WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 10, 1276-1294. Review Article ISSN 2277– 7105

DNA DAMAGING AGENT USED IN CANCER CHEMOTHERAPY

Shubhangi Vidhate*, Nita Yenare, Dakshata Vayeda and Bhushan Mundhe

Kasturi Shikshan Sanstha College of Pharmacy Shikrapur, 412208.

Article Received on
20 July 2020,

Revised on 10 August 2020,
Accepted on 31 August 2020,

DOI: 10.20959/wjpr202010-18611

***Corresponding Author**
Shubhangi Vidhate
Kasturi Shikshan Sanstha College of Pharmacy
Shikrapur, 412208.

ABSTRACT

DNA-damaging agents have a long history of use in cancer chemotherapy. The full extent of their cellular mechanisms, which is essential to balance efficacy and toxicity, is often unclear. Chemotherapeutics target rapidly dividing cancer cells by directly or indirectly inducing DNA damage. Upon recognizing DNA damage, cells initiate a variety of signaling pathways collectively referred to as the DNA damage response (DDR). Novel anticancer compounds are continually being developed in the hopes of addressing these limitations; however, it is essential to be able to evaluate these compounds for their mechanisms of action. This review covers the current DNA-damaging agents used in the clinic, discusses their limitations, and describes the use of chemical genomics to uncover new information about the DNA damage response network and to evaluate novel DNA-damaging compounds.

KEYWORDS: ATM, ATR, DNA damage, Chemotherapy, Cisplatin.

INTRODUCTION

DNA damaging agents are widely used in oncology to treat both hematological and solid cancers. Some commonly used modalities include ionizing radiation, platinum drugs (cisplatin, oxaliplatin, and carboplatin), cyclophosphamide, chlorambucil, and temozolomide. By modifying the chemical structure of nucleic acid, these agents induce apoptosis to subsequently eliminate cancer cells from the body. The DDR is a crucial signaling pathway that serves to coordinate the necessary series of biochemical and cellular events in response to both exogenous and endogenous induced DNA damage. The clinical use of DNA damage-inducing therapies remains a mainstay in the treatment of cancer. Targeting the rapidly dividing cancer cells with genotoxic agents has demonstrated clinical utility and more recently, it has become apparent that the DDR impacts the response to these therapies both in

terms of anti-cancer activity and toxicity to non-cancer cells. Thus, to begin to understand why different cancer types respond to various DNA damage therapies, the detailed mechanisms involved in the initiation of the DDR in response to these therapies is essential and recently has begun to be addressed. There are two major response available to a cell upon DNA damage, such as chemotherapeutic drug action, to arrest the cell cycle and repair the damage, to initiate a pathway to apoptosis (programmed cell death).The major limitation of the cytotoxic anti-cancer drugs is the tumor non-specific action, and the suppression of all rapidly dividing cells. Because the desired drug actions (damage to rapidly proliferating cancer cells) and the adverse effects (damage to rapidly proliferating healthy cells) are identical in targets and mechanisms, they are not separable. DNA damaging drugs interfere with transcription and reduplication. Affected cells respond with cell cycle arrest or programmed cell death. DNA damaging drugs are mutagenic, teratogenic, and carcinogenic. Adverse effects are exerted on rapidly proliferating cells (skin—hair loss, gastrointestinal tract—nausea and vomiting, bone marrow—anemia causing fatigue/leukopenia causing infections/thrombocytopenia causing bleeding).[1,2]

DNA DAMAGING AGENT

Table 1: Properties of DNA-Damaging Compounds Used in the Treatment of Cance Alkylating agent.

