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DNA Damage Response and Cancer Metastasis: Clinical Implications and Therapeutic Opportunities

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Cite this chapter as: Yin M, Hong F, Wang Q-E. DNA Damage Response and Cancer Metastasis: Clinical Implications and Therapeutic Opportunities. In: Sergi CM, editor. *Metastasis*. Brisbane (AU): Exon Publications. Online first 2022 Mar 13.

Doi: https://doi.org/10.36255/exon-publications.metastasis.dna-damage-response

Abstract: The DNA damage response (DDR) system is critical to maintain genomic integrity and guard against DNA damages. DDR alterations, resulting from DDR gene mutations or epigenetic modifications, have been involved in cancer initiation, progression, and treatment response. However, the role of DDR alterations in cancer metastasis has not been well characterized. Recently, there is increasing evidence of an important role of DDR in regulating multiple facets of cancer metastatic process. In this chapter, we summarize current knowledge of the interplay among DDR alterations, tumor genomic evolution, tumor microenvironment remodeling and emergence of treatment resistance, which ultimately leads to tumor progression and metastasis development. We discuss several pre-clinical models of DDR gene alterations and cancer metastatic predisposition, and clinical evidence of potential DDR involvement in metastasis. We further discuss its

In: Consolato M. Sergi, editor. *Metastasis*. Exon Publications, Brisbane, Australia. ISBN: 978-0-6453320-2-5. Doi: https://doi.org/10.36255/exon-publications.metastasis

clinical relevance in metastatic cancer management, such as the utilization of DDR defects as a biomarker and therapeutic target.

Keywords: cancer metastasis; cancer treatment resistance; DNA damage response; immune-checkpoint inhibitor therapy; tumor microenvironment remodeling

INTRODUCTION

The human genome consists of over 3 billion base pairs of DNA sequence, which is under constant assault from a wide variety of endogenous and exogenous genotoxins. It is estimated that tens of thousands of DNA lesions occur in each cell per day (1). If they are not repaired or are repaired incorrectly, these lesions can block genome replication and transcription, or lead to mutations or genome aberrations that threaten cell or organism viability. The DNA damage response (DDR) system, comprising DNA repair and cell-cycle checkpoint pathways, consists of over 200 proteins, which evolve to sense, signal, and repair DNA lesions to maintain genomic stability. At least eight major DNA repair pathways have been identified, including mismatch repair (MMR), base-excision repair (BER), nucleotide-excision repair (NER), trans-lesion synthesis (TLS), homologous recombination (HR), non-homologous end joining (NHEJ), Fanconi anemia (FA) and the direct DNA repair (Figure 1) (2). Those pathways work independently but also crosslink with each other as redundant mechanisms to remove all kinds of DNA lesions.

Mounting evidence suggests a critical role of DDR defect in human cancer development. Patients of several hereditary diseases resulting from DNA repair defects, such as xeroderma pigmentosum (XP), ataxia-telangiectasia (AT) and Fanconi anemia, have a dramatically increased risk of cancer (3–5). Germline mutations of some critical DDR genes, such as *BRCA1* and *BRCA2*, lead to familial clustering of multiple cancer types (6, 7). Single nucleotide polymorphisms of

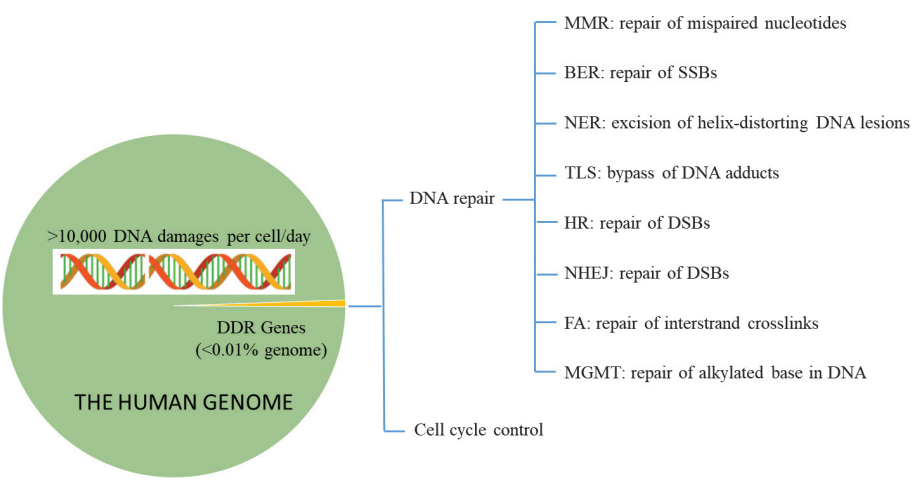

Figure 1. Human genome and DNA damage response pathways.

