monotherapy to selectively kill cancer cells with a defect in the DNA damage response or DNA repair. Synthetic lethal interactions between a tumor defect and DNA repair pathway can be used to identify novel treatment strategies.

High levels of DNA damage cause cell-cycle arrest and cell death. Furthermore, DNA lesions that persist into the S phase of the cell cycle can obstruct replication fork progression, resulting in the formation of replication-associated DSBs. Evidence is also building that the DDR is not only invoked but also dysfunctional at an early stage in the development of neoplasia. Markers of DSBs, such as nuclear γH2AX foci (a histone phosphorylation event that occurs on chromatin surrounding a DSB), are markedly elevated in some precancerous lesions [14, 17]. The activation of oncogenes such as MYC and RAS stimulates the firing of multiple replication forks as part of a proliferative program. These forks rapidly stall, collapse, and form DSBs because they exhaust the available dNTP pool or because multiple forks collide on the same chromosome. Regardless of the mechanism, stalled and collapsed forks normally invoke the DDR and cell-cycle checkpoints that enable DNA lesions to be repaired before mitosis takes place. For precancerous lesions to progress to mature tumors, it is thought that critical DSB signal transduction and cell-cycle checkpoint proteins, such as ataxia telangiectasia (ATM) and ATM-Rad3 related (ATR), and the master "gatekeeper" protein p53 become inactivated. With these DDR components rendered dysfunctional, collapsed forks are not effectively repaired, and cells proceed through the cell cycle with DNA lesions intact, increasing the chance of mutagenesis [14, 17].

Common types of DNA damage that interfere with replication fork progression are chemical modifications (adducts) of DNA bases, which are created by reactive drugs that covalently bind DNA either directly or after being metabolized in the body. These *alkylating agents* are grouped in two categories: *monofunctional alkylating agents* with one active moiety that modifies single bases and *bifunctional alkylating agents* that have two reactive sites and cross-link DNA with proteins or, alternatively, cross-link two DNA bases within the same DNA strand (intrastrand cross-links) or on opposite DNA strands (interstrand cross-links). Interstrand cross-links pose a severe block to replication forks.

Despite the adverse side effects caused by alkylating agents on the bone marrow and other normal tissues, drugs such as cyclophosphamide, ifosfamide, chlorambucil, melphalan, and dacarbazine remain some of the most commonly prescribed chemotherapies in adults and children with various solid and hematological malignancies, particularly in combination with anthracyclines and steroids in multi-agent

regimens. The repair of alkylated lesions is thought to be quick, with the majority of lesions probably being repaired within 1 h. If the lesions are removed before the initiation of replication, the efficiency of alkylating agents in killing the tumor is significantly reduced. Thus, modulation of DNA repair that clearly influences the efficacy of alkylating agents is often explained by increased expression and/or activity of DNA repair proteins.

Antimetabolites, such as 5-fluorouracil (5FU) and thiopurines, resemble nucleotides, nucleotide precursors, or cofactors required for nucleotide biosynthesis and act by inhibiting nucleotide metabolism pathways, thus depleting cells of dNTPs. They can also impair replication fork progression by becoming incorporated into the DNA [56].

An alternative approach of interfering with replication is to target specific DDR components. Topoisomerase inhibitors, such as irinotecan (a topoisomerase I inhibitor) and etoposide (a topoisomerase II inhibitor), could be considered as the first generation of DDR targeted agents [45]. Topoisomerases are a group of enzymes that resolve torsional strains imposed on the double helix during DNA transcription and replication. They induce transient DNA breaks to relax supercoiled DNA or allow DNA strands to pass through each other [57]. Etoposide and irinotecan that inhibit this function leave DNA breaks across the genome. Topoisomerase II poisons cause DSBs, and topoisomerase I poisons cause positive supercoils in advance of replication forks and replication-associated DSBs [57].

PARP inhibitors as targeted therapy: PARP inhibitors are the next generation of DDR inhibitors.

It has been reported that expression levels of DNA repair genes are frequently associated with chemotherapy sensitivity and prognosis in BC subtypes. The poly(ADP-ribose) polymerase-1 (PARP1), one of the best characterized nuclear enzymes of the 17-member PARP family, participates in the repair of DNA SSB via the base excision repair pathway.