Drug	Mode of Action	Major Side Effects	Mechanisms of Resistance
Cisplatin	DNA crosslinker	nephrotoxicity; neurotoxicity; ototoxicity	decreased uptake; increased efflux; enhanced replication bypass; increased DNA repair capacity; increased DNA damage tolerance; failure of death pathways
Carboplatin	DNA crosslinker	myelosuppression	
Oxaliplatin	DNA crosslinker	neurotoxicity; pulmonary toxicity; hepatotoxicity	
Methotrexate	prevents DNA synthesis by inhibiting dihydrofolate reductase (DHFR)	myelosuppression; pulmonary toxicity; gastrointestinal toxicity; hepatotoxicity; nephrotoxicity; neurotoxicity	increased DHFR expression; mutations in folate transporter genes
Doxorubicin	topoisomerase II poison	cardiotoxicity; myelosuppression; neurotoxicity	P-glycoprotein-mediated MDR; decreased topoisomerase II expression; enhanced DNA repair; decreased activity due to increased glutathione levels
Daunorubicin	topoisomerase II poison	cardiotoxicity; myelosuppression	

In the 1960s and 1970s, there was a surge of interest in developing anticancer compounds that react chemically with DNA. These included compounds that directly modify DNA bases, intercalate between bases, or form crosslinks in DNA. The nitrogen mustards studied by Goodman and Gilman act by directly alkylating DNA on purine bases, leading to stalled replication forkprogression and subsequentcell death via apoptosis. Derivatives of nitrogen mustards were developed, including the DNA alkylators cyclophosphamide, chlorambucil, and melphalan, all of which are currently used in clinical therapeutics. Other examples of DNA-alkylating agents used in cancer treatment include nitrosoureas (e.g., carmustine, lomustine, and semustine) and triazenes (e.g., dacarbazine and temozolomide). Natural products which alkylate DNA bases were also discovered around this time, such as mitomycin C and streptozotocin. These compounds and several of the alkylators mentioned above crosslink DNA on opposite strands of the double helix (interstrand crosslinks), resulting in a more potent effect against cancer cells compared to monofunctional alkylation. For example, carmustine (N,N0-bis(2-chloroethyl)-nitrosourea) binds to the N1 of guanine on one DNA strand and the N3 of cytosine of the opposite strand to form interstrand crosslinks, which block DNA replication and can cause cell death if not repaired. The discovery of the alkylating-like platinum agents had a significant positive impact on anticancer drug research. Cis-diamminedichloroplatinum(II) (cisplatin), was discovered by accident in the 1960s, when a magnetic field generated by platinum electrodes was shown to block E. coli cell divisio. Cisplatin, as its name implies, contains a platinum core with two chloride leaving groups and two amine nonleaving groups. After cell entry, aquation of the chloride groups allows the platinum to bind guanine residues and, to a lesser extent, adenine residues to form adducts on DNA. When two platinum adducts form on adjacent bases on the same DNA strand, they form intrastrand crosslinks. The structures of these platinum-DNA adducts have been solved at atomic resolution using X-ray crystallography and nuclear magnetic resonance. Inspired by the efficacy of cisplatin, platinum-based analogs have been developed, including carboplatin and oxaliplatin, which also act by forming DNA crosslinks but have different pharmacological properties, decreased side effects, and increased efficacy against different tumors. In particular, platinum compounds have been very successful in the treatment of solid tumors. Indeed cisplatin therapy can cure over 90% of all testicular cancer cases and also has good efficacy in the treatment of ovarian, bladder, head and neck, and cervical cancers. Current efforts to develop cisplatin analogs are aimed at reducing toxicity to nontargeted tissues, which results in dose-limiting toxicities, such as nephrotoxicity and neurotoxicity. The spectrum of different platinum compounds under development is broad, and platinum

compounds have also encouraged the synthesis and testing of other metal-containing compounds for use in chemotherapy.[3,4]