DDR genes contribute to variations of individual DNA repair capacity and correlate with cancer susceptibility in large populations (8–10). Moreover, cancer genome harbors a broad range of DNA damages (mutations, deletions, copy number changes, etc.), suggesting substantial dysregulation of DDR system in all cancer types (11).

Cancer-related morbidity and mortality are primarily driven by metastasis, a phenomenon that cancer cells break away from where they first formed (primary cancer), travel through the blood or lymph system, and form new tumors (metastatic tumors) in other parts of the body. Metastasis can be viewed as an evolutionary process, arising from a small fraction of tumor cells that overcome stringent physiological barriers as they separate from their original environment and developmental fate. It is a long-standing question about how cancer cells acquire metastatic abilities and what the predisposition factors are. Recent researches reveal that there are genetic determinants of cancer metastasis: the genetic alterations that mediate tumor cell invasion, intravasation, survival in circulation, extravasation into parenchyma, and colonization of vital organ (12). Such cancer metastasis models argue the necessity of the downregulation of damage surveillance mechanisms and an increase in genetic and epigenetic instability to achieve uncontrolled proliferation and the adaptability associated with aggressive tumors. The DDR system prevents the accumulation of mutations and DNA aberrations, thus blocking the harmful genomic changes required by metastasis.

CORRELATION OF DDR WITH CANCER METASTASIS

Current knowledge suggests both genetic and epigenetic alterations as the primary etiologies of DDR defect in cancer. The mutator phenotype hypothesis suggests that mutations occur randomly throughout the genome, and among these would be mutations in genes that guarantee the fidelity of DNA replication and DNA repair (13). Deletions, insertions, and rearrangements are commonly recognized as harmful mutations in DDR genes. The role of single-nucleotide substitutions is more difficult to determine, which may have little impact on DDR functions (passenger mutations) or substantially alter DDR functions (driver mutations). Epigenetic alterations refer to functionally relevant modifications to the genome that do not involve a change in the nucleotide sequence, such as DNA methylation and histone modification. Epigenetic alterations in DNA repair genes can cause reduced expression of DNA repair proteins and deficient DNA repair. Regardless, cells with DDR defect lose cancer avoidance mechanisms, thereby allowing accumulation of initiator mutations and accelerated DNA damage production from replication stress, eventually leading to further genomic instability and DDR downregulation. The vicious cycle persists in the entirety of cancer life.

Pre-clinical evidence of DDR gene alterations and cancer metastatic predisposition

DDR gene alterations can modulate cancer metastasis cascade, including cell motility, migration, invasion, anoikis resistance and anchorage-independent growth. *In vitro* transwell migration assays and wound healing assays showed that

DDR defects might lead to reduced cancer cell migration and invasion ability, as observed in multiple studies by inhibition of *ATR*, *ATM*, *BRCA1*, *KPNA2*, *NBS1*, *MRE11*, *TIN2*, *MLH1*, *WEE1* and *PARP* (14–19). These likely result from cell cycle arrest and decreased transcription of genes involved in migration (e.g., MMP), triggered by unrepaired DNA damages. It may also be mediated by the non-canonical roles of DDR genes beyond DNA repair. For example, although ATM is a master controller of DDR to double-strand breaks (DSB), it can also regulate cancer cell survival and motility through Akt pathway (20). However, conflicting data do exist to correlate DDR alterations with increased cancer migration/invasion, which is largely attributed to gain-of-function mutations. For example, several mutant p53 have been shown to drive cancer cell migration across different contexts via inducing epithelial–mesenchymal transition (EMT), promoting integrin recycling, or suppressing the anti-invasive gene *CCN-5/WISP2* (21–24). Data are more controversial regarding the impact of DDR gene alteration on anoikis and anchorage-independent growth, which is usually measured by colony formation assay in soft agar. Inhibition of different DDR gene function has been correlated with both reduced and increased colony formation (25–27). Another study showed that anchorage-independent growth ability might correlate with particular gene expression signatures beyond DDR genes (28). It is possible that different DDR alterations may cause divergent expression of genes involved in anchorage-independent growth, leading to different phenotypes.

In xenograft models, cancer cells with silenced or altered DDR genes are allowed to form a primary tumor in the site of injection and then escape into lymphatic or blood circulation (spontaneous metastasis model) or are injected directly into mouse tail vein and allowed to circulate (experimental metastasis model) in immunocompromised mice. So far, data from xenograft models have reported both increased and decreased metastatic potentials related to different DDR gene defect (23, 29–31). In contrast, genetically engineered mouse models are more consistent in correlating DDR defects with increased metastatic potential (32–35). Thus, the current preclinical models, in general, have limited abilities to study DDR defects. Metastasis in humans is a highly complicated and individualized multi-step process that is impacted by host, environment, and treatment-related factors. No preclinical model has been able to faithfully reproduce these complex interactions.

Clinical evidence of potential DDR involvement in metastasis

Clinical evidence supports potential DDR involvement in cancer metastasis development, which can be elaborated by the role of inherited or acquired DDR defect. Prostate cancer is a good example to show that a high frequency of germline DNA-repair gene mutations is not exclusive to an early-onset cancer phenotype, but also to clinically and histologically aggressive disease. In a study of 692 metastatic prostate cancer patients who were unselected for family history of cancer, inherited DDR mutations were detected in approximately 4.6% of patients with localized prostate cancer, 11.8% of patients with metastatic prostate cancer, and less than 1% of the general population through sequencing assay of 20 DNA repair genes (36). Additionally, multiple studies showed that localized prostate cancer with certain germline DDR mutation (e.g., *ATM* and *BRCA1/2*) had aggressive tumor biology, rapidly failing local therapy, higher risk of nodal and distant

metastasis, and poor survival outcomes, independent of Gleason score, stage, PSA, or age at diagnosis (37–39). Although those results did not establish a causative relationship between DDR mutations and prostate cancer metastasis, inherited DDR defects at least are enriched in metastatic prostate cancer patients.

Somatic aberrations of DNA repair pathway, acquired during cancer development, are prevalent in metastatic samples from different cancer types. Limited data exist to infer a correlation between acquired DDR defect and metastasis. *TP53* gene is the most frequently altered DDR gene and is dominated by somatic mutations (*Germline mutations* in *TP53* cause Li-Fraumeni syndrome). Exploration of 617 metastatic breast cancers uncovered *TP53* as one of the nine frequently altered genes in the metastatic setting, compared with early-stage disease (40). Direct comparison between 1897 primary and 1133 metastatic NSCLC tumors using GENIE (AACR Project GENIE Consortium, 2017) cohort revealed *TP53* mutation as the single most significant mutation in metastatic tumors after adjustment to false discovery rate (41). In a separate investigation of *TP53* mutation landscape in metastatic head and neck cancer, *TP53* mutation rate was lower in metastases than in primary tumors; however, missense mutations in the DNA binding region were significantly enriched in metastases and were associated with a common fragile site in chromosome 11, leading to amplification and overexpression of genes with established role in metastasis (42). For other DNA repair gene mutations, current evidence points to similar biologically functional changes and impact on clinical outcomes (e.g., treatment response and survival) between the same somatic and germline DDR alterations (43). It is reasonable to speculate that acquired DDR defect arising in the early cancer stage may contribute to metastasis to a similar degree as the germline mutations.

MECHANISM OF THE EFFECT OF DDR DEFECT ON TUMOR PROGRESSION

The DDR defect is an important mechanism involved in cancer survival and progression. Cancer cells compete with each other, and surrounding healthy cells, for nutrition and proliferation. They also inhabit a harsh and hostile microenvironment characterized by hypoxia, poor nutrition, and immune cell infiltration (44), and confront toxic anti-cancer therapies. Hence, cancer cells need to make quick changes (evolution) to adapt to those challenges. The genomic instability caused by DNA repair defect enables genetic variation and provide the basis for cancer evolution.

DDR alterations and tumor genomic evolution

Cancer is a genetic disease, that is, the manifestation of cancer phenotypes is rooted in cancer genomic alterations. Genomic evolution could be the outcome of clonal dynamics that lead to the expansion of pre-existing subclones or the outcome of the emergence of new subclones during propagation (45), which can be captured and visualized by analysis of the phylogenetic tree. Tumor genomic evolution can be estimated by mutation rate, defined as the probability of a cell acquiring a mutation in a gene. It was shown that metastatic cells have higher

mutations rates than non-metastatic cancer cells (40). According to the mutator phenotype hypothesis, accelerated mutation rates can be observed in cells with reduced ability to detect and/or repair DNA damage, failure of genomic surveillance mechanisms, and increased susceptibility to DNA damage by exogenous and endogenous carcinogens (46). This concept is supported by robust evidence from tumor genomic sequencing data. An analysis of DDR genes across 9,125 PanCanAtlas samples showed significant correlations between DDR gene alteration and overall tumor mutation burden (11). Mutations in *MSH2* or *MSH6* of the MMR pathway or in the exonuclease domain of the DNA polymerase epsilon (*POLE*) gene can cause a hypermutation phenotype (47). Genomic scarring with large-scale genome instability has been attributed to HR deficiency (48). While DDR defects can cause negative consequences, such as genomic instability or cell death, these genomic changes importantly enable genetic diversity. For example, V(D)J (Variable-Diversity-Joining) recombination and meiosis exploit DNA damage response to promote adaptive immunity and the exchange of genetic materials from parental homologs, respectively (49, 50). Overall, DDR defect is a driver for the molecular and phenotypic heterogeneity seen within tumors and facilitates tumor cell populations to evolve under selective pressure.

DNA damages and tumor microenvironment remodeling

A tumor and its microenvironment, including blood vessels, immune cells, the extracellular matrix, signaling molecules and other cells around the tumor, form an ecosystem, which constantly interact and influence each other. The influence of tumor microenvironment on cancer genome has been extensively discussed in the literature (51, 52). In brief, the hostile microenvironment typically inhibits DNA synthesis, replication, and repair efficiency, leading to increased genomic instability. In this section, we focus on how DNA damages alter tumor microenvironment to impact tumor growth and metastasis, both positively and negatively.

First, persistent DNA damages can stimulate inflammation, while chronic inflammation is a driving force that speeds cancer metastasis (53). Inflammation is the immune system's response to harmful stimuli in order to remove injurious stimuli and initiate the healing process. Accumulation of DNA damage due to DDR defects can be viewed as a harmful event by living cells and promote inflammatory responses. This process is not fully understood but could relate to two mechanisms: (i) damaged DNA can leak into the cytosol, triggering the induction of specific cytosolic DNA sensors and release of inflammatory molecules (54); and (ii) overwhelming DNA damages can lead to tumor cell senescence and autophagy, which are strong sources of inflammation (55, 56). Second, DDR defects and the unrepaired DNA damages are important risk factors for tumor angiogenesis, which is critical for tumor growth and metastasis. Tumor angiogenesis is usually regulated by hypoxia-induced HIF-1 alpha and angiogenic growth factors such as vascular endothelial growth factor (VEGF) and platelet derived growth factor (PDGF).

Studies have shown that phosphorylation of H2AX at S139, known as γH2AX, triggered by active ATM kinase during double stranded DNA breaks can interact with HIF-1α to maintain its stability and nuclear accumulation, thereby facilitating HIF-1α/hypoxia signaling activation and predicting metastatic outcome (57). Homologous recombination deficiency such as BRCA1 loss can lead to

upregulation of pro-angiogenic factors such as VEGF through transcriptional reprogramming in tumor cells via stimulator of interferon genes (STING) (58). Third, DDR defect and related DNA damages, on the other hand, may inhibit tumor metastatic potential by recruiting tumor infiltrating lymphocytes (TIL) to the microenvironment. High TIL infiltration is an independent, positive predictive factor of low tumor invasion, reduced lymph node and distant metastasis in many cancers (59, 60). In conclusion, the interplay between DNA damages and tumor microenvironment is one of the determining factors involved in metastatic process.

DDR and cancer treatment resistance

Acquired resistance to cancer therapy remains the leading cause of treatment failure and contributes to cancer metastasis. Enhanced DDR plays a critical role in the resistance of treatments designed to induce cell death by direct or indirect DNA damages, such as chemotherapy and radiotherapy. Cisplatin is one of the most widely used DNA-damaging agents. It binds and distorts DNA helix by promoting intrastrand and interstrand cross-links between purine bases, leading to stalled replication forks and double-strand breaks formation. In a murine lung cancer model, prolonged cisplatin treatment led to elevated expression of DDR proteins and increased DNA repair capacity, which were considered the predominant mechanism of cisplatin resistance *in vivo* in this model (61). Additionally, there were increased genomic instability with higher-grade tumor histology in cisplatin-treated mice, compared with control mice. Hence, long-term cisplatin treatment can promote tumor progression likely from increased and inaccurate repair of cisplatin-induced DNA damages.

Likewise, radiotherapy kills cancer by producing catastrophic DNA strand breaks, while cancer cells can acquire radioresistance following multiple rounds of irradiation. Several groups have highlighted a relationship between DNA repair and radioresistance. Upregulated DNA damage reaction was observed in multiple studies of cancer radioresistance (62–64) and was the intrinsic mechanisms associated with radioresistance of cancer stem cells (65). In a cellular model, acquired radioresistance was the result of alterations in DDR mediated by cyclin D1 overexpression, resulting in forced progression of S-phase and DDR activation (66). Overall, it appears that an improved DDR in cancer cells is a general adaptation to increased replication stress and increased oxidative damage already in the untreated state, which further increases after administration of DNA-damaging agents.

The role of DDR deficiency in mediating cancer treatment resistance is more controversial. Cancer cells with related DDR defect are typically more vulnerable to DNA-directed cancer therapies. However, DDR is a highly redundant and diverse mechanism which can use alternative proteins or pathways to compensate the defect. As an example, HR and NHEJ are mechanistically distinct DNA repair pathways that contribute substantially to DSB repair. Mammalian cells normally use HR pathway for error-free DNA repair. When HR is defective, NHEJ becomes the predominant pathway for error-prone DNA repair, contributing to genomic instability (67). Overtime, cancer cells become reliant on the alternative DNA repair proteins/pathways and grow resistance to the initial DNA damaging therapies. Notably, DDR defect can be directly involved in resistance of non-DNA

targeted therapies. In an analysis of 887 tumors from breast cancer patients who received endocrine treatment, single-strand DNA damage repair defect (loss of *CETN2*, *NEIL2*, or *ERCC1* genes) was found to be a novel endocrine therapy resistance driver that disrupts estrogen receptor (ER) regulation of the cell cycle through dysregulation of G1–S transition (68). DDR defect may contribute to half of ER+ breast cancer patient deaths within the first 5 years after diagnosis (68).

Additionally, the impact of DDR defect on cancer treatment response can be modulated by a variety of factors, such as tumor types, DDR gene expression level, and other genetic alterations. *ATM* is a frequently mutated DDR gene in malignancy. A previous study suggests that ATM acts as a binary switch that dictates the effect of p53 activation on tumor response to chemotherapy in lung and breast cancer (69). Inhibition of ATM promotes chemoresistance in cancer cells retaining functional p53 and sensitizes tumors with deficient p53 to chemotherapy. Moreover, some studies suggest that only cancer cells with complete ATM functional loss are sensitive to chemotherapy, while residual ATM function from ATM knockdown is sufficient to rescue cells from cytotoxic treatment (70). Those results demonstrate a complex role of DDR alterations in cancer treatment response and argues for more in-depth studies on the mechanisms.

DDR DEFECT AND METASTATIC CANCER MANAGEMENT

DDR defect is a "double-edged sword". It drives the development of cancer by fostering DNA mutation but also provide cancer-specific vulnerabilities that can be exploited therapeutically. Metastatic cancers are enriched with DDR gene alterations and genomic errors from inaccurate repair. Several treatment approaches have been developed to utilize such information to guide cancer management, including targeting the existing DDR defect for genotoxic agents, creating artificial DDR defect by specific DDR inhibitors, or aiming increased immunogenicity for immune-checkpoint inhibitor therapy.

Targeting existing DDR defects for genotoxic treatment

Traditional chemotherapy and radiotherapy are typical examples of DNA damaging therapies that are especially effective in cancers with related DDR defects. In the absence of efficient DNA repair, such treatments can produce overwhelming DNA damages that compromise critical cellular functions and jeopardize cell viability. Genotoxic cancer therapy can be grouped into five categories based on the mechanisms of action and types of DNA damages induced, which correspond to one or several DNA repair pathways (Table 1). Overall, cancer therapy produces a variety of damages to chromosomal DNA and presents a considerable challenge for DDR.

In general, cancers with defects in particular DNA repair mechanisms are especially sensitive to related DNA damaging therapies, and therefore have better clinical outcomes. Studies have shown that patients with *BRCA*, *ERCC2* or *ATM/RB1/FANCC* mutations are sensitive to platinum-based chemotherapy (71–73), likely because those genes are critical in HR or NER pathways. Epigenetic silencing of the O6-methylguanine-DNA methyltransferase (*MGMT*) gene by promoter

TABLE 1 Genotoxic cancer treatment and related DDR pathways

Agent Type	Drug Examples	Types of DNA Damage	Primary Repair Pathways	Sensitivity to DNA Repair Defect
Platinum	Cisplatin Carboplatin Oxaliplatin	interstrand and intrastrand cross-links	NER and HR	sensitive to primary repair defect; resistant to MMR defect (except oxaliplatin)
Alkylating agents	Temozolomide (monofunctional methylating agent)	Base alkylation and monofunctional DNA adducts	MGMT and MMR	sensitive to MGMT defect; resistant to MMR defect
	Nitrogen mustards (bifunctional alkylating agent)	interstrand and intrastrand cross-links	NER, HR, FA and TLS	sensitive to primary repair defect; resistant to MMR defect
Antimetabolites	5-Fluorouracil	Misincorporates into DNA	BER and MMR	sensitive to BER defect; resistant to MMR defect
Topoisomerase inhibitors	Irinotecan (Top I inhibitor) Etoposide (Top II inhibitor)	blocking cleavage and ligation step of DNA helix, causing strand breaks or stalled/collapsed DNA replication forks	HR, NHEJ and FA	sensitive to primary repair defect; resistant to MMR defect
Ionizing radiation		SSBs and DSBs	HR and NHEJ	sensitive to primary repair defect; little impact from MMR defect

methylation has been associated with better response to temozolomide (an alkylating agent) because the *MGMT* gene encodes a DNA-repair protein that removes alkyl groups from the O^6 position of guanine, an important site of DNA alkylation (74). Preclinical models and human tissue studies showed that BRCAness, SLFN11, and RB1 loss predicted response to topoisomerase I inhibitor in breast cancer, likely because of DNA repair defects (75).

Paradoxically, although MMR deficient cancers often have a more favorable prognosis compared with their MMR-proficient counterparts (76), MMR deficiency seems to result in resistance to chemotherapy, including platinum (cisplatin and carboplatin) (77, 78), alkylating agents (79), inhibitors of topoisomerases (80), and antimetabolites (81, 82). Two models have been proposed to explain the

paradoxical findings with MMR deficiency. The first model suggests that strand-specific MMR engages in a futile DNA repair cycle when it encounters DNA lesions in the template strand. As long as the lesion persists, insertion of new bases in the nontemplate strand fails to resolve the mismatch, and this futile cycling activates DNA damage signaling pathways to induce cell cycle arrest and apoptosis (83). The second model suggests that hMutSα/hMutLα, as a sensor for DNA damage in mammalian cells, directly triggers DNA damage signaling by recruiting ATM or ATR/ARTIP to the lesion, which activates checkpoint response (84). Both models provide a reasonable explanation for increased DNA damage tolerance and drug resistance in MMR-deficient cells. Overall, DDR defect can be a useful predictor of genotoxic treatment response in metastatic cancer management.

Creating artificial DDR defect by DDR inhibitors

Although DDR defect is a hallmark of genomic alterations in metastatic cancer, cancer cells still need at least a basic level of DNA repair capacity to avoid catastrophic genomic disruption and collapse in order to survive and replicate. Some cancer cells are "addicted" to a particular DDR pathway (redundant repair mechanisms) to partially maintain the genomic stability if the primary DDR pathway is already inactivated by genetic or epigenetic changes. By targeting the salvage DDR pathway, cancer cell killing can be achieved through a mechanism known as synthetic lethality (Figure 2). Additionally, artificial DDR inhibition can open opportunities for enhanced efficacy of other treatment modalities, such as genotoxic agents as described above. These features make DNA repair mechanisms a promising target for novel cancer treatments. A variety of DDR inhibitors have been developed, which target key protein kinases in different DDR pathways. So far, only PARP inhibitors (PARPi) have been approved for metastatic cancer management and hence we use PARPi as a prototype drug to discuss the working mechanisms, and therapeutic opportunities of DDR inhibitors.

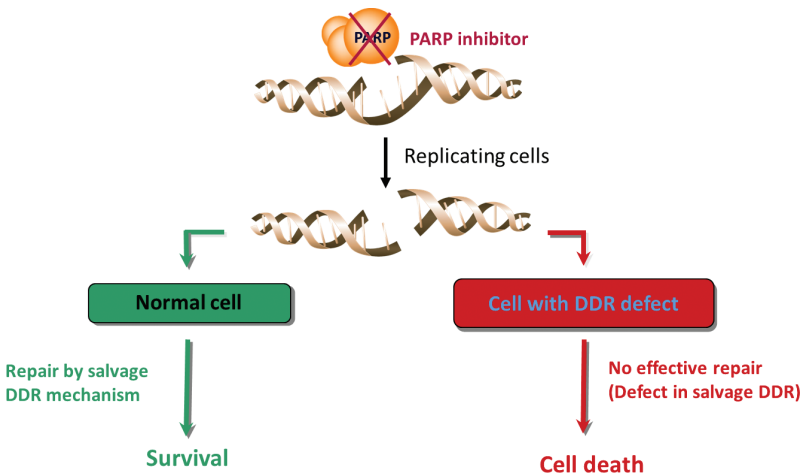

Figure 2. **Mechanisms of synthetic lethality using PARP inhibitor as an example.**

PARP proteins are involved in detection and initiation of single-strand break (SSB) repair through generating a polymeric adenosine diphosphate ribose (poly ADP-ribose) chain (PARylation), a molecular signal for recruitment of the other DNA-repairing enzymes. PARPi induces stalled replication forks by trapping the inactive PARP protein on DNA and/or inhibiting single-strand break repair, leading to more deleterious double-strand breaks (DSBs) during DNA replication. Four PARP inhibitors (olaparib, niraparib, rucaparib, and talazoparib) have been FDA-approved for metastatic prostate, pancreatic, breast, and ovarian cancer as a monotherapy in select patients based on "synthetic lethality" mechanisms. In brief, patients were selected either by clinical biomarker, such as platinum treatment response (suggesting HR defect) or by genetic biomarker, such as *BRCA* mutation and HR-deficient genomic scars. Additionally, PARPi-based combination therapy has also been extensively studied in metastatic cancer treatment, which can be classified into three mechanisms: (i) promoting DNA damage and subsequent dependence on PARP-mediated DDR; (ii) introducing other anticancer mechanisms that can synergistically work with PARPi; and (iii) inhibiting critical DNA repair proteins located in the redundant pathways to create artificial synthetic lethality partners of PARPi. Examples of combination therapies being studied include cytotoxic chemotherapy, immune checkpoint inhibitors, DNA damage checkpoint inhibitors, radiation or radionucleotides, and kinase inhibitors (e.g., PI3K, AKT, mTORC1/2, MEK). In addition to PARP inhibitors, other DDR inhibitors are also being developed and are under active clinical investigations, such as ATM inhibitor, ATR inhibitor, WEE1 inhibitor, Chk1/2 inhibitor, MGMT inhibitor, DNA-PK inhibitor, POLQ inhibitor, and Rad51 inhibitor (Table 2).

Aiming increased tumor immunogenicity for immune-checkpoint inhibitor therapy

Tumor mutation burden (TMB), defined as the total number of somatic mutations per coding area of a tumor genome, has been considered an emerging biomarker predictive of response to immune checkpoint inhibitors across tumor types. Tumor cells with high TMB has been associated with increased expression of tumor-specific neoantigens, which can be recognized and eliminated by the host immune system. There is strong evidence to correlate DDR defects with higher TMB, likely because an inability to repair DNA damage results in the accumulation of mutations. Clinically, MMR deficiency has been approved as an indication for immune checkpoint inhibitors (CKI) treatment in metastatic colorectal cancer (pembrolizumab, nivolumab, and dostarlimab) and other advanced cancers (pembrolizumab and dostarlimab) by FDA. Presence of other DDR gene alterations has also been associated with increased TMB and better response and survival outcomes of CKI treatment in metastatic NSCLC (85), urothelial cancer (86) and GI cancer (87). In our studies, we further demonstrated an upward trend of higher TMB and CKI response by increased numbers of DDR gene alterations in advanced urothelial cancer (88), suggesting an accumulative effect of DDR alterations on genomic instability and treatment outcomes.

Independent of TMB, DDR defect may sensitize cancers to CKI therapy by several other mechanisms. DDR deficient tumors were found to have increased

TABLE 2 Select DDR inhibitors

DDR Inhibitor	Manufacturer	IC50 (Catalytic Inhibition)	Synthetic Lethality Partner	Development Stage
PARP inhibitor				
Olaparib	AstraZeneca	6 nM	PTEN, ATM, ATR, BARD1, BRCA 1/2, ERBB2, FANCCD2, MRE11A, NF1, RAD51C, PALB2	FDA approved
Rucaparib	Clovis Oncology	21 nM		FDA approved
Niraparib	Tesaro	60 nM		FDA approved
Talazoparib	Pfizer	4 nM		FDA approved
Veliparib	Abbott Laboratories	30 nM		Approval pending
ATM inhibitor				
CP-466722	Pfizer	410 nM	ATR, PARP, TOP1, PTEN, TP53	Stopped
KU-55933	AstraZeneca	13 nM		Stopped
KU-60019	AstraZeneca	6.3 nM		Clinical evaluation
KU-59403	AstraZeneca	3 nM		Stop development
AZ31	AstraZeneca	46 nM		Preclinical
AZ32	AstraZeneca	6.2 nM		Preclinical
AZD0156	AstraZeneca	0.58 nM		Preclinical
AZD1390	AstraZeneca	0.78 nM		Clinical evaluation
ATR inhibitor				
M6620	Vertex Pharmaceutical	0.2 nM	ATM, ARID1A, PARP, CHEK1, MLH1, TP53	Clinical evaluation
M4344	Vertex Pharmaceutical	13 nM		Clinical evaluation
AZD6738	AstraZeneca	1 nM		Clinical evaluation
BAY1895344	Bayer	7 nM		Clinical evaluation
Wee1 inhibitor				
Adavosertib	AstraZeneca	5.2 nM	CHEK1, STED2, CCNE1	Clinical evaluation
Debio 0123	Debiopharm	2.2 nM		Clinical evaluation
PD0166285	Pfizer			Preclinical
PD0407824	Pfizer	97 nM		Preclinical

immune cell infiltration and chemokine production, due to cGAS/STING pathway activation instead of neoantigen production (89). DDR deficiency has also been correlated with upregulated PD-1 and PD-L1 expression (90, 91), and priming an anti-tumor microenvironment through modulating both the innate and adaptive immune system (92). In addition, multiple phase I/II trials have shown

good tolerance and improved clinical outcomes (e.g., objective response) when combining checkpoint inhibitors with DDR inhibitors (93, 94). The TOPACIO/Keynote-162 trial studied niraparib + pembrolizumab in 55 patients with metastatic triple-negative breast cancer irrespective of *BRCA* mutation status or PD-L1 expression. Immunogenomic profiling of tumor tissue samples identified two determinants of response: mutational signature reflecting HR defect and interferon-primed exhausted CD8 + T-cells. Presence of one or both of these features was associated with improved outcome, while concurrent absence yielded no response (95). All those results highlight increased tumor immunogenicity and enhanced CKI treatment potential in DDR defective tumor cells.

LIMITATIONS

Although there are strong scientific and clinical evidence to implicate DDR defects with cancer progression and metastasis, there are significant knowledge gaps and barriers to utilize such information in clinical practice. First, the majority of published investigations are correlation studies, lack of mechanism details. It is plausible that DDR defect primarily contributes to metastasis by allowing harmful alterations of cancer genome, some of which are metastasis driver genes (genes involved in cancer cell circulation, homing, penetration, or colonization of distant organs). However, genetic changes largely arise randomly in the entire genome, causing intratumoral and intertumoral genomic heterogeneity. As a result, patients with similar DDR defects may have different tumor genomic alteration profile and clinical outcomes. For example, *ATM* mutations have been implicated in both favorable and unfavorable chemotherapy response and prognosis in various cancer types (96–98). Even for the same cancer type (e.g., urothelial cancer), *ATM* mutations were found to correlate with good and bad prognosis in different study reports (71, 99).

Second, the DDR system is a complicated system, consisting of hundreds of proteins with distinct and connected biological functions. Some genes may play a more critical role in DDR and tumor metastatic process than others, while cumulative alterations of multiple DDR genes may produce more devastating outcomes than single gene alterations. Hence, it is extremely difficult to develop a scientific model with hundreds of variables to assess their roles in cancer progression.

Third, it is critical to develop rapid functional tests and computational algorithms based on sequencing data that can reliably determine biological consequences of related DDR gene alterations. Currently, DDR defects are primarily inferred from presence of pathogenic alterations within DDR genes or presence of genomic scars, such as loss of heterozygosity (LOH), telomeric allelic imbalance (TAI), and large-scale state transitions (LST). However, such information can only partly inform the qualitative functional status and provide no quantitative measurement of how DDR gene or related DNA repair pathway is impacted. Thus, it is difficult to apply to individuals for precision medicine. For example, genetic alterations can occur at the entire length of the whole *ATM* gene (lack of hotspots). ATM inhibitors were not successful in clinical trials so far probably because we lack knowledge of functional impact associated with each alteration and hence are unable to select appropriate patients. For another example, olaparib has been

approved in metastatic castrate-resistant prostate cancer with HR gene mutations. However, it showed a 40% objective response rate in patients with *BRCA 1/2* alterations and a less than 10% ORR in those with other HR gene alterations. Hence, *BRCA* alterations seem to be more harmful than other HR gene alterations, but we lack efficient tools to measure such differences.

Fourth, Although DDR defect can be utilized as a biomarker or a target to increase treatment efficacy in metastatic cancer management, it may be more attractive to block cancer progression and dissemination by restoring DDR function at the early cancer stage, thus impeding accumulation of harmful genomic alterations. Subsequently, cancer may lose metastatic potential and can be eradicated by surgery or radiation. However, such a concept has yet to be translated into reality.

CONCLUSION

DDR defect is an intrinsic feature of tumor cells that participates not only in cancer initiation, but in cancer progression and metastasis from multiple facets (Figure 3). Increased DDR gene alterations and genomic instability are hallmarks of metastatic evolution. DDR function status can be utilized as a useful predictive/prognostic biomarker, and a valid target in metastatic cancer management. However, most published evidence is correlative in nature and lacks cause-effect relationships between DDR genes and cancer metastasis. More in-depth research is required to understand the functional consequences of various DDR alterations, the molecular mechanisms involved in DDR-driven metastatic process, and their impact on treatment response and outcomes across different therapies.

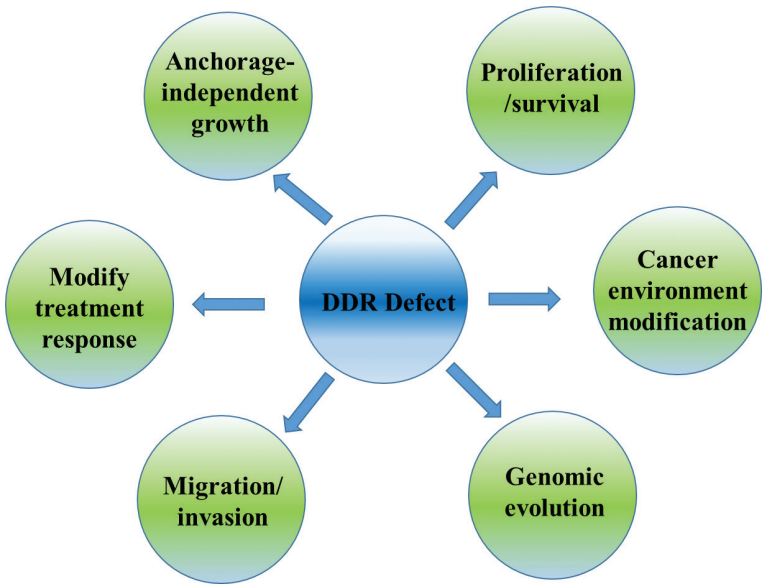

Figure 3. **Potential mechanisms of DDR defect in cancer progression and metastasis.**

Conflict of Interest: The authors declare no potential conflicts of interest with respect to research, authorship and/or publication of this manuscript.

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