PARP1 and PARP2 catalyze the polymerization of ADP-ribose moieties onto target proteins (PARsylation) using NAD^+ as a substrate, releasing nicotinamide in the process. This modification often modulates the conformation, stability, or activity of the target protein [45]. The best understood role of PARP1 is in SSBR, a form of BER. PARP1 initiates this process by detecting and binding SSBs through a zinc finger in the PARP protein. Catalytic activity of PARP1 results in the PARsylation of PARP1 itself and the PARsylation of a series of additional proteins, such as XRCC1 and the histone H1 and H2B; when PARP activity is inhibited, SSBR is compromised [45].

The PARP inhibitors have been shown a substantial efficacy for hereditary BRCA1/2-related and triple-negative BC (TNBC) therapy [58–60]. Meanwhile, there are reports demonstrating that PARP inhibitors might be also active in non-hereditary BC cells lacking mutations in BRCA1 or BRCA2 [60, 61]. From a historical perspective, PARP-1 inhibitors entered the arena as promising co-adjuvant components of standard chemo-and radiotherapy regimens. Later, the discovery that tumor cell lines bearing deficiencies or mutation in DNA-repair genes (e.g., BRCA1 or BRCA2) do not tolerate PARP-1 inhibition fuelled the application of PARP inhibitors as single agent therapies in breast and ovarian BRCA-mutated cancer settings. More recently, the discovery of new potential combinative synergisms (e.g., PI3K, NAMPT, and EFR inhibitors) as well as the broadening of "synthetic lethality" context (e.g., PTEN and ATM mutations, MSI colorectal cancer phenotypes and Ewing's sarcomas) in which the inhibition of PARP-1 can be therapeutically valuable has further raised interest in this target.

PARP inhibitors were designed to imitate the nicotinamide portion of NAD^+ with which they compete for the corresponding PARP-1 binding site. PARP inhibition probably works by allowing the persistence of spontaneously occurring SSBs or by inhibiting PARP release from a DNA lesion. Whichever is the case, both of these DNA lesion types could credibly stall and collapse replication forks, potentially creating lethal DSBs [45]. Recent data propose an indirect mechanism, according to which PARP1 activity would be dispensable for BER sheer execution and would be rather engaged to seize potentially detrimental SSB intermediates and to promote their resolution. Recently, PARP1 contribution to SSB repair has also been extended to MMR and NER. In normal cells, the effects of PARP inhibition are protected by HR, which repairs the resultant DSB. However, effective HR is reliant on functioning BRCA1 and BRCA2, so when these genes are defective—as they are in tumors of germline BRCA-mutant carriers—DSBs are left unrepaired, and potent PARP inhibitors can cause cell death. BRCA1 plays a role in both the G1/S and G2/M cell-cycle checkpoint regulation in response to DNA damage, again preserving genomic integrity. Moreover, the sensitivity to PARP inhibitors seems to be defined more by the BRCA genotype of a cancer cell than by its tissue of origin. Breast, ovarian, and prostate cancers with BRCA mutations all seem to be profoundly sensitive to these drugs.

As early as in 1980, Durkacz and colleagues used the still immature, low-potency PARP inhibitor 3-aminobenzamide (3-AB) to derail DNA damage repair and enhance the cytotoxicity of dimethyl sulfate, a DNA alkylating agent [62].

The first clinical trial in pts was initiated in 2003 and allowed safety, pharmacokinetic, and pharmacodynamic evaluation of the PARP inhibitor AG014699 (*rucaparib* [63]) in combination with temozolomide (TMZ), a DNA alkylator and methylator, in advanced solid tumors [64]. However, the subsequent phase II study in melanoma [65], as well as additional independent clinical trials, featured a common (albeit not universal) shortcoming of combinatorial strategies with PARP inhibitors, namely, enhanced toxicity. Myelotoxicity was the main dose-limiting concern in the face of variable