Dna Target Site For Alkylating Agent

Base	Target	Drug	Class	Recognition sequence
Guanine	N7	Melphalan	Nitrogen mustard	
		Cyclophosphamide	Phosphoramide mustards	
		Temozolomide	Triazene	
		Cisplatin	Platinum drugs	
		Fotemustine	Nitrosourea	3′ end of guanine tracts
Guanine	N1	Fotemustine	Nitrosourea	
Guanine	N2	Ecteinascidin-743	Minor groove binding antibiotics (G/C preference)	AGC, CGC, TGG
Guanine	N3	Duocarmycin A	Cyclopropylpyrroloindole antibiotics	5′-GCAATTGCGCAATTGC-3′
Guanine	O6	Temozolomide	Triazene	
		Dacrbazine	Triazene	
		Laromustine	Hydrazine	
Guanine		Duocarmycin A	Cyclopropylpyrroloindole antibiotics	5′-CGCGTTGGGAG-3′
		Mithramycin A	Aureolic acid minor groove binders	
Adenine	N3	Duocarmycin A	Cyclopropylpyrroloindole antibiotics	
		CC-1065	Cyclopropylpyrroloindole antibiotics	5′-d(A/G)NTTA-3′
				5′-dAAAAA-3′
		Adozelesin, carzelesin, bizelesin	Minor groove binding antibiotics (A/T preference)	5′-(A/T)(A/T)A-3′
		Tallimustine	Minor groove binding antibiotics (A/T preference)	5′-TTTTGA-3′
Adenine	N7	Cisplatin	Platinum drugs	
Adenine	N1	(Uncommon)		
Cytosine	N3	Fotemustine	Nitrosourea	
Cytosine	N1	(Uncommon)		

Nitrogen mustard	Examples	Adverse effects	Drug interactions
1st generations	Mechlorethamine Nitrogen mustard N-oxide	Nausea and vomiting, Substantial Granulocytopenia, Lymphocytopenia, Thrombocytopenia, Anaphylactic reactions or bleeding, Hyperuricemia, fatigue, hair loss, Jaundice, vertigo, tinnitus and hearing loss occur infrequently. Carcinogenic.	Turmeric may decrease the effect of mechlorethamine. Aspirin or salicylate containing medicines decreses platelet count can increase the risk of bleeding.
2nd generation	Chlorambucil Melphalan Bendamustin Spiromustin	Myelosuppression, Severe neutropenia, gastrointestinal distress, central nervous system damage, skin reactions, infertility, carcinogenic. Myelosuppression bone marrow suppression nausea and vomiting	Hypersensitivity intake of salicylic acid Aspirin should be avoided as it could intensify any bleeding problems. Interactions with cimetidine, steroids, and cyclosporine are possible
3rd generation	Uramustin	nausea and vomiting, diarrhea, and dermatitis nervousness, irritability, and depression	anti-gout agents
Steroid coupled	Estramustin phosphate DHEA mustard Prednimustin	allergic reactions, with hives, difficulty breathing, and blood clots	calcium-rich food

Platinum compound	Example	Adverse effecys	use
1st generation	Cisplatin	gastrointestinal and renal toxicity Angina pectoris and myocardial infarction Ototoxicity	to treat small cell lung cancers, sarcomata, lymphomata, germ cell tumors, and gestational trophoblastic tumors. Further, it is applied to the palliation of bladder, cervical, nasopharyngeal, esophageal, and head and neck cancers.
2nd generation	Carboplatin, nedaplatin, iproplatin.	Thrombocytopenia Neutropenia leukopenia	ovarian carcinoma, lung cancers, and head and neck cancers
3rd generation	Oxaliplatin, picoplatin, spiroplatin.	nausea and vomiting diarrhea, neutropenia, or fatigue	treatment of advanced colorectal cancer, malignancy

Propertise of Alkylating agent

Alkylating agents attach alkyl groups to DNA bases, leading to DNA fragmentation.

Alkylating agents cause intra- and inter-strand DNA cross-links.

Alkylating agents can induce mispairing of nucleotides, leading to mutations.

Alkylating agents exert cytotoxicity in all phases of the cell cycle.

Nitrogen mustards and chlorethylnitrosoureas have a preference for guanine-N7 alkylation.

The mustard pharmacophore is –N(CH 2 CH 2 Cl)2.

The two arms of mustard drugs can cross-link DNA strands.

Nitrogen mustard also damages DNA through the production of reactive nitrogen species.

First generation nitrogen mustards cannot be taken orally.

In second generation nitrogen mustards, electron withdrawing aromatic radicals reduce the reactivity and permit oral use.

In third generation nitrogen mustards, the pyrimidine nucleus carrier for the mustard pharmacophore permits oral administration.

Steroid-coupled nitrogen mustards combine the effect of the alkylating agent with a steroid for selective uptake or combined anti-cancer action.

In phosphoramide mustards, the base nitrogen mustard is administered as non-toxic prodrug that is actively transported into the cancer cells and enzymatically converted into its cytotoxic forms.

Nitrosoureas are alkylating agents that cross-link DNA.

Due to their lipophilicity, they cross the blood-brain barrier.

Pulmonary toxicity is a common adverse effect of nitrosoureas.

Acute leukemia or bone marrow dysplasia can be long term complications.

Ethylene imines and methylmelamines are prodrugs that require metabolic activation.

Methylmelamines release the weakly alkylating formaldehyde upon demethylation.

Methylated melamines are extensively demethylated by hepatic mixed-function oxidases. Their anti-tumor activity decreases with a decreasing number of methyl groups.

Upon aquation of a ligand, platinum drugs become highly reactive, allowing them to coordinate DNA bases.

Complexes with labile leaving groups are toxic, compounds with stable leaving groups are inactive.

Modifications in the chloride leaving group affect pharmacokinetics; modifications in the ligand affect efficacy and spectrum of activity.

Most platinum compounds cannot be taken orally.

Early generation platinum drugs have severe adverse effects. Recent generation platinum drugs reduce toxicity and avoid cross-resistance.

Drug resistance may be caused by platinum efflux, detoxification through thiols, apoptosis resistance, or enhanced DNA repair.

Antibiotics

Among the research programs related to World War II was a large scale screening of bacterial and fungal fermentation products by pharmaceutical companies to isolate and produce antibiotics suitable for treating wound infections. Some of the agents under investigation were also examined for their anti-tumor effects. The program was largely based on observations with penicillin, which had initially been thought to have tumor suppressing properties. In particular, Streptomyces species are a genus of soil bacteria, from which numerous antibiotic and antifungal compounds have been derived. The antibiotic actinomycin D, extracted from Streptomyces, was an early product of the large scale screening endeavor. Introduced by Sidney Farber into the clinic in 1954, actinomycin D24 had substantial anti-cancer effects and therefore found considerable use in the treatment of pediatric tumors through the 1960s. This drug established feasibility and led to a continued search for more active anti-tumor antibiotics. The effort has yielded a series of compounds that are in common use today. Anti-neoplastic antibiotics fall into two broad functional classes. Cyclopropylpyrroloindoles, minor groove DNA binding antibiotics, and aminoquinones alkylate DNA. Polycyclic aromatic antibiotics (anthracyclins, anthracenediones, anthrapyrazoles) and enediynes generate free radicals through redox cycling by a mechanism that depends in part on cellular iron; the generated reactive oxygen species damage the DNA of cancer cells. In addition, polycyclic aromatic antibiotics inhibit Topoisomerase 2.[5]

Cyclopropylpyrroloindoles

The cyclopropylpyrroloindole analogs (drug names ending on -zelesin) contain a cyclopropyl group, which mediates sequence selective N3 adenine covalent adduct formation. These DNA minor groove binding compounds do not react with single stranded DNA, RNA, or protein.

Properties

The cyclopropyl groups in cyclopropylpyrroloindole antibiotics mediate N3 adenine covalent adduct formation in a sequence selective fashion.

Myelosuppression is the dose limiting toxicity.

Adozelesin, bizelesin, and carzelesin alkylate the N3 of adenines at the 3′ end of the DNA sequence motif 5′-(A/T)(A/T)A-3′.[14]

Low doses of adozelesin, bizelesin, or carzelesin induce cell cycle arrest, high doses induce apoptosis.

P21 is crucial to sustained bizelesin induced G2/M arrest.

The minor groove of DNA is frequently unoccupied and vulnerable for chemical attack.

Some minor groove binding antibiotics prefer A/T-rich sequences, others prefer G/C rich sequences.

Antibiotic binding may displace essential transcription factors and interfere with gene expression.

Antibiotics that bind sequence specifically, non-covalently, and reversibly to the minor groove may serve as carriers for alkylating moieties.

The pyrrole-amide backbone of distamycin A, which binds 4–5 adjacent A/T base pairs, serves as a sequence selective vehicle for the delivery of DNA alkylating functions.

Tallimustine alkylates N3 adenines in the target sequence 5′-TTTTGA-3′.

Brostallicin is activated upon binding to Glutathione, catalyzed by Glutathione-S-Transferase (GST). It may thus treat cancers that are resistant to other forms of chemotherapy.

PNU-151807 abolishes Cyclin/CDK kinase activity. Its cytotoxicity is not affected by disruption of P53 function or loss of DNA repair.

In the presence of divalent cations, aureolic acid antibiotics form dimers that non-intercalatively bind to the DNA minor groove in high-GC-content regions.

Upon binding to DNA, the aureolic acid chromophores form hydrogen bonds with NH2 residues of guanines.

Mithramycin A binds to C/G-rich tracts as a dimer and blocks SP-1 family transcription factors.

Ecteinascidin-743 effects guanine N2 alkylation.

Aminoquinones

Quinone containing alkylating drugs have a quinone moiety, which can be reduced, and an alkylating group, which can form covalent bonds with a variety of cellular components. The oxidation state of the quinone moiety modulates the activity of the alkylating element. In many of these agents, reduction of the quinone is required for induction of the alkylating

activity. The quinone element may also contribute directly to cytotoxicity through the formation of reactive oxygen species during redox cycling.

Properties

Mitomycins and streptonigrins comprise the group of anti-cancer aminoquinone antibiotics.

In quinone containing alkylating drugs, the quinone moiety is subject to redox cycling, while the alkylating group can form covalent bonds with cellular components.

The mitomycins require intracellular activation of their alkylating groups by reducing enzymes (NADPH:Cytochrome P450 Reductase, NADH:Cytochrome b5 Reductase, DT-Diaphorase, or Xanthine Dehydrogenase).

Mitomycin C has effectiveness against hypoxic cells, which are resistant to radiation.

Streptonigrin forms complexes with DNA and Topoisomerase 2 and undergoes redox cycling, resulting in DNA damage.

Polycyclic aromatic antibiotics

The anti-tumor activity of polycyclic aromatic compounds is associated with their ability to intercalate into DNA. The planar 3-ring structure accounts for the biological characteristics of these molecules. Anthracyclines, anthracenediones and anthrapyrazoles are groups of agents in this class of drugs.

Properties

Anthracyclines, anthracenediones and anthrapyrazoles are related anti-neoplastic antibiotics. They share a polycyclic aromatic core structure.

The 2 main modes of anthracycline action comprise Topoisomerase inhibition and generation of reactive oxygen species.

Because first generation anthracyclines are associated with myocardial dysfunction and alopecia, second generation drugs attempt to reduce these adverse effects.

Because of enhanced total body clearance, epirubicin can be used at high cumulative doses without increased cardiotoxicity.

Oligosaccharide anthracyclines induce hematopoietic differentiation.

The diaminoalkyl groups are crucial for the biological activity of anthracenediones. Anthracenediones may require metabolic activation before effectively intercalating into DNA.

Anthracenediones may act as anti-oxidants by inhibiting conjugated diene formation from linoleic acid.[19]

Other antibiotics

Actinomycins were first described by Selman Waksman and H.B. Woodruff in 1940[20]. Actinomycin D <Dactinomycin, Cosmegen, Oncostatin K, Actinomycin IV, Actinomycin C1> was isolated from the soil bacterium Streptomyces parvulus. It is a polypeptide antibiotic containing an aminoquinone chromophore.

Actinomycin D specifically intercalates to GC-rich sites of DNA. It inhibits transcription by binding to the DNA at transcription initiation complexes and preventing elongation by RNA Polymerase.

As actinomycin D can bind DNA duplexes, it can interfere with DNA reduplication. The agent acts in a cell cycle non-specific manner.

Actinomycin D causes single strand DNA breaks, possibly via a free radical intermediate or an interaction with Topoisomerase 2.

Properties

Actinomycin D suppresses transcription, interferes with DNA reduplication, and inhibits Topoisomerase 2.

Bleomycin is inactivated by Aminohydrolase (widely expressed except in skin and lungs, both of which are targets of bleomycin toxicity). The presence of Hydrolases in tumor cells is the primary mechanism of resistance to bleomycin.

Fostriecin is a Topoisomerase 2 inhibitor and acts as an inhibitor of several Protein Phosphatases.

Topoisomerase inhibitors

Otherwise identical loops of DNA having different numbers of twists are topoisomers (Gr. τόπος = place, ἰσομερής = equal parts). They cannot be interconverted by any process that does not involve the breaking of DNA strands. Topoisomerases (Gyrases) (type 1: EC 5.99.1.2, type 2: EC 5.99.1.3) are enzymes that catalyze and guide the unknotting of DNA. They can cause transient single strand (Topoisomerase 1) or double strand (Topoisomerase 2) DNA breaks that are resealed after changing the twisting status of the double helix. Topoisomerases wrap around the DNA and make cuts permitting the helix to spin. Once the DNA is relaxed, the enzymes reconnect the broken strands. Thus, Topoisomerase activity releases the tension generated by the winding of DNA and facilitates transcription and reduplication. Topoisomerase inhibitors interfere with this process.[6]

Topoisomerase 1 Inhibitors

Topoisomerase 1 is a 100 kD monomeric protein that makes a single cut in the DNA duplex and relieves transcription-associated torsional strain. No energy cofactor is required to carry out this reaction. Drugs may stabilize the Topoisomerase 1/DNA complex (referred to as the "cleavable complex"), thus blocking DNA relegation and converting the enzyme into a DNA damaging agent. Topoisomerase 1 inhibitors exhibit S phase cytotoxicity and G2/M cell cycle arrest. Zubrod had a particular interest in natural products, and established a broad program for collecting and testing plant and marine sources. Although this was a controversial project, it led to the discovery of the camptothecins in 1966.[21]

Camptothecin/Topoisomerase 1/DNA complexes inhibit reduplication and trigger apoptosis. Camptothecin also inhibits RNA synthesis.

Irinotecan is an S phase specific agent.

Camptothecins exist in a pH dependent equilibrium between their active lactone and inactive ring-opened conformations.

The stability of the lactone ring at physiological pH is a determinant of activity for all camptothecin analogs.

Topotecan and irinotecan are substrates for the efflux pump ABCB1. Camptothecins are substrates for ABCG2.

Indolocarbazoles with one glycosidic linkage are Topoisomerase inhibitors, indolocarbazoles with two glycosidic bonds are Protein Kinase inhibitors.

The sugar moieties of indolocarbazoles increase their water solubility.

Indolocarbazoles are substrates for efflux pumps, which may constitute a mechanism of drug resistance.

Lamellarins may be second-line-of-defense drugs for multi-drug resistant tumor cells.[22]

Indenoisoquinolines trap Topoisomerase 1-DNA cleavage complexes in unique genomic sites.

Indenoisoquinolines are seldom substrates for the multi-drug resistance efflux pumps.[23]

Bisindenoisoquinolines induce Topoisomerase 1-DNA cleavage complexes and Topoisomerase 2-DNA cleavage complexes. They lead to cell cycle arrest in G1.

Topoisomerase 2 Inhibitors

Type 2 Topoisomerases are enzymes that change the topology of DNA by introducing transient double strand breaks to from a cleavage complex, through which other DNA strands are passