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Targeting DNA Damage and Repair (DDR) Pathways: Advances in Understanding and Therapeutic Implications

*Mudasir Rashid, Rumaisa Rashid, Deverapalli M, Farjana N, Swetha MA, Brim H and Ashktorab H**

Department of Medicine and Cancer Center, Howard University College of Medicine, USA

Abstract

DNA Damage and Repair (DDR) is a complex and dynamic process that takes care for damaging events occurs in all living organisms. DDR pathways are essential for maintaining genome integrity and preventing the accumulation of mutations that can lead to cancer development.

This review comprehensively covers the different DDR pathways includes Base Excision Repair (BER), Nucleotide Excision Repair (NER), Homologous Recombination Repair (HRR), Non-Homologous End Joining (NHEJ), and Mismatch Repair (MMR); key genes involved in DDR pathway (*BRCA1/2*, *ATM*, *CHEK1/2*, *MSH2/6*, *ATR*, *MDM2*); DNA damaging agents (endogenous and exogenous), advancement in targeting the Inhibitors (*PARP*, *ATM* and *CHEK1* Inhibitors) against *DDR* genes; and their limitations. Furthermore, detailed challenges and promise of using *DDR* Inhibitors and future prospective were discussed. Based on the current evidence further research is required to overcome limitations of *DDR* inhibitors and their specific uses for specific cancer.

Keywords: DNA Damage and Repair (DDR) pathways; Key *DDR* genes, *DDR* Inhibitors

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Correspondence:
Hassan Ashktorab, Department of Medicine and Cancer Center, Howard University College of Medicine, Washington DC, USA

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Introduction

Overview of DNA damage and repair

DNA is one of the most vital molecules containing all the genetic information required for life processes [1]. Every day, DNA in human cells undergoes thousands to millions of damaging events either caused by endogenous (internal metabolic processes) or exogenous factors such as UV radiation, exposing to genotoxic chemicals and errors during DNA replication resulting into various types of DNA aberrations. However, When the nuclear proteins detect any damage, they initiate the repairing process by attaching the protein complexes to the lesion and the targets (like p53) are then phosphorylated by signal transducers, mediators, and effector proteins arresting the cell cycle at the G1/S, intra-S, or G2/M checkpoints [2]. The mutation or lesion may pass to the next generations if left unrepaired before mitosis and to start an apoptotic signaling cascade if the DNA damage is above threshold, it can prompt changes like chromosomal aberrations, malignant transformation (including immortal traits and the initiation of uncontrolled division of cells as shown in and ultimately cell death. DDR and cell cycle check point pathways are frequently dysregulated in malignancies, leading to increase in mutagenesis and genomic instability which aids in the development of the diseases like cancer predisposition and neurodegeneration. However, various kinds of DNA damaging agents which contribute to DNA damage based on their source, origin and nature have been discovered and need to be repaired are discussed below in detail (Figure 1 and Supplementary Table 1A-1C).

Classification of DNA Damage Agents

DNA damaging agents are generally categorized into: Clastogens which leads to chromosomal breakage x and the induction of Micronuclei (MN) by generation of acentric chromosome fragments and Aneugens which causes the integration of whole chromosomes in MN by creating aneuploidy, and disrupts cell growth and the mitotic spindle apparatus [3-5].

Based on its origin, DNA damage can be divided into two primary groups: (A) Endogenous DNA damage includes

(I) Replication errors, DNA base mismatches and topoisomerase-DNA complexes- high fidelity replicative polymerases and other DNA polymerases, including Tdt, PrimPol, and REV1, can perform lower-fidelity DNA synthesis during DNA replication which results in DNA errors.

Supplementary Table 1A: Environmental Agents.

Environmental Agents:				
Agent	**Type**	**Mechanism**	**Pathway**	**References**
UV	Formation of pyrimidine dimers	Absorption of UV energy by DNA bases	NER	[32,219]
Ionizing radiation	DSBs	Induction of free radicals and ionization	DSBR	[24,220]
Alkylating agents	Alkylation of DNA bases	Covalent addition of alkyl groups	BER	[34, 35,37]
Platinum-based drugs	DNA interstrand crosslinks	Formation of covalent crosslinks	DNA Interstrand Crosslink Repair	[221,222]
Topoisomerase inhibitors	Topoisomerase-mediated DNA damage	Inhibition of DNA topoisomerases	Topoisomerase-mediated DNA damage repair	[223,224]

UV: Ultraviolet Radiation; DSBR: DNA Double-Strand Break Repair; NHEJ: Non-Homologous End Joining; BER: Base Excision Repair; DICR: DNA Inter-strand Crosslink Repair; SSBs: DNA single-strand breaks

Supplementary Table 1B: Chemical Agents.

Chemical Agents:				
Agent	**Type**	**Mechanism**	**Pathway**	**References**
H_2O_2	Oxidative damage to DNA bases	Generation of ROS	BER	[225,226]
Cisplatin	DICR	Covalent binding to DNA	DICR	[221,222]
Bleomycin	SSBs	Induction of DNA cleavage by bleomycin	DSBR	[227,228]
MMS	Alkylation of DNA bases	Covalent addition of alkyl groups	BER	[229,230]
Camptothecin	DNA topoisomerase I inhibition	Inhibition of topoisomerase I	Topoisomerase-mediated DNA damage repair	[231,232]

H_2O_2: Hydrogen Peroxide; MMS: Methyl Methane Sulfonate; ROS: Reactive Oxygen Species; DSBR: DNA Double-Strand Break Repair; NHEJ: Non-Homologous End Joining; BER: Base Excision Repair; DICR: DNA Inter-strand Crosslink Repair; SSBs: DNA single-strand breaks

Supplementary Table 1C: Biological Agents.

Biological Agents:				
Agent	**Type**	**Mechanism**	**Pathway**	**References**
HP	Induction of DNA double-strand breaks	Activation of host DNA-damaging enzymes	DSBR	[55,233]
HPV	Integration of viral DNA into host genome	Viral integration into host DNA		[234,235]
EBV	Induction of DNA double-strand breaks	Activation of host DNA-damaging enzymes	DSBR	[236,237]
HIV	Integration of viral DNA into host genome	Viral integration into host DNA	NHEJ	[238,239]
MV	Induction of DNA double-strand breaks	Activation of host DNA-damaging enzymes	DSBR	

HP: *Helicobacter Pylori*; HPV: *Human Papillomavirus*; EBV: *Epstein-Barr Virus*; HIV: *Human Immunodeficiency Virus*; MV: *Measles Virus*; DSBR: DNA Double-Strand Break Repair; NHEJ: Non-Homologous End Joining

PMID: 18626473. Additionally, the activities of topoisomerase enzymes are other sources of endogenous DNA damage [6-9]. However, the Mismatch Repair (MMR) pathway increases replication fidelity by more than 100-fold by fixing the rare faults that has evaded proofreading by replication polymerases.

(II) Spontaneous base deamination- base deamination, in which DNA's Cytosine (C), Adenine (A), Guanine (G), and 5-methylcytosine (5 mC) lose their exocyclic amine and gets converted into Uracil (U), Hypoxanthine (H), Xanthine (X), and Thymine (T), respectively, is a primary cause of spontaneous mutagenesis in human cells [9]. Base deamination rate in DNA can generally be increased by external exposure to UV light, intercalating agents, nitrous acid, and sodium bisulfite in addition to endogenous deamination sources [10]. Base Excision Repair (BER) is the main and significant cellular repair pathway triggered by lesion specific DNA glycosylases, *via* which deamination products are primarily repaired within cells [11].

(III) Abasic sites- when the N-glycosyl bond connecting the nitrogenous base and the sugar phosphate backbone hydrolyzes spontaneously or is broken by a DNA glycosylase to produce an intermediate in the BER pathway. About 10,000 abasic sites are produced daily in human cells during the removal of uracil from the DNA by uracil-DNA glycosylase and their synthesis is positively influenced by high temperatures and extreme pH conditions [12,13]. However, the Abasic sites are naturally unstable and quickly transform into Single Strand Breaks (SSBs) because of a β-elimination reaction that attacks the 3′ phosphodiester link of the remaining deoxyribose [9]. Whereas the BER and Nucleotide Excision Repair (NER) pathways are largely responsible for repairing AP sites [14].

(IV) Oxidative DNA damage- occurs due to DNA lesions including, DNA SSBs, and DSBs [15]. An essential class of DNA damaging agents which can directly cause a wide variety of DNA damages are Reactive Oxygen Species (such as Superoxide Radicals ($\bullet O_2$), Hydrogen Peroxide (H_2O_2), and Hydroxyl Radical ($\bullet OH$) [16], they are continually produced by cells as a result of endogenous metabolism, infection/inflammation, and/or exposure

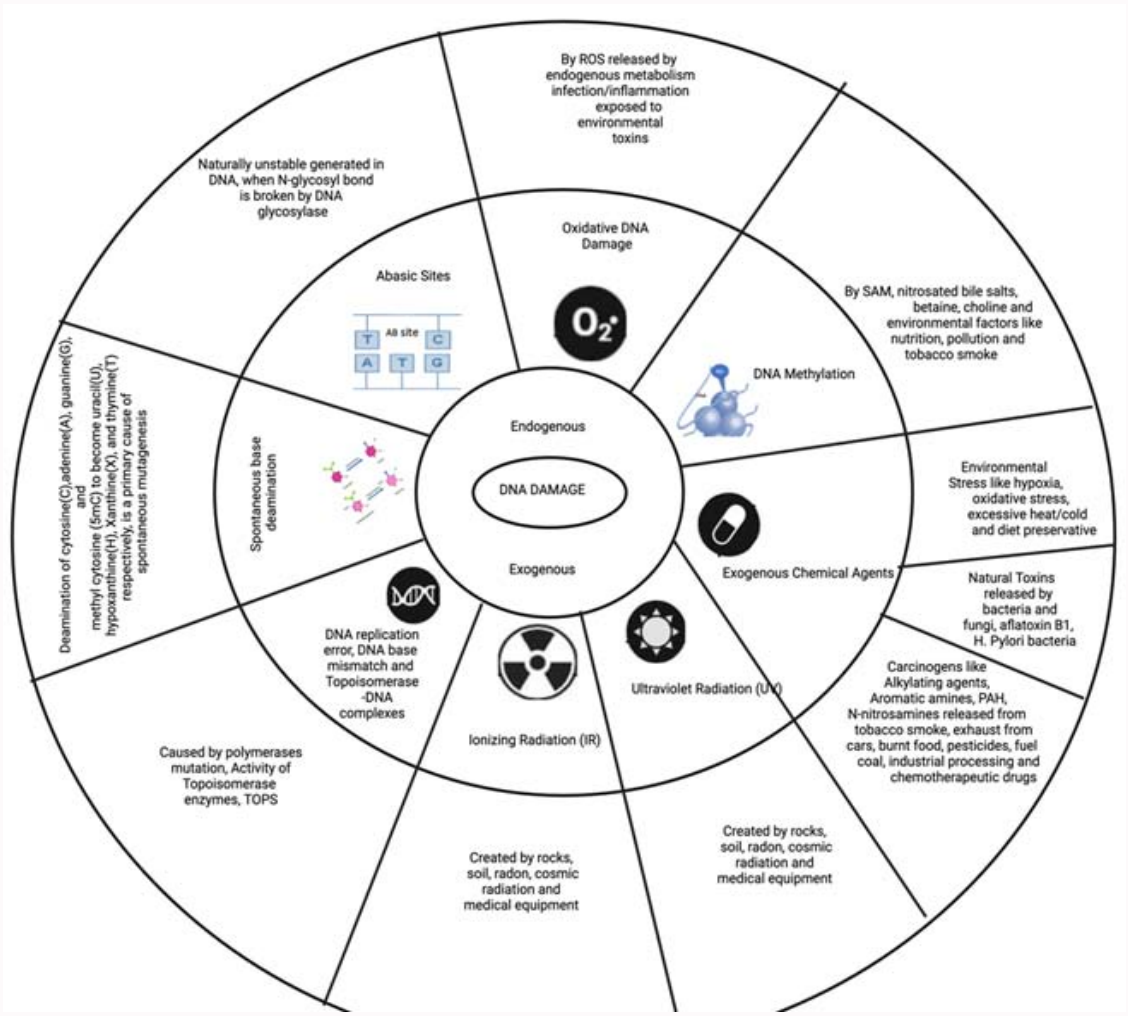

Figure 1: Classification of different types of DNA damages: The Endogenous DNA damages are classified in 5 sections, the first one is based on replication errors, DNA base mismatches-and topoisomerase and DNA complexes which are caused by polymerases mutation, Activity of topoisomerase enzymes, TOP1. The second section is based on DNA methylation, which is come from SAM, Endogenous nitro sated bile salts, betaine, choline, and environmental factors like nutrition, pollution, and tobacco smoke. The next one is based on Oxidative DNA damage caused by the superoxide radicals (•O2), hydrogen peroxide (H2O2), and the hydroxyl radical (•OH). The fourth one is based on Abasic sites which are naturally unstable generated in DNA, when N-glycosyl bond connecting the nitrogenous base and the sugar phosphate backbone hydrolyzes spontaneously or is broken by a DNA glycosylase. The last section is based on Spontaneous base deamination; Cytosine (C), Adenine (A), Guanine (G), and 5-Methyl Cytosine (5mC) lose their exocyclic amine to become Uracil (U), Hypoxanthine (H), Xanthine (X), and Thymine (T), respectively, is a primary cause of spontaneous mutagenesis; Exogenous DNA damages are classified in 3 different sections, i.e., Ionizing Radiation (IR), Ultraviolet (UV) radiation and Exogenous chemical agents. The Ionizing Radiation is created from rocks, soil, radon, cosmic radiation, and medical equipment. The UV radiation is emitted by sunlight, laboratory research, and UV-A stimulates by endogenous (porphyrins and flavins) and exogenous (psoralens, tetracycline, promazine, and methylene blue) photosensitizers. The chemical agents include carcinogens like alkylating agents, aromatic amines, Polycyclic Aromatic Hydrocarbon (PAH), N-nitrosamines, hormone estrogen; other agents come from natural toxins and environmental stress. Alkylating agents are released from Food substances, tobacco smoke, burning biomass, industrial processing, and chemotherapeutic drugs; aromatic amines are found in cigarettes, fuel, coal, industrial colors, pesticides, and routine high-temperature cookery; PAH is generated from cigarettes smoke, exhaust from cars, burnt food, and incomplete combustion of organic matter and fossil fuels. Natural toxins are typically released by bacteria or fungus, Aflatoxin B1, *H. pylori* bacteria. Finally, environmental stress consists of hypoxia, oxidative stress, and excessive heat or cold.

to environmental toxins. The •OH radicals formed during the Fenton reaction between H_2O_2 and Fe^{2+} are the most reactive of these ROS species and can damage DNA, proteins, and lipids [17]. Additionally, the highly reactive endogenous aldehydes produced because of oxidative stress and cell processes such lipid peroxidation and glycation can cause DNA damage by directly interacting with DNA to generate aldehyde-derived DNA adducts [18]. An extensive network of DNA repair processes is aided by Base Excision Repair (BER), Transcription-Coupled Repair (TCR), Global Genome Repair (GGR), Mismatch Repair (MMR), Trans-Lesion Synthesis (TLS), Homologous Recombination (HR), and Non-Homologous End-Joining (NHEJ) pathways to repair oxidative DNA damage [19].

(V) DNA methylation: During methylation processes, methyl transferases use S-Adenosylmethionine (SAM) as a methyl donor whereas the endogenous nitro-sated bile salts, betaine, choline, and environmental factors like nutrition, pollution, and tobacco smoke are among the other methylating substances [20]. Additionally, the 6-methylguanine and the associated residues O4-methylthymine and O4-ethylthymine are extremely mutagenic and result in G:C:A:T and T:A:C:G transition mutations, respectively. While the N7-methylguanine residue is essentially nontoxic unless it experiences a spontaneous cleavage to produce an AP site, N3-methyladenine is only partially harmful since it inhibits DNA synthesis [9]. Intriguingly, the O6-methylguanine DNA damage also initiates

a futile and cytotoxic cycle of MMR through aberrant base pairing with other residues [21,22]. Methylated DNA bases are a significant contributor to spontaneous DNA damage when left unrepaired [9]. The spontaneous deamination of 5-methylcytosine residue is responsible for the high frequency of C-to-T conversions reported in the p53 tumor-suppressor gene.

Exogenous DNA damage

Exogenous substances can cause DNA damage when exposed to them. DNA damage brought on by the following carcinogens: (1) Radiation/physical agents (ionizing radiation, UV light); (2) Chemical agents (particulate matter, aristolochic acid, nitrosamines, heterocyclic aromatic amines, mycotoxins, and polycyclic aromatic hydrocarbons) [23].

(I) Radiation/physical agent: Ionizing Radiation (IR)- is present in our environment, which is made up of alpha, beta, gamma, neutrons, and X-rays. It is created by a variety of sources, including rocks, soil, radon, cosmic radiation, and medical equipment. IR can harm DNA directly or indirectly, like by radiolyzing the surrounding water to produce a clump of extremely reactive Hydroxyl Radicals (•OH) [24]. X-rays and radioisotopes are two examples of how IR is used in medicine for diagnostic purposes. The very largest portion of the total annual dosage to humans who are not occupationally exposed to IR from other sources during their everyday work activity is made up of natural radiation and radioactivity in the environment, together with diagnostic medical exposure. IR also produces SSB'S with a distinct signature, where the DNA breaks feature 3′ phosphate or 3′-phosphoglycolate ends rather than 3′-OH ends, in addition to producing base lesions. The double strand break is a significant radiation-induced lesion that results from numerous damaged sites being located closely together on both DNA strands [25]. IR-induced double strand breaks can be repaired by the HR pathway even if they are harmful [26].

(II) Ultraviolet (UV) radiation: The primary cause of skin cancer in people is UV radiation which is one of the components presents in the sunlight and ranges from 190 nm to 400 nm in wavelength (i.e., UV-C (190 nm-290 nm), UV-B (290 nm-320 nm), and UV-A (320 nm-400 nm).) responsible for different kinds of DNA damages [27]. At 260 nm, most of the UV light is absorbed by DNA, after which the photo-absorption drastically decreases. DNA damage may have various negative effects, including cell death, mutagenesis, photoaging, and cancer, in contrast to that sunshine, particularly UVB, is important for the synthesis of vitamin D and is essential for human health. The chromophores found in skin cells are capable of directly absorbing UVA and UVB photons which leads to the formation of potential DNA damaging agents known as ROS [28]. Sunlight contains 5.1% UV-A, 0.3% UV-B, 62.7% visible light, and 31.9% infrared since the ozone layer primarily filters out harmful UV-C radiations which cause the DNA damage by creating covalent bonds between two neighboring pyrimidines [27]. Due to its maximum DNA absorption, UV-C is frequently utilized in laboratory research because it generates more photoproducts than UV-A and UV-B radiation, which are also medically relevant UV wavelengths that can damage DNA [29]. By stimulating endogenous (porphyrins and flavins) and exogenous (psoralens, tetracycline, promazine, and methylene blue) photosensitizers, UV-A damages DNA by causing DNA adduct formation through photooxidation processes [30-32]. Direct reversal of UV-damaged bases, NER, Inter-Strand Crosslink (ICL) repair, translesion synthesis and HR, are all methods for repairing UV lesions either by fixing the lesions or help cells to tolerate their presence [9,18]. IR can harm DNA directly or indirectly, for example, by radiolyzing the surrounding water to produce a clump of extremely reactive Hydroxyl Radicals (•OH).

(III) Chemical agents:

Alkylating agents: The main sources of exogenous alkylating agents are food substances, tobacco smoke, burning biomass, industrial processing, and chemotherapeutic drugs [33]. Methyl Methanesulfonate (MMS), Ethyl Methanesulfonate (EMS), N-Methyl-N′-nitro-N-nitrosoguanidine (MNNG), and Methylnitrosourea (MNU) are the most prevalent alkylating chemicals that are frequently utilized in laboratories [34,35]. Sulfur and nitrogen mustards, which were initially used in World War I and have subsequently been utilized in several other conflicts, including the one currently raging in Syria, are other traditional examples of alkylating agents. In contrast to monofunctional alkylating agents, which only carry one reactive group, mustards are bifunctional because they contain two reactive groups and have the capacity to react with two distinct DNA locations. These bifunctional processes produce DNA-protein crosslinks and intra- and interstrand crosslinks, which stop DNA metabolite activity [9,36]. To protect against alkylation-induced cell death or mutation, numerous biological mechanisms, such as direct DNA damage reversal, BER, and Mismatch Repair (MMR), react to alkylation damage [37].

Aromatic amines: The main sources of aromatic amines are cigarettes, fuel, coal, industrial colors, pesticides, and routine high-temperature cookery [38,39]. Aromatic amines are changed into carcinogenic (ester and sulfate) alkylating agents by the P450 monooxygenase system, which targets the C8 position of guanine [40]. In human cells, the NER pathway is known to repair C8-guanine adducts [41].

Polycyclic Aromatic Hydrocarbon (PAH)- are polycyclic aromatic hydrocarbons with two or more aromatic rings, and are inert, nonpolar, and pervasively carcinogenic environmental agents. Smoke from cigarettes, exhaust from cars, burnt food, and incomplete combustion of organic matter and fossil fuels are typical causes [42,43]. To produce reactive intermediates that react with DNA, PAHs rely on the liver's P-450 system [44]. Dibenzo[a,l]pyrene is the most powerful PAH in terms of its ability to cause cancer in humans. If TLS polymerases do not obstruct them, excision repair pathways like NER and BER typically heal the PAH DNA damages [45-47]. Other important reactive electrophiles that harm DNA are N-nitrosamines, which are strong carcinogens and tobacco smoke byproducts, can also be found in preserved meats and have been found to be linked to cancers of the esophagus, stomach, and nasopharynx [48-50]. The hormone estrogen, which is often used in hormone replacement therapy and increases the risk of cancer over time when used continuously, is the last noteworthy component [51,52]. Epidemiological and clinical trial research show that using estrogen and progesterone together, as opposed to estrogen alone, increases the risk of breast cancer and other health problems [52].

Biological agents

(I) toxins- A family of genotoxic and carcinogenic substances known as "natural toxins" are typically used by bacteria or fungus as part of defensive mechanisms [53]. Contaminated grains, oilseeds, spices, tree nuts, milk, and milk derivatives cause exposure to both humans and animals [54]. The best biological agent includes

Aflatoxin B1 which is the most potent liver carcinogen among the naturally occurring aflatoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus* [24]. DNA is also harmed by oxidative stress brought on by *H. pylori* bacteria, which causes genetic instability. In addition, *H. pylori* itself has been linked to neoplastic transformation and genetic instability due to epigenetic alterations and DNA damage [55]. The original genome annotation indicated that *H. pylori* lacked numerous HR genes involved in processing DNA damage intermediates (recBCDFO), as well as mismatch repair (mutHLS1), which suggested that this organism had a high mutation rate that may have contributed to its genetic diversity [56]. Other DNA damaging virus is HPV. Multiple HPV-mediated illnesses, such as genital warts, precancers, and malignancies of the cervix, anus, penis, vulva, vagina, and head and neck, including cancers of the oropharynx, can develop because of chronic infections [57].

(II) Environmental stress- DNA damage in human cells has been linked to environmental stressors like hypoxia, oxidative stress, and excessive heat or cold [9]. Additionally, it has been demonstrated that these stresses lead to mutation at trinucleotide repeats, which is connected to the alt-NHEJ DNA repair mechanism and the emergence of neurological diseases [58,59]. Other common biological product usage is now more frequently linked to DNA damage. For instance, Bisphenol A (BPA) and Butyl Paraben (BP), which are used in the production of food, beverages, pharmaceuticals, and cosmetics, have been associated with sperm cell DNA damage [9]. There is evidence that the dietary additives phosphoric acid, brilliant blue, sodium benzoate, and potassium sorbate, as well as the preservatives sodium benzoate, potassium benzoate, and sodium sorbate, can damage DNA [60-63].

DNA Damage Response and Repair Pathways

DNA Damage response and repair pathways

It has been noted that DNA damage may occur due to various factors, including exposure to chemicals or radiation, as well as errors that occur during replication. Therefore, to maintain genomic integrity and prevent the accumulation of mutations, cells have developed a complex and dynamic network to detect and repair DNA damage and ensure the rectification of DNA lesions that occur in all living organisms known as DNA Damage Response (DDR) pathways. DDR protects genome stability, by coordinating through several networks of pathways ensuring the correct transmission of genetic material at any stage of DNA replication, repair and recombination, cell cycle checkpoint, and chromosome segregation [5]. When these pathways perfectly function, the damage is successfully detected and accurately repaired and intern restores the normal functioning of cells. However, sometimes the unrepaired DNA damage accumulates within non-replicating cells and might result in ageing [64-66], due to mutation or dysregulation of the genes involved in DDR process which can lead to genomic instability and contribute to developing cancer and other diseases [67]. DDR comprises several DNA repair pathways (DR, BER, NER, HR, NHEJ, FA, MMR, ALT-EJ), damage tolerance processes, and cell cycle checkpoints which shows several effects, such as chromatin remodeling, cell cycle modulation, gene expression, and repair [5,67,68]. The molecular components of the induced DDR pathways are typically classified into three major groups: "sensors," "transducers," and "effectors," which mediate eventual outcomes such as repair, apoptosis, and immune clearance are discussed below in detail (Figure 2 and Supplementary Table 2).

(I) Direct Repair Pathway (DR): Most DNA damage repair pathways remove damaged lesions by breaking the phosphodiester backbone, excising the damaged base, and resynthesizing a segment of DNA using a complementary template and error-prone DNA polymerases. The DR pathway removes DNA and RNA damage without excision and resynthesis, thereby making this repair pathway error-free [69]. DR maintains genomic integrity by protecting DNA mainly from radiation and alkylation damage such as UV-induced DNA damage. The primary consequence of UV light exposure on DNA is the induction of damage by forming pyrimidine dimers when adjacent pyrimidine bases on the same DNA strand become covalently linked by the generation of a cyclobutene ring. Specifically, this ring structure is formed by the saturation of the double bonds between carbon atoms 5 and 6 in the pyrimidine bases. The resulting cyclobutene pyrimidine dimers perturb the normal DNA helical structure, potentially disrupting vital processes such as DNA replication and transcription. If left unaddressed, these pyrimidine dimers can give rise to mutations and other genetic alterations, thereby contributing to the development of skin cancer and other UV-related pathologies. One mechanism of repairing UV-induced pyrimidine dimers is the direct reversal of the dimerization reaction known as photoreactivation because energy derived from visible light is utilized to break the cyclobutene ring structure to restore the pyrimidine bases in normal state in DNA.

Another form of direct repair deals with damage resulting from the reaction between endogenous and exogenous forms of alkylating agents and DNA. The most frequent lesions brought on by alkylating substances are N1-methylguanine (1meG), O6-methylguanine (O6meG), N7-methylguanine (7meG), N3-methylguanine (3meG), N3-methylcytosine (3meC), N1-methyladenine (1meA), and N3-methyladenine (3meA) [69]. The O6-methylguanine causes methylation of guanine at its O6 position, generating complementary base pairs with thymine rather than cytosine. An enzyme (known as O6-methylguanine methyltransferase) that transfers the methyl group from O6-methylguanine to a cysteine residue in its active site can repair this injury by removing the mutagenic chemical modification, and hence restores the original guanine and is found in both prokaryotes, Eukaryotes (humans) [5]. Previous studies have shown that low levels of DR proteins contribute to elevated cancer risk progression and are important determinants of therapeutic response. Numerous *DR* genes, such as *ALKBH3* and *MGMT*, often undergo changes, primarily through epigenetic silencing [70-74]. Studies suggest that the absence of MGMT (O6-Methylguanine Methyltransferase) is associated with point mutations in KRAS, observed in colon and gastric cancers, as well as in p53 of non-small cell lung cancer and astrocytic tumors [75]. Additionally, MGMT promoter methylation is frequently observed in various cancer types, including glioma, lymphoma, breast, and retinoblastoma [75-78]. Other reports have shown that *ALKBH2* and *ALKBH3* genes are often overexpressed in certain cancers, such as non-small cell lung carcinoma, prostate adenocarcinoma, and pancreatic adenocarcinoma and the downregulation of *ALKBH2* contributes to the development and progression of various cancers, such as gastric cancer. Altered DNA damage repair pathways are often targeted with anti-cancer agents to enhance a favorable tumor response through synthetic lethality. Cancer cells lacking DR pathways can be targeted with alkylating agents. However, many cancers overexpress DR enzymes, rendering them resistant to alkylating agents. Therefore, using inhibitors to inactivate MGMT or ALKBH proteins in tumors

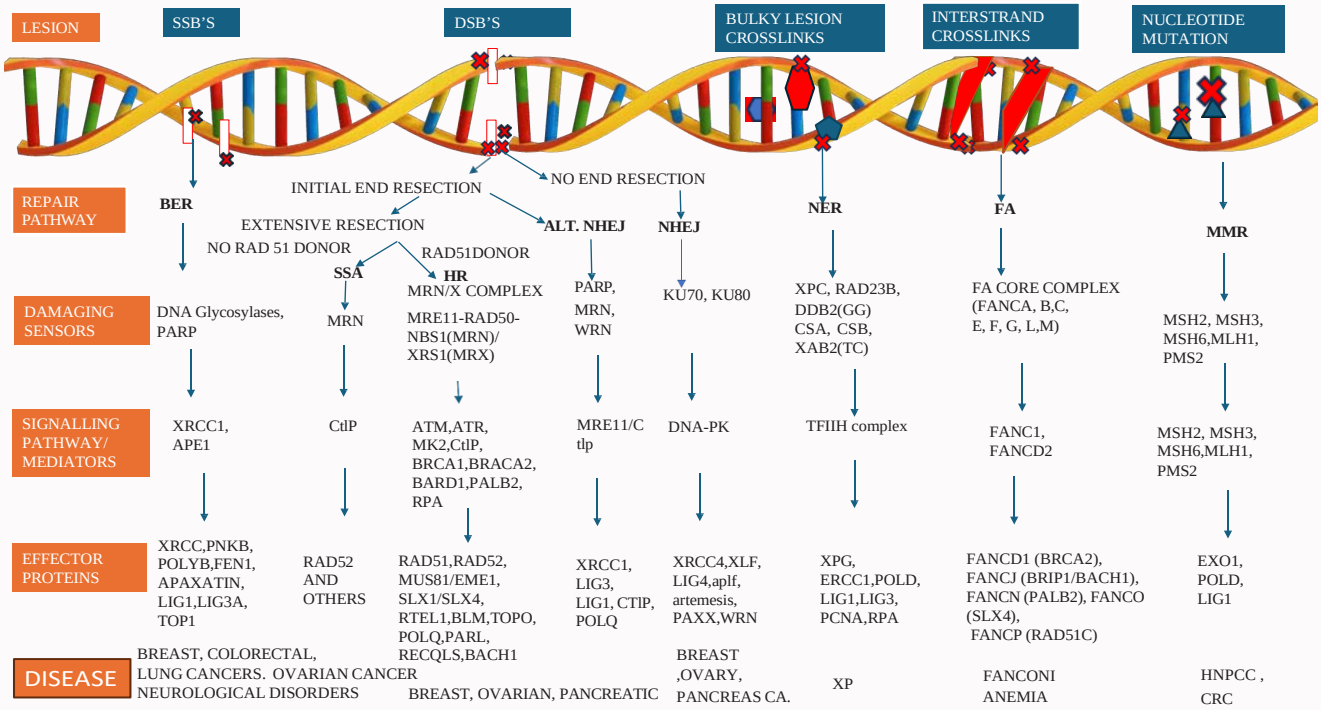

Figure 2: Summery of DDR pathways and associated diseases: The Key DDR signaling components in mammalian cells are the damaging sensors, mediators and effectors protein involved in various pathways, and alteration in any pathway associated diseases.

Supplementary Table 2: DDR pathways associated genes their function and associated diseases.

DDR pathways associated genes their function and associated diseases			
DDR	**Gene**	**Function**	**Link Cancer Types**
BER	*XRCC1*	Scaffolding protein for BER	Breast, lung, ovarian, gastric, bladder, colorectal
	APEX1	AP endonuclease	Breast, lung, colorectal
	POLB	DNA polymerase beta	Breast, colorectal, lung
NER	*XPA*	Damage recognition factor	Skin, lung, bladder, breast
	ERCC1	Endonuclease for NER	Lung, colorectal, gastric, pancreatic
	XPC	Damage recognition factor	Skin
	XPB	Helicase for NER	Skin
HR	*BRCA1*	Tumor suppressor, DDR	Breast, ovarian, pancreatic
	BRCA2	Tumor suppressor, DDR	Breast, ovarian, pancreatic
	PALB2	BRCA2 co-factor	Breast, ovarian, pancreatic
NHEJ	*XRCC4*	Ligase for NHEJ	Breast, gastric, colorectal, lung
	DNA-PKcs	Kinase for NHEJ	Lymphoma
	Ku70/Ku80	Damage recognition factors for NHEJ	Breast, gastric, lung, colorectal
MMR	*MLH1*	MMR protein, DDR	Colon, endometrial, gastric
	MSH2	MMR protein, DDR	Colon, endometrial, gastric
	MSH6	MMR protein, DDR	Colon, endometrial
	PMS2	MMR protein, DDR	Colon, endometrial

DDR: DNA Repair Pathway; BER: Base Excision Repair; NER: Nucleotide Excision Repair; NHEJ: Non-Homologous End Joining; MMR: Mismatch Repair; HR: Homologous Recombination

is helpful in increasing the response to alkylating agents.

(II) Base Excision Repair Pathway (BER): BER is an essential DNA repair pathway that maintains genomic integrity by repairing damages from oxidative, alkylating, and deamination genotoxic activities. It plays a critical role by identifying and excising small base adducts inappropriate or oxidized bases, and DNA single-strand breaks generated by several groups of environmental agents or their metabolic intermediates, followed by the precise replacement of the damaged DNA segment with the correct nucleotides [79-81]. Moreover, BER operates through two common pathways: Depending on the size of the repair patch i.e., short patch BER, which repairs

single nucleotide areas, while long patch BER repairs areas involving two or more nucleotides. The BER repair process involves several steps like first DNA glycosylases recognize and excise damaged or modified bases, creating an abasic site. PARP1 and PARP2 act as sensors for Single-Stranded Breaks (SSBs) and recruit BER factors like XRCC1 to the damaged site. XRCC1 then recruits the APE1 nuclease, which cleaves the abasic site to generate a 3' OH and a 5' deoxyribose phosphate (dRP) terminus in the DNA strand. End processing follows, where RFC recruits a complex of PCNA and DNA-Polδ/ε to displace and resynthesize 2 to 8 nucleotides around the damaged site. FEN1, an endonuclease, cleaves the displaced oligonucleotide and then ligase I seals the resulting single-strand break by gap filling and DNA synthesis with the correct nucleotides. These steps collectively ensure the effective repair of DNA lesions in the BER pathway [81-83].

The short-patch repair pathway in BER involves several core proteins which includes an initiating DNA glycosylase, AP-endonuclease APE1, DNA Polymerase β (Pol β), and DNA Ligase I or III (LIG1/3), poly (ADP-ribose) Polymerase 1 and 2 (PARP1 and PARP2), XRCC1, Flap Endonuclease FEN1, and associated factors. PARP1 and PARP2 act as sensors and signal transducers for lesions, while XRCC1 plays a role in coordinating the repair process [79]. Long-patch repair occurs mainly in proliferating cells, involving proteins such as DNA polymerase δ/ε, Proliferating Cell Nuclear Antigen (PCNA), FEN1, and LIG1. These proteins facilitate the processing and repair steps following glycosylase activity and strand cleavage by APE1 [67,84].

The repair of uracil-containing DNA is an excellent example of BER, in which single damaged bases are recognized and removed from the DNA molecule. Uracil can be found in DNA through two distinct processes. Firstly, during DNA replication, uracil (in the form of dUTP Deoxyuridine Triphosphate) can occasionally replace thymine. Secondly, uracil can arise in DNA *via* cytosine deamination. Additionally, DNA glycosylases can identify and eliminate other abnormal bases, such as hypoxanthine (formed by adenine deamination), pyrimidine dimers, alkylated purines other than O6-alkylguanine, and bases damaged by oxidation. However, if there are imbalances in BER proteins in human cells, BER intermediates such as Single-Strand Breaks (SSBs) and Double-Strand Breaks (DSBs) can accumulate, which can contribute to genomic instability [83,85]. Multiple studies have indicated that the modification of *BER* genes is linked to a variety of diseases, including cancer, neurological disorders, and the aging process.

(III) Nucleotide Excision Repair Pathway (NER): NER is a major DNA repair pathway in mammals that preserves genomic integrity by eliminating various helix-distorting DNA lesions caused by environmental mutagens such as UV irradiation and specific bulky platinum-based chemotherapeutic agents [86-90]. NER operates by excising damaged nucleotides *via* dual incisions that flank the lesion site. This results in the release of a concise single-stranded DNA fragment, typically spanning 22 to 30 nucleotides in mammalian cells, encompassing the damaged region. NER includes the following three key steps: (1) recognition of DNA damage, involving initial recognition and verification; (2) dual incisions and removal of the damaged section; and (3) gap filling, encompassing repair synthesis and DNA ligation to restore the integrity of the DNA strand. The two sub-pathways of NER include: Global Genome NER (GG-NER) which can occur throughout the genome and is initiated by the GG-NER specific factor XPC-RAD23B, sometimes assisted by UV-DDB while Transcription-Coupled NER (TC-NER) specifically targets lesions on the transcribed strand of active genes and is initiated by RNA polymerase stalled at a lesion and requires TC-NER-specific factors CSA, CSB, and XAB2 [86,91-95]. Both pathways rely on the core NER factors to complete the excision process and restore the damaged DNA [86]. In both GG-NER and TC-NER, the final step involves recruiting the TFIIH complex which comprises of two ATPase/helicase subunits, XPB and XPD, with XPB playing an essential role in both transcription and NER processes. XPB and XPD act as a helicase and unwind a 30-nucleotide fragment surrounding the damaged site. This unwinding activity enables further processing of the DNA lesion and facilitates the subsequent steps of NER, leading to the repair of the damaged DNA region [96]. After DNA unwinding, the XPF/ERCC1 and XPG complexes are recruited, exhibiting nuclease activity at the 5' and 3' ends of the DNA lesion. Excision Repair Cross-Complementing Protein 1 (ERCC1) is crucial for this excision step and is involved in the precise cleavage of the damaged DNA strand [5]. The damaged site is then resynthesized by complexes involving DNA Polymerase δ/ε (DNA-Polδ/ε), Replication Factor C (RFC), Proliferating Cell Nuclear Antigen (PCNA), or alternatively, DNA-Polδ/ε and XRCC1. These complexes are responsible for synthesizing and filling in the missing DNA segment, ensuring the restoration of the damaged site and the SSB is sealed by ligase I or IIIa [90]. In recent findings, a novel class of enzymes known as Alkyltransferase-Like (ATL) proteins has been discovered, which can redirect bulky O6-alkylguanine lesions towards the NER pathway.

The key proteins involved in NER are sensors elongating RNA polymerase, XPC-HR23B and DDB1/2, XPA and XPE, XPF/ERCC1 and XPG, CSA and CSB, TFIIH complex contains helicases XPB and XPD, DNA Polymerase k and other DNA polymerases, PCNA (Proliferating Cell Nuclear Antigen) and RPA (Replication Protein A). Ligase I and III Together, these proteins form a complex network that enables the recognition, verification, incision, and repair of DNA lesions through the nucleotide excision repair pathway [67]. Another Xeroderma Pigmentosum group C protein (XPC) is extensively studied due to its vital role in the initial steps of identifying DNA damage and activating the NER pathway [5,67,86,97]. Deficiencies in NER factors, including XPC, lead to a range of inherited diseases with distinct phenotypes. Cockayne Syndrome (CS) is characterized by growth abnormalities, neurological impairments, and premature aging. Ultraviolet Sensitive Syndrome (UV-SS) results in heightened sensitivity to UV light. These diseases underscore the importance of NER in maintaining genomic integrity and protecting against the harmful effects of DNA damage, offering valuable insights for potential treatment strategies [98,99].

(IV) Homologous Recombination Pathway (HR): HR pathway is involved in the exchange of genetic information between allelic sequences; it is a mechanism that repairs a variety of DNA lesions in mitosis and chromosomal pairing and exchange during meiosis, including DSBs), single-strand DNA gaps, stalled replication forks, inter-strand crosslinks and sites of meiotic recombination and abortive topoisomerase II action [67,100] HR acts mainly in the S and G2 phases of the cell cycle and is a conservative process and tends to restore the original DNA sequence to the site of damage when an intact sister chromatid is available as a template.

HR is largely error-free and begins with the resection of a DSB by nucleases and helicases. The MRN complex is crucial in recognizing

and binding to the free DNA ends resulting from DSBs. By acting as a DNA damage sensor, it facilitates the recruitment and activation of other repair factors necessary for HR. The complex components, MRE11, RAD50, and NBS1 (or XRS1 in yeast), coordinate key steps in HR, including DNA end processing, resection, and strand exchange. Once DNA synthesis is initiated in HR, at least three different routes can be taken. In the Double-Strand Break Repair (DSBR) model, the second end of the DSB can be engaged to stabilize the D-loop structure, resulting in the formation of a double-Holliday Junction (dHJ). The dHJ can then be resolved to produce crossover or non-crossover products or dissolved to generate non-crossover products exclusively. Alternatively, the invading strand in the D-loop can be displaced and annealed with its complementary strand through gap repair or associating with the other end of the DSB. This mode is known as Synthesis-Dependent Strand Annealing (SDSA) and is preferred during mitosis. In meiosis, crossovers are formed by resolving dHJs *via* the DSBR mechanism, while non-crossovers primarily result from the SDSA mechanism. In the third mode, the D-loop structure can assemble into a replication fork, leading to the replication and copying of the entire chromosome arm. This process is called Break-Induced Replication (BIR) which is more commonly observed when there is only one DNA end available, either due to the loss of the other end or in telomerase-deficient cells during telomere lengthening. The HR process provides various pathways that allow for diverse options in DNA repair and replication, ultimately resulting in the accurate restoration of DNA integrity essential for maintaining genomic stability in various cellular contexts. The essential protein components involved in HR include RAD51, RAD51-related proteins (such as XRCC2, XRCC3, RAD51B, RAD51C, RAD51D, DMC1, RAD52, RAD54, BRCA2, RPA, and FEN1). Additionally, several factors facilitate the HR process, such as the MRN complex (MRE11-RAD50-NBS1), CtIP, BRCA1, and the ATM signaling pathway. These factors are crucial for DNA end processing, strand resection, and the regulation of HR [67].

HR pathway disruptions promote genomic instability and contribute to cancer initiation and progression. Heterozygous germline mutations in HR genes significantly elevate cancer risk. Disabling mutations in BRCA1, BRCA2, ATM, CHEK2, RAD50, RAD51C, and others are frequently found in various cancers such as lung cancer, ovarian carcinomas, pancreatic ductal adenocarcinoma, and Chronic Lymphocytic Leukemia (CLL) [5,96].

(V) Non-Homologous End Joining Pathway (NHEJ): NHEJ is a collection of pathways involved in maintaining the integrity of the genome in which two DNA DSB ends are re-joined by apposition, processing, and ligation without using extended homology to guide repair [101,102]. NHEJ comprises two main subtypes: Classical-NHEJ also known as "canonical NHEJ" or c-NHEJ relies on specific factors essential for V (D) J recombination. These factors include the KU70/80 heterodimer (KU), XRCC4, Ligase IV, and DNA-PKcs [103,104], On the other hand, alternative-NHEJ (alt-NHEJ) or "backup NHEJ" is distinct from classical-NHEJ, which operates independently of the factors mentioned earlier. Alt-NHEJ, also known as "Microhomology-Mediated End Joining" (MMEJ), frequently leads to deletions at the repair junction and utilizes microhomology regions [101,105-107].

Although NHEJ is active throughout the cell cycle and is favored in G1 cells, importantly, c-NHEJ is still possible in S/G2 and remains a predominant repair pathway for DSBs in mammalian G2 [79,108-110]. During the process of NHEJ, the DNA DSB ends are safeguarded against 5' end resection and maintained in proximity by a protein complex called the Ku70-Ku80 heterodimer (Ku). NHEJ facilitates the direct joining of the DSB ends, but this repair mechanism is prone to errors. As a result, it often leads to the occurrence of mutations, small insertions, deletions, and substitutions at the break site and other alterations in the repaired DNA sequence. Additionally, if DSBs arise from different genome regions, NHEJ can result in translocations where the broken ends are incorrectly fused together [108].

Upon initiation of NHEJ, the non-catalytic subunits Ku70 and Ku80 come together to form a heterodimer, which plays a crucial role in detecting and attaching to the broken ends of the DNA [111,112]. The Ku70-Ku80 complex facilitates the binding and activation of DNA-PKcs. One notable outcome of impaired classical-NHEJ (c-NHEJ) is an elevation in chromosomal mutagenesis associated with Double-Strand Breaks (DSBs). Inefficient V(D)J recombination, a process reliant on c-NHEJ, is accompanied by an increased susceptibility to developing B- and T-cell lymphomas due to the persistence of unresolved DSBs. The formation of medulloblastoma, a type of brain tumor, is also observed in cases where c-NHEJ function is compromised [113-115].

(VI) Fanconi Anaemia Pathway (FA): FA is a rare autosomal or x-chromosomal recessive human genetic disease that was first described by Guido Fanconi in 1927 which is a genetically heterogeneous instability disorder caused by mutations in genes regulating replication-dependent removal of inter-strand DNA crosslinks [115,116]. The FA pathway is thought to coordinate a complex mechanism that enlists elements of three classic DNA repair pathways, namely HR, NER, and mutagenic trans-lesion synthesis, in response to genotoxic insults and is essential for the repair of DNA Inter-Strand Crosslinks (ICLs) [117], and shares components, such as BRCA2 and PALB2, with these pathways. It employs a unique nuclear protein complex that ubiquitinates FANCD2 and FANCI, which coordinates multiple DNA repair activities required to resolve ICLs [116,117]. The FA pathway, also called the FA-BRCA pathway, is a fundamental DNA repair pathway, involved with 22 genes, i.e., *FANCA*, *FANCB*, *FANCC*, *FANCD1*, *FANCD2*, *FANCE*, *FANCF*, *FANCG*, *FANCI*, *FANCJ*, *FANCL*, *FANCM*, *FANCN*, *FANCO*, *FANCP*, *FANCQ*, *FANCR*, *FANCS*, *FANCT*, *FANCU*, *FANCV*, and *FANCW* [118-122]. The proteins in the FA pathway can be grouped into three functional subgroups- the FA core complex, the FA-ID complex, and downstream FA proteins. The FA core complex comprises eight proteins (FANCA, B, C, E, F, G, L, M) [103,116,123]. FANCM is a crucial component and activates the FA pathway by forming a heterodimeric complex with FAAP24 [103,123]. FANCM plays a vital role as a critical element in initiating the FA pathway through its association with FAAP24 to form a FANCM/FAAP24 complex which identifies the ICL and subsequently brings in other components of the FA core complex to help in stabilizing replication forks stalled at the ICL site and triggers cell cycle checkpoints mediated by ATR/Chk1 [103,123]. Finally, the FA core complex essentially constitutes a multi-subunit E3 ubiquitin ligase complex, which ultimately leads to the mono-ubiquitination of the FA-ID complex, composed of FANCD2 and FANCI [103,123]. Its primary function is to facilitate the mono-ubiquitination of the FA-ID complex, consisting of FANCD2 and FANCI. FANCL mediates the process of mono-ubiquitination and holds significant regulatory importance within the FA pathway. This step is crucial as it enables the recruitment of the FA-ID complex to the site of the ICL [103,123]. The FA-ID complex

enables ICL repair through the downstream FA proteins *FANCD1* (*BRCA2*), *FANCJ* (*BRIP1*/*BACH1*), *FANCN* (*PALB2*), *FANCO* (*SLX4*), and *FANCP* (*RAD51C*) [103,123]. The FA pathway includes familial breast cancer predisposition genes commonly associated with HR pathway dysfunction, highlighting a notable overlap between the two pathways. In the initial stages of ICL repair, the nucleases FAN1 and SLX4 are recruited to the mono-ubiquitinated FA-ID complex through specific ubiquitin-binding zinc finger four domains. Subsequently, MUS81/EME1 and the NER nucleases XPF/ERCC1 are involved in further cleavage events, facilitating the unhooking of the cross-link, and allowing for its elimination. The unhooking of the ICL results in the generation of a DSB at the site of a stalled replication fork. This DSB is subsequently repaired through the HR pathway, which involves the faithful and accurate restoration of DNA using the intact sister chromatid as a template [103,123]. To facilitate the repair of the DSB resulting from the unhooking of the ICL, specialized DNA polymerases called Translesion Synthesis (TLS) polymerases come into play which can bypass the damaged crosslinked regions of the DNA strand by incorporating nucleotides opposite the lesions, allowing the replication process to continue past the crosslinked sites. By doing so, TLS polymerases generate an intact DNA strand that can serve as a template for the subsequent HR-mediated DSB repair process which ensures the accurate and efficient restoration of DNA integrity following the ICL repair [103,123].

In the final steps of the FA pathway, the NER machinery is recruited to the site of the repaired ICL where it eliminates any remaining DNA adducts or lesions to ensure complete restoration of DNA integrity. After that the resulting gap in the DNA strand is sealed and repaired by the activity of specific DNA polymerases thereby restoring the continuity of the DNA strand [103,123,124]. Several studies showed that the breast cancer susceptibility genes *BRCA1* and *BRCA2*, also known as *FANCS* and *FANCD1*, respectively, are involved in the FA pathway and carriers with inherited heterozygous mutations with high risk for developing breast and ovarian cancer, Acute Myeloblastic Leukemia (AML) [116,118]. Biallelic mutations in Fanconi anemia genes lead to bone marrow failure and susceptibility to Acute Myeloid Leukemia (AML), solid tumors, congenital abnormalities, and infertility.

(VII) Mismatch Repair Pathway (MMR): The MMR pathway plays a critical role in the DDR by addressing DNA replication and recombination errors. It deals primarily with dNTP misincorporation and the formation of 'insertion and deletion' loops (Indels) that form during DNA replication. The MMR system serves as a mechanism for identifying and repairing these indels and misincorporations of bases that may arise during DNA replication and recombination [125]. Furthermore, it also plays a role in repairing certain types of DNA damage beyond replication errors, contributing to the overall maintenance of genomic stability. The MMR pathway prevents permanent mutations in cell divisions. Hence, if there is a flaw or defect in the MMR process, it would increase the spontaneous mutation rate and has the responsibility of reducing the number of replications linked to errors. These errors cause base 'mismatches' in the DNA sequence (that is, non-Watson-Crick base pairing) that distort the helical structure of DNA and so are recognized as DNA lesions. Upon detecting such a distortion or mismatch, a series of events is triggered to rectify the error which includes the removal or excision of the newly synthesized DNA strand containing the mismatched site, followed by the resynthesis of DNA to replace the excised portion.

The essential genes involved in this MMR pathway include MutS alpha (detection of minor mismatches, formed by MSH2/MSH6), the MutSβ complex (detection of significant mismatches and insertion loops, formed by MSH2/MSH3), and MutL homolog genes, such as MSH2 and MLH1 [79]. It has been suggested in eukaryotes that the lagging strand undergoes a process where transient nicks or breaks occur. These nicks are coated by a protein called the β-sliding clamp PCNA, with the assistance of a Replication Factor C (RFC). The connection between the MutS protein and the PCNA/RFC complex is facilitated by a complex called MutLα, which consists of MLH1 and PMS2 proteins [125]. The precise mechanism of this connection is not entirely understood. Once the MutS/MutLα complex binds to the PCNA/RFC complex, it recruits the exonuclease Exo1 followed by DNA Polymerase δ (Polδ), Exonuclease 1 (hExo1) enzyme involved in synthesizing and completing the DNA strand, thereby repairing the damaged region [125]. The interaction between hExo1 and several MMR proteins, including MutL and the DNA lesion recognition proteins MutSα and MutSβ, regulates the exonucleolytic activity [126,127].

Deficiencies in the MMR system led to an elevated rate of spontaneous mutations and play a role in multistage carcinogenesis [128]. Most human cancers, whether hereditary or non-hereditary, are linked with the inactivation of MMR in the cells, and some DNA damage demands the MMR mechanism to function for cell cycle arrest and programmed apoptosis. Therefore, MMR has a vital role in the DDR pathway to eradicate the seriously damaged cells and suppress both mutagenesis in the short term and tumorigenesis in the long term [5,129]. Several reports have shown that the alteration of MutSα/MutSβ (*MMR* genes (*MSH2*, *MSH6*, *MLH1*, *PMS2*) results in microsatellite instability or Elevated Microsatellite Alterations at Selected Tetranucleotide repeats (MSI or EMAST) genotypes and have been associated with multiple cancers like colorectal cancer glioblastomas, lymphomas, stomach, urinary tract, ovaries, and endometrium pathogenesis [130-136].

(VIII) Single Strand Annealing Pathway (SSA): SSA represents a DNA DSB repair pathway that utilizes homologous repeat sequences located on either side of a DNA DSB, which undergoes annealing and bridges the ends of DSBs [107,137]. Consequently, the outcome of DSB repair *via* SSA involves flanking repeats which entails a deletion rearrangement occurring between the homologous repeats resulting in a relatively high mutagenic effect and loss of genetic information [137]. The functional analysis of various factors has provided evidence that DSB end resection, which involves the creation of single-stranded DNA (ssDNA) with a 3' end, plays a crucial role in the SSA process [138,139]. Based on the previous evidence, it is suggested that CtIP, a protein known as CtBP-interacting protein, a key end resection factor, is crucial for the dependence of SSA [140-142]. In contrast, there are factors known to inhibit end resection, and their presence has been found to suppress SSA. This includes the involvement of specific components in the DDR pathway, such as H2AX, RNF168, 53BP1, and RIF1. Studies have shown that these factors play a role in preventing excessive end resection and thereby negatively regulating SSA occurrence [138,141,143-145].

Studies have shown that chromosomal translocations, which are known to play a significant role in various types of cancer, can occur through SSA. When RAD52, a vital regulator of SSA, is inhibited, the proliferation of cells lacking BRCA1/2 is reduced. This finding has relevance in cases of hereditary breast and ovarian cancer. Numerous

cancer-related genes, such as BRCA1 and MLL (Myeloid/Lymphoid or Mixed-Lineage Leukemia; KMT2A), contain multiple copies of the Alu element, the most common transposon found in the human genome, representing around 10% of its sequences [107]. Improper recognition of multiple DSBs occurring in different chromosomes by SSA can occur due to Alu repeats flanking these DSBs. This can result in significant chromosomal rearrangements, such as reciprocal translocations, which are believed to be crucial in the development of many cancers, particularly in leukemias and lymphomas [146].

In summary, SSA can potentially contribute to cancer transformation due to its association with increased genomic instability and the induction of chromosomal translocations. Additionally, inhibiting SSA can lead to the demise of cancer cells that are deficient in other Double-Strand Break Repair (DSBR) pathways, remarkably Homologous Recombination Repair (HRR) with impaired RAD51 or BRCA1/2. Consequently, SSA represents a valuable component of synthetic lethality-based cancer therapy, providing a promising approach for targeted treatment [107].

(IX) Alternative End Joining Pathway (ALT-EJ): In addition to SSA, another DSB repair pathway is also initiated by end resection known as ALT-EJ. It is a repair mechanism that involves the annealing of short homologous repeats called microhomology, which are located on either side of a DSB. It shares similarities with SSA as both pathways involve annealing flanking repeats to bridge a double-strand break. Due to this similarity in mechanism, ALT-EJ is sometimes referred to as micro-SSA as well as Microhomology Mediated End Joining (MMEJ) [139,147-151]. The mending of double-strand breaks through ALT-EJ; specifically, the mechanism known as MMEJ, becomes more apparent in mammalian cells lacking functional NHEJ. For instance, processes like class switch recombination, typically reliant on NHEJ elements, proceed *via* an ALT-EJ route when the NHEJ pathway is inactive. Similarly, cells with impaired Homologous Recombination (HR) increasingly rely on a-EJ pathways to fix double-strand breaks [152-155]. Due to the significance of end resection in ALT-EJ, it was commonly hypothesized that the repair of DSB's through Single-Strand Annealing (SSA), MMEJ, and other end-joining pathways primarily takes place in cells during the S and G2 phases. This assumption is rooted in the activation of end-resection processes during these phases. The initiation of all ALT-EJ pathways, much like HR, is instigated by the end resection process and involves a subset, if not the entirety, of the factors that constitute the HR end resection machinery. The ALT-EJ pathways also exhibit similarities to NHEJ in that they bring together the DNA ends without requiring a homologous template for guidance. Nevertheless, they diverge in utilizing distinct levels of sequence homology to align the DNA molecules. In both Homologous Recombination (HR) and Alternative End Joining (ALT-EJ), the initiation of end resection is prompted by the Mre11/Rad50/Nbs1 (MRN) complex and CtIP. Then, PARP-1 facilitates the rapid recruitment of MRN and CtIP to the DSB end, where CtIP enhances MRN's endonuclease activity, leading to an internal single-strand break in the 5′ strand. A pivotal stage in all ALT-EJ pathways involves bringing the DNA ends into proximity. In the case of the other ALT-EJ pathways, various proteins have been proposed to link DNA ends and align them through microhomologies like PARP-1, MRN, and Pol θ. More recently, a DNA polymerase from the A-family, Pol θ, has been identified as a pivotal element in ALT-EJ. Pol θ can search for and align microhomologies, thereby linking DNA ends. This alignment might also occur independently of MRN when long-range exonucleases like Exo1 and DNA2 resect the ends. The finalization of double-strand break repair *via* alternative end-joining pathways requires the LigIIIα-XRCC1 complex to ligate the termini after end processing [156-158]. The repair of DSBs by ALT-EJ is inherently mutagenic, potentially giving rise to chromosomal translocations and intra- and inter-chromosomal deletions and insertions [156]. DSBs in cancer cells are often inaccurately repaired *via* the Alternative Non-Homologous End Joining (Alt-NHEJ) pathway, resulting in genomic instability. This instability has the potential to initiate oncogenic transformation and progression of cancer. Paradoxically, these events also present vulnerability in cancer that can be strategically exploited through a synthetic lethality approach for therapeutic purposes. Moreover, they offer potential biomarkers for the effectiveness of immunotherapy. Alt-NHEJ was also involved in human Neural Crest Stem Cells (NCSCs), contributing to the neoplastic transformation driven by the pro-tumorigenic activity of MYCN in neuroblastoma precursors [159].

DDR Gene, Their Malfunction, and Associated Inhibitors

Several lines of evidence have shown that in a normal system, DNA damage is detected and repaired by various sensors and different repair pathways such as BER, NER, MMR, and HR to ensure accurate repairing, preserving the integrity of the genetic material [9,129,160-162] and to prevent the accumulation of mutations [5,9,163]. In contrast, deficiencies in the DDR pathways, in which several genes are involved, have been found to get frequently mutated resulting in the accumulation of mutations, and altered protein function, leading to defects in DNA repair and genomic instability which is one of the hallmarks for multiple diseases like cancer [5,159,163-166]. Some of the most well-known *DDR* genes (*BRCA1/2*; *ATM*; *CHEK2*; *TP53*; *XRCC1*; *ERCC1*; *MSH2/6*; *PALB2*; *RAD51*; *XRCC1*) briefly discussed below along with their associated mechanisms of action and associated inhibitors are mentioned in detail in (Supplementary Table 3).

1a. Breast Cancer Associated gene 1/2 (*BRCA1/2*): Studies have demonstrated that tumor suppressor genes such as *BRCA1/2* are involved in the maintenance of DSBs ensuing genomic stability *via* the HR pathway [167,168]. Several mounting pieces of evidence have shown that somatic and germline genetic predisposition of *BRCA1* and *BRCA2* with loss-of-function mutations is often associated with an increased risk of breast, ovarian, prostate, and pancreatic cancers [169-180]. Individuals with *BRCA1* mutations have a 50% to 80% chance of developing breast cancer by the age of 70 [181-183].

1b. PARP inhibitors: Reports have shown that Poly ADP-Ribose Polymerase (PARP) inhibitors are vital in function as they are implicated in the BER pathway and act by blocking the activity of PARP enzymes *via* inhibiting the repair process of ssDNA breaks leading to dsDNA breaks and subsequent accumulation of DNA damage and cell death [184-186]. PARP inhibitors (PARPi) are clinically applicable for treating cancers as they bind to the active site of PARP molecules and compete with NAD+ and are more effective in treating tumors with mutations in HR genes like *BRCA1* and *BRCA2* and make cancer cells vulnerable to PARP inhibition [187-190]. Other examples of PARP inhibitors include, Olaparib (used in treating breast and ovarian cancer); Talazoparib (used in treating breast cancer); Rucaparib (used in treating ovarian cancer); Niraparib (used in treating ovarian and fallopian tube cancer) and Veliparib are currently being evaluated in clinical trials for the treatment of

Supplementary Table 3: DDR pathway inhibitors their mechanism and limitations.

DDR pathway inhibitors their mechanism and limitations				
	Drugs	**In tumor Activity**	**Limitation**	**Resistance**
PARP Inhibitors	Olaparib, Talazoparib, Rucaparib, Niraparib, Veliparib	Bind to active site of PARP molecules and compete with NAD+ inhibiting the repair process of ssDNA and dsDNA breaks	Drug resistance, lack of biomarkers, limited data long term safety and side effects such as nausea, fatigue and anemia, myelodysplastic syndrome or AML	Drug metabolism or uptake alterations in DDR pathways and epigenetic changes
ATM Inhibitors	AZD0156,M4344,KU-60019, CEP-8983	Dysregulation of ATM kinases	Drug resistance, lack of biomarkers, Off-target effects such as impaired glucose metabolism and maintaining immune system and possess some toxic effects on normal cells like bone marrow and GI tract.	Mutations in ATM or activation of alternative DDR pathways
Checkpoint Kinase I Inhibitors	Prexasertib, GDC-0575, SRA737, PF-477736, MK-8776, AZD7762 and CHIR-124	Dysregulate of CHK I Kinase enzyme	Drug resistance, lack of biomarkers, limited data long term safety and limited efficacy using as monotherapy with optimum dose	Mutations in CHEK 1/2, epigenetic changes altered the expression and activity of CHEK 1/2
MMR gene Inhibitors	Pembrolizumab and nivolumab	Targets PD-1 receptor on T cells can block the signals from cancer cells		Immune checkpoint inhibitors in MSI-H/dMMR tumors, loss of neoantigens in tumors, which are mutated peptides that are presented on the tumor cell surface, upregulation of complementary immune checkpoints like TIM-3 and LAG-3, accumulation of genetic alterations in the genes like JAK1/ JAK2 or ß2M
ATR Inhibitors	Berzosertib, VE-821/22, BAY 1895344, AZD6738, VX-970 (M6008)	Inhibit activity of ATR Kinase	Drug Resistance, limited efficacy, limited predictive biomarkers and toxic effects on normal cells especially on bone marrow and GI tract.	Mutations in ATR or activation of alternative DDR Pathways.
MDM2 Inhibitors	Nutilin-3, RG7388, AMG-232, DS-3032b, 8242	Targets an E3 ubiquitin ligase MDM2 protein	Drug resistance limited predictive biomarkers	Mutations in MDM2/p53 or activation of alternative pathways that bypass the MDM2-p53 axis.
MMR gene Inhibitors	Pembrolizumab and nivolumab	Targets PD-1 receptor on T cells can block the signals from cancer cells		Immune checkpoint inhibitors in MSI-H/dMMR tumors, loss of neoantigens in tumors, which are mutated peptides that are presented on the tumor cell surface, upregulation of complementary immune checkpoints like TIM-3 and LAG-3, accumulation of genetic alterations in the genes like JAK1/ JAK2 or ß2M
ATR Inhibitors	Berzosertib, VE-821/22, BAY 1895344, AZD6738, VX-970 (M6008)	Inhibit activity of ATR Kinase	Drug Resistance, limited efficacy, limited predictive biomarkers and toxic effects on normal cells especially on bone marrow and GI tract.	Mutations in ATR or activation of alternative DDR Pathways.
MDM2 Inhibitors	Nutilin-3, RG7388, AMG-232, DS-3032b, 8242	Targets an E3 ubiquitin ligase MDM2 protein	Drug resistance limited predictive biomarkers	Mutations in MDM2/p53 or activation of alternative pathways that bypass the MDM2-p53 axis.
DNA-PK Inhibitors	VX-984, M3814	Against DNA-dependent protein kinase enzyme (DNA-PK) which is responsible for restoring DSB in DNA		
MGMT Inhibitors	O6-Benzylguanine and Lomeguatrib	DNA damage caused by alkylating agents can be restored by using MGMT gene which helps to repair it.		

several types of cancer, including ovarian and lung cancer and as a combination therapy with other treatments [191,192].

2a. Ataxia-Telangiectasia Mutated (ATM): Previous studies have shown that *ATM* gene is involved in DSBs through DDR, NHEJ and HRR pathways [193-195]. Several studies have reported that loss-of-function mutations in *ATM* increase the risk for wide range of cancers, including colorectal, lung, breast, lymphoma, and leukemia, uterine and prostate cancer [196-200]. *ATM* inhibitors are currently being investigated in preclinical and early-phase clinical studies for various cancer types [201-204].

2b. *ATM* inhibitors: Reports have shown that *ATM* inhibitors show promising results by blocking the activity of the ATM kinase, which is one of the critical proteins in the DDR pathway. Dysregulation of ATM kinases has been linked to multiple cancers, including leukaemia, lymphoma, and breast cancer [201-203] because the specific *ATM* inhibitors can prevent cancer cells from repairing DNA damage, which in turn induces cell cycle arrest and ultimately leads to cell death. These inhibitors have revealed encouraging outcomes under the preclinical and clinical studies i.e., phase I/II clinical trials, such as AZD0156 (by AstraZeneca) and M4344 (both *ATM* and *ATR* by Merck) both are used for treating lymphomas, solid tumors and leukemias. Whereas KU-60019 and CEP-8983 (by Cephalon) are used for solid tumors and lymphomas respectively. However, further studies are warranted to fully understand their safety and efficacy in treating cancer.

3a. Checkpoint Kinase1/2 (*CHEK1/2*): Is vital in regulating and repairing the DNA damage or replication stress by controlling the S-phase and G2/M checkpoint replication through DDR pathway, where it causes the cell to pause and repair the damage by replication process. Reports have shown that inherited mutations in these genes have been identified as an increased risk factor for families with a history of breast and/or ovarian cancer. On the contrary, somatic mutations can lead to a loss or reduction of *CHEK2* function, which can impair the DNA damage response and increase genomic instability as found in various diseases like ovarian, breast, prostate, and colon cancers [205-208]. Likewise, alteration and deregulation in the expression of *CHEK1* gene have also been linked to an increased risk of certain types of cancer like leukemia, breast, and lung cancer. The *CHEK1* and *CHEK2* are currently being studied as therapeutic potential targets for cancer therapy and tumors with defects in other DNA repair pathways, like *BRCA1/2* mutations. However, their exact role in cancer development is not clear, as the inhibition of these proteins may sensitize the cancer cells to DNA damaging agents, such as chemo and radiation therapy by preventing the activation of DNA repair pathways. Extensive research is required to uncover their therapeutic and risk factors for better cancer therapy.

3b. *CHK1* inhibitors: *CHK1* inhibitor hinders the functioning of the *CHK1* kinase enzyme causing DNA damage and cell cycle arrest, ultimately to cell death. It is vital for enhancing the effectiveness and success of multiple cancer treatments like breast, ovarian, and lung cancer. *CHK1* inhibitors, such as Prexasertib, GDC-0575, SRA737, PF-477736, MK-8776, AZD7762, and CHIR-124, have shown synergistic functions as a novel therapeutic treatment either alone or in combination with other chemo or radiotherapy regimes. These inhibitors along with PARP inhibitors are effective against multiple cancers like ovarian, pancreatic, and lung cancer. However, reports have revealed the antagonistic effect of these inhibitors like causes accumulation of DNA damage, toxicity, and affects normal cell cycle development which makes them limited for certain cancers and decrease their efficiency for all patients and leads to the development of resistance *via* several mechanisms. In one of the possible mechanisms, it upregulates the alternative DDR pathways that can balance the loss of *CHEK1/2* function. An additional mechanism is the development of mutations in *CHEK1/2* or other genes that can bypass the need for *CHEK1/2* activation in DNA repair. Furthermore, the genetic and epigenetic changes which cancer cells can undergo may alter the expression and activity of *CHEK1/2*, and even can reduce their effectiveness [209]. Resistance mechanisms, combination therapies with other DNA-damaging agents, targeted therapies, or immunotherapies are being investigated. Based on the genomic and molecular profiles of the patients' novel strategies and biomarkers are being explored to recognize the patients who benefitted from the treatment of these therapies. Therefore, extensive research is required to uncover their safety and efficacy for cancer treatment.

4a. MutS Homolog 2/6 (MSH2/6): Helps in preserving the genome integrity by fixing the errors which occur during the replication of DNA *via* the DNA MMR pathway [129,210]. Additionally, germline and somatic mutations in *MSH2* and *MSH6* genes may cause defective MMR which results in the accumulation of DNA damage leading to Microsatellite Instability (MSI), a type of short repetitive DNA sequences genomic instability linked with Hereditary Non-Polyposis CRC (HNPCC), also known as Lynch syndrome, and sporadic CRC [211-214] which may predisposes * individuals to develop CRC, as well as other cancer types such as ovarian, endometrial, pancreatic, gastric, and urinary tract cancers [215,216]. *MSH2* and *MSH6* deficiencies are targets for cancer therapy, particularly for those tumors that exhibit MSI high.

4b. *MMR* genes inhibitor: Pembrolizumab and nivolumab are FDA-approved immunotherapeutic drugs for patients with Microsatellite Instability-High (MSI-H) or Mismatch Repair Deficient (dMMR) tumors caused by the defects in the DNA MMR system, due to the mutations in *MMR* genes (*MSH2*, *MSH6*, *MLH1*, and *PMS2*) and therefore exhibit a high level of genomic instability by producing neoantigens substances produced by tumor cells due to the accumulation of DNA damage recognized by the immune system. These immune checkpoint inhibitors target the Programmed Cell Death of protein 1 (PD-1) receptor on T cells which block the signals that cancer cells use to evade immune detection by activating the immune system, allowing T cells to attack the tumor. Clinical studies have reported that these drugs can induce durable results and improve patient outcomes with MSI-H/dMMR tumors, in the case of several cancer types like colorectal, endometrial, gastric, and others. Although pembrolizumab and nivolumab have shown remarkable clinical activity in MSI-H/dMMR tumors and not all MSI-H/dMMR tumors respond equally to immune checkpoint inhibitors, some patients can still develop resistance to these therapies. The mechanisms of resistance to immune checkpoint inhibitors in MSI-H/dMMR tumors are not fully understood, but several studies have suggested potential mechanisms. One of the proposed mechanisms of resistance is the loss of neoantigens in tumors, which are mutated peptides that are presented on the tumor cell surface and recognized by the immune system as foreign. However, loss of neoantigens due to tumor evolution or treatment-induced selective pressures can lead to resistance to these drugs, which can limit the detection and killing of tumor cells by the immune system [217].

Another resistance mechanism responsible for resistance to immune checkpoint inhibitors involves the upregulation of complementary immune checkpoints, like TIM-3 and LAG-3, which can suppress T cell activation and function and deletion of expression of Major Histocompatibility Complex (MHC) molecules essential for tumor antigen presentation on T cells. Further possible mechanism is the accumulation of genetic alterations in the genes like JAK1/JAK2 or β2M and other conditions like the composition of the tumor microenvironment, tumor heterogeneity and the immunosuppressive effects of chemo and radiotherapy in some patients is linked with the resistance to pembrolizumab and nivolumab. The immune detection can be evaded by tumors by constructing an immunosuppressive

microenvironment, categorized by the presence of regulatory T cells, immune suppressive cells, and other myeloid-derived suppressor cells. Hence, to overcome resistance and increase anti-tumor immunity, these immune suppressive cells in combination with pembrolizumab and nivolumab can be targeted for better outcomes. Also, targeting tumor-associated macrophages or stromal cells in the tumor microenvironment may lead to increased efficacy of immune checkpoint inhibitors in MSI-H/dMMR tumors. However, further research is required to uncover the mechanisms of resistance and to develop strategies to improve treatment outcomes.

5a. Ataxia telangiectasia and Rad3-related protein (ATR): is stimulated by ss DNA and stalled replication forks, during DNA damage in the DDR pathway on activation, ATR phosphorylates downstream targets, like CHK1, which in turn activates the G2/M checkpoint to inhibit cell cycle progression and stimulate DNA repair. ATR pathway is important for retaining stability of genome and preventing the mutation accumulation, however defects in the ATR pathway has been linked to several cancer types, including lung, breast, and ovarian cancer.

5b. ATR inhibitors: inhibit the activity of the ATR kinase, and is activated in response to DNA damage, preventing DNA repair leading to cell cycle arrest and ultimately to cell death *via* DDR pathway. ATR inhibitors are currently being investigated in clinical trials for various cancer types. Some examples of ATR inhibitors being evaluated in pre-clinical and clinical trials include: Berzosertib (M6620) for the treatment of solid tumors and hematological malignancies. VE-821/22 has shown promise in preclinical studies for the treatment of ovarian and lung cancer. BAY 1895344 is for the treatment of solid tumors. AZD6738 for the treatment of several types of cancer, including lung, pancreatic, and ovarian cancer. VX-970 (M6008) for the treatment of advanced solid tumors [218].

6a. MDM2 (Mouse Double Minute 2 Homolog): is a negative regulator of the tumor suppressor protein p53, which is activated in response to DNA damage and allows p53 to accumulate and activate the DDR pathway which induces cell cycle arrest, DNA repair, or apoptosis, on binding to p53 and promotes its ubiquitination and degradation, thereby inhibiting its activity, depending on the severity of the damage. It's important in ATM/ATR signaling as it interacts with ATM and ATR kinases and controls their activity in response to DNA damage and similarly in HR pathway, it controls the activity of the RAD51 by inhibiting it's binding to DNA or inducing its degradation.

6b. MDM2 inhibitors: are the drugs which target an E3 ubiquitin ligase MDM2 protein that regulates the tumor suppressor protein p53. The inhibition of MDM2 by these drugs may result in an increase in the p53 levels which induces apoptosis in cancer cells. Various examples of MDM2 inhibitors are currently under various preclinical and clinical trials for the treatment of a variety of cancers like solid tumors, Acute Myeloid Leukemia (AML) etc. include: Nutlin-3, RG7388 (idasanutlin), SAR405838, AMG-232, DS-3032b, MK8242. Conversely, several lines of evidence have demonstrated that MDM2 inhibitors have potential in preclinical and clinical outcomes, however; furthermore, research is required to uncover the safety and efficacy in the field of cancer treatment.

7a. Topoisomerases: are enzymes involved in the regulation of topology and structure by catalyzing the breaking and rejoining of DNA strands. Two types of topoisomerases involved in the DDR pathway: Type I and type II are vital for the replication, recombination, and transcription of DNA. Type I topoisomerase enzyme is involved in single strand cut, whereas type II allows dsDNA cuts by unwinding and relaxing DNA supercoiling before resealing it. Previous studies have shown that dysregulation of type I and type II enzymes may induce the accumulation of dsDNA breaks, which can result in genomic instability leading to the progression and development of cancer. Thus, inhibiting topoisomerases will prove to be a therapeutic potential target for the treatment of cancer, as it can induce DNA damage and apoptosis in cancer cells.

7b. Topoisomerase inhibitors: Topoisomerase enzymes are vital in the DNA transcription and replication process for relieving the torsional strain produced when DNA strands detach. Topoisomerase inhibitors are the drugs which inhibit the normal function of topoisomerases by preventing them from repairing DNA damage resulting in the accumulation of DNA damage and cell death. Topoisomerase inhibitors (I & II) are extensively used in treating various types of cancers. Such as topoisomerase I inhibitors Topotecan and Irinotecan are used to treat ovarian, lung cancer and CRC. Whereas topoisomerase II inhibitors Etoposide a used to treat lymphoma, lung, and testicular cancer. Doxorubicin for treating leukemia, and breast and bladder cancer and Mitoxantrone is for prostate cancer, leukemia, and lymphoma.

8a. DNA-dependent protein kinase (DNA-PK): A (ser/thr) protein kinase is stimulated in response to DSBs in binding the broken ends of the DNA strands and facilitates other proteins in the repairing process *via* the DDR NHEJ pathway. However, inhibition of DNA-PK has been explored as a therapeutic strategy as cancer cells are more reliant on the NHEJ signaling pathway for DNA repair than normal healthy cells.

8b. DNA-PK inhibitors are inhibitors (VX-984 and M3814) designed against DNA-dependent Protein Kinase enzyme (DNA-PK) which is responsible for restoring DSB in DNA *via* the NHEJ pathway. They have been tested in different clinical trials as a potential therapeutic drug for various cancer types.

9a. O-6-methylguanine-DNA methyltransferase (MGMT): is important in the DDR pathway for repairing O-6-methylguanine adducts in DNA damage initiated by alkylating agents, as they limit the efficiency of alkylating agents by removing them from DNA before damage. However, the Inhibition of MGMT function due to promoter methylation is being seen in various cancers like CRC, gliomas, and lung cancer, resulting in reduced DNA repair capacity and increased sensitivity to alkylating agents with better response to chemotherapy and improved patient outcomes. Therefore, strategies to inhibit or downregulate MGMT expression are being explored as potential therapies to enhance the efficacy of alkylating agents in cancer treatment.

9b. MGMT inhibitors: The DNA damage caused by alkylating agents can be restored by using the *GMT* gene which helps to repair it. O-6-benzylguanine and Lomeguatrib are the MGMT inhibitors clinically tested as potential therapeutic targets for treating Glioblastoma.

Perspectives of DDR Research

Although extensive research is being conducted on DDR signaling pathways, we still lack a comprehension of how these pathways operate in several oncogenic circumstances. There are

various unmet questions, like (i) why there is a loss of specific DDR pathways in certain cancer types; and (ii) whether the effect of DDR defects differs in different tissues and cell types. (iii) The interaction concerning oncogenic stress and the DDR is inadequate, and (iv) it is unclear whether the oncogenic stress in cancer cells determines which DDR pathways to lose or whether its loss determines which oncogenic events to acquire. A detailed comprehension of various sources of genomic instability and the effect of DDR defects in different oncogenic contexts is important for the development of potential therapeutic strategies to persuade synthetic lethality.

Furthermore, it is worth noting that the wiring of DDR pathways can differ in various tissues and cell types, and under different selective pressures, the loss of specific DDR pathways in cancer cells or selective pressure during tumor evolution and cancer therapy could alter the DDR network's wiring. A better understanding of the DDR network's rewiring in cancer cells is essential for targeting DDR pathways and their impact on tumor microenvironments in cancer cells and overcoming resistance to DDR-targeted drugs. Future research using patient samples, *in vivo* models, and single-cell analysis has the potential to significantly enhance our understanding of genomic instability in tumors and improve the efficacy of DDR-targeted therapy. Here are some future directions for DDR research and their potential impact on clinical applications can be improved like developing novel DNA repair pathways; identifying genetic biomarkers and developing personalized treatment; developing combination therapies; understanding drug resistance mechanisms; gene editing; nanoparticles for targeted delivery and developing non-invasive diagnostic tests.

DDR research is a rapidly evolving area, which relies on developing more effective and specified cancer treatments and has the promise to develop and identify novel diagnostic tools and new therapeutic targets to significantly impact clinical applications. However, extensive research is required to completely recognize the safety and efficacy of these initial therapies, and personalized treatment approaches, and to interpret these findings into clinical practice to improve the outcome of cancer patients.

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