Table 68.2 PARP Inhibitors under investigation

PARP inhibitor	Cancer type
Veliparib	Ovarian, breast, gastric, colorectal, and pancreatic tumors and a range of other solid tumors
Niraparib (Nira, MK4827)	Ovarian cancer and BRCA+ breast cancer
Olaparib (Ola, AZD2281)	Ovarian, breast, gastric, colorectal, and pancreatic tumors and a range of other solid tumors
Iniparib (BSI-201)	Breast cancer, ovarian cancer, lung cancer, glioma, glioblastoma
Rucaparib (AG014699)	Breast and other solid tumors
BMN-673	Ovarian, breast, gastric, colorectal, and pancreatic tumors and a range of other solid tumors
CEP9722	Lymphoma, breast, ovarian cancer
E7016	Melanoma
AZD-2641	Solid tumors
INO-1001	Melanoma, breast cancer
E7449	Melanoma, breast cancer, ovarian, B-cell malignancies

response rates. The need to reduce the dosage of either chemotherapy or PARP inhibitor (or both) to overcome excessive toxicity raises obvious questions about the real contribution of PARP inactivation to combinatorial regimens.

Currently, almost eight PARP inhibitors are at different stages of clinical investigation, targeting several tumor types either as single agents or in combination (Table 68.2).

Veliparib (Veli, ABT-888) is a potent, oral inhibitor of PARP-1 and PARP-2 [66]. It is orally bioavailable and crosses the blood-brain barrier. Veli potentiated the cytotoxic effect of TMZ in several human tumor models. ABT-888 was investigated in an innovative phase 0 trial, the first such study in oncology [67]. The primary study endpoint was target modulation by the PARPi. There is an extensive clinical trial program associated with this agent with 32 ongoing clinical trials of Veli in combination with cytotoxics in ovarian, breast, colorectal, prostate, liver cancers, neurologic malignancies, and leukemias. In a phase II study [68], combined ABT-888 and TMZ is active in metastatic BC (MBC). Exploratory correlative studies including BRCA mutation analysis are underway to determine predictors of response. The dose and schedule of Veli suggest the clinical activity seen is not likely due to Veli alone but rather to the combination. Promising antitumor activity was observed in pts with BRCA mutations.

Olaparib (Ola, AZD2281) also inhibits PARP-1 and PARP-2 at nanomolar concentrations. Preclinical studies have largely concentrated on investigations of synthetic lethality in BRCA1 or BRCA2 defective models or combinations with platinum in these models. The first clinical study of PARP inhibition in BRCA-mutant cancers was with this agent. In this phase I study which enrolled 60 pts, Ola doses were escalated from 10 mg daily for 2 of every 3 weeks to 600 mg twice daily [69]. Olaparib is one of the most investigated PARP inhibitors through clinical trials either as monotherapy [70, 71] or in combination with other anticancer drugs [72–77]. There is general agreement that 400 mg b.i.d. is the maximum tolerable dose of Ola. At this dose, Ola exhibited an acceptable safety profile. Most common adverse effects reported are of Grade 1/2 type, such as procedural pain, nausea, and other gastrointestinal symptoms of mild to moderate intensity, and thus are manageable. An important outcome of combination phase I trials results is the general tolerance of Ola when given in combination with bevacizumab [74], cediranib [75] and liposomal doxorubicin [77]. Ola-paclitaxel combination against TNBC [76], as well as the Ola-CDDP combination against breast or ovarian cancer in pts carrying germline BRCA1/BRCA2 also report partial efficacy. In both studies, dose-limiting hematological toxicities were neutropenia and thrombocytopenia.

Five phase II trials were conducted with Ola alone. As with the phase I clinical trials for Ola, despite inherent differences in the study design, cancer types, patient variability, and evaluation protocols, important similarities are evident in the outcomes of these phase II clinical trials. A study in pts with confirmed BRCA1 or BRCA2 mutations and recurrent ovarian cancer [78] yielded the objective response rate (ORR) of 33% for Ola 400 mg b.i.d. In pts with BRCA1 or BRCA2 mutations and advanced BC, ORRs were significantly higher (41%) for the 400 mg dose [79]. In another study conducted at this dose level [80], TNBC pts with or without BRCA mutations failed to show any objective response (OR). Interestingly, in the same study, a very strong ORR of 41% was obtained for ovarian cancer pts with BRCA1 or BRCA2 mutations; pts without the BRCA1 or BRCA2 mutations also responded at a robust ORR of 11% [80]. In summary in phase II clinical studies, 40% of pts with breast or ovarian cancer with germline BRCA mutations had a favorable response to the drug. This is a particularly high response given that the pts in these trials had been heavily pretreated and had become resistant to a range of chemotherapies [45, 64].

INO-1001 is an isoindolinone derivative and is being developed for both oncological and cardiovascular indications. Preclinical studies demonstrate its protective effect in models of cardiac dysfunction and reversal of TMZ resistance in MMR-defective xenografts. This agent is being developed in oncology in melanoma and glioma and as a single agent in cancer for BRCA1- and BRCA2-deficient tumors. In phase I trials, INO-001was tested alone or in combination with TMZ [83]. Pharmacokinetic analyses indicate lack of interactions between TMZ with INO1001 and establish a “safe to administer” dose of the combination for further evaluation of the efficacy of INO1001 against

advanced melanoma. However, outcomes of some clinical trials are less encouraging.

CEP9722 in phase I trials was tested alone or in combination with TMZ [84]. These dose escalation phase I trials established what the authors call an "adequately tolerated" dose for these compounds. Thus, while no neutropenia and other hematological toxicities were noticed, dose-dependent PARP inhibition was also not observed, with only limited clinical activity.

Niraparib (Nira, MK4827) is a potent inhibitor of PARP-1 and PARP-2 that is currently in phase III clinical trials for ovarian cancer and BRCA+ BC. In a phase III, randomized, open-label, multicenter, controlled trial, Nira was compared versus physician's choice in previously treated, HER2-negative, germline BRCA mutation-positive BC pts. MK4827 (in a 2:1 ratio) is administered once daily continuously during a 21-day cycle. Physician's choice will be administered on a 21-day cycle. Health-related quality of life will be measured. The safety and tolerability will be assessed by clinical review of adverse events (AEs), physical examinations, electrocardiograms (ECGs), and safety laboratory values.

Iniparib (BSI-201) is an anticancer agent with PARP inhibitory activity in preclinical models. Although the full mechanism of its antitumor activity is still under investigation, iniparib enhances the antiproliferative and cytotoxic effects of carboplatin and gemcitabine in vitro models of TNBC. Phase I–Ib studies of iniparib alone and iniparib in combination with chemotherapy in pts with advanced solid tumors have shown iniparib to have mild toxicity, with no maximal dose reached in terms of side effects. O'Shaughnessy et al. [59], in a phase II trial, evaluate whether iniparib could potentiate the antitumor effects of gemcitabine and carboplatin with acceptable toxicity levels. A total of 123 pts were randomly assigned to receive gemcitabine (1000 mg per square meter of body-surface area) and carboplatin (at a dose equivalent to an area under the concentration-time curve of 2) on days 1 and 8—with or without iniparib (at a dose of 5.6 mg per kilogram of body weight) on days 1, 4, 8, and 11—every 21 days. Primary end points were the rate of clinical benefit (CB) (i.e., the rate of OR [complete or partial response] plus the rate of stable disease (SD) for ≥6 months) and safety. Additional end points included the ORR, progression-free survival (PFS), and overall survival (OS). The addition of iniparib to chemotherapy improved the CB and OS of pts with metastatic TNBC without significantly increased toxic effects. On the basis of these results, a phase III trial adequately powered to evaluate overall survival, and progression-free survival is being conducted.

In summary, there are many differences in the studies evaluating anticancer activity of PARP inhibitors used alone or in combination with one or more anticancer agents. While there are many differences in the studies, some common observations should be noted with particular emphasis on various enzymatic activities associated with this multi-domain group of proteins as it applies to developing new anticancer agents and/or regimens. Specifically, the discovery of activation of PARP-2 and PARP-3 by phosphorylated DNA ends mimicking substrates or intermediates in various DNA repair pathways is quite important. These observations shed new light on the molecular functions of different PARPs. Additionally, better understanding of the substrate specificity of individual members of the PARP family will allow researchers to further refine inhibitor chemistry and minimize adverse effects of drugs currently under evaluation. Another area of considerable potential for research and development of PARP inhibitors as first-line anticancer drugs is their application to personalized medicine. Targeted therapy is rapidly becoming a hallmark of a number of anticancer drugs.

Platinum chemotherapies: Cisplatin, carboplatin, and oxaliplatin have become three of the most commonly prescribed chemotherapeutic drugs used to treat solid cancers in pts [57]. Platinum resistance, either intrinsic or acquired during cyclical treatment, is a major clinical problem as additional agents that can be added to therapy in order to circumvent tumor resistance do not currently exist. Platinum chemotherapy is now being tested with PARP inhibition clinical trials. The rationale for combining PARP inhibition with platinum chemotherapy is based on preclinical observations that PARP inhibitors preferentially kill neoplastic cells and induce complete or partial regression of a wide variety of human tumor xenografts in nude mice treated with platinum chemotherapy [57]. For example, Veli has been shown to potentiate the regression of established tumors induced by cisplatin, carboplatin therapy in rodent orthotopic, and xenografts models [57]. However, the biological mechanisms of chemo-sensitization of cancer cells to platinum chemotherapy by PARP inhibition remain to be resolved.

Ionizing radiation and radiomimetic agents such as bleomycin cause replication-independent DSBs that can kill non-replicating cells. In addition, such treatments can also rapidly prevent DNA replication by activation of cell-cycle checkpoints to avoid formation of toxic DNA replication lesions [57].

Targeting microsatellite instability (MSI): MSI is a marker of defective MMR. The predictive value of MMR status as a marker of response to 5fluorouracil, irinotecan, and other drugs is still controversial. Two large retrospective analyses from several randomized trials confirmed the detrimental effect of a 5 fluorouracil-based adjuvant therapy in stage II colorectal patients [81–83], not applicable to stage III patients [84]. These latter authors, however, reported that MSI stage III tumors harboring genetic mutation in the MMR genes seem to benefit from the 5 fluorouracil adjuvant therapy. These data imply that molecular differences within the MSI subgroup influence the response to 5 fluorouracil. Combination therapy with methotrexate (MTX) and PARP inhibitors may be effective against tumors with MMR mutations. MTX elevates ROS and DSBs and the combination of

MMR mutation and PARP inhibition may attenuate repair and induce growth arrest or apoptosis [85–87].

Targeting gene expression of cell cycle and DNA repair components: Resveratrol, a phytoalexin produced by plants such as the Japanese knotweed, prevents hypermethylation of the BRCA1 promoter [88] and may be effective for TNBC or basal subtype BC. Other natural compounds, like genistein and lycopene, can alter DNA methylation of the glutathione S transferase p1 (GSTP1) tumor suppressor gene.

Targeting centrosome abnormalities: Griseofulvin, an antifungal drug that suppresses proliferation in tumor cells without affecting non-transformed cells, declusters centrosome, although the precise mechanisms behind the drug's action remain unknown [36]. In a similar fashion, depletion of a kinesin-like motor protein can selectively kill tumor cells with supernumerary centrosomes [36]. Finally, the PARP inhibitor PJ34 also declusters super numerary centrosomes without deleterious effects on spindle morphology, centrosome integrity, mitosis, or cell viability in normal cells [89–90].

Conclusion

Genomic instability plays a critical role in cancer initiation and progression. The fidelity of the genome is protected at every stage of the cell cycle. In cancer, the presence of aneuploid or tetraploid cells indicates the failure of one or many of these safety nets. The resultant genomic heterogeneity may offer the cancer "tissue" a selection advantage against standard of care and emerging therapies. Understanding these safety nets, and how they are bypassed in cancer cells, may highlight new and more specific mechanisms for cancer prevention or therapeutic attack. The therapeutic targeting of genomic instability may check and inhibit other enabling characteristic of tumors cells, such as replicative immortality, evasion of antigrowth signaling, and tumor promoting inflammation. To this end, vitamins, minerals, and antioxidants, such as vitamin B, vitamin D, carotenoids, and selenium, as well as nutraceuticals, such as resveratrol, have shown remarkable plasticity in elucidating antitumor responses. In addition to alleviating genomic instability, these compounds are known to inhibit proliferative signaling, attenuate oncogenic metabolism, and block inflammation.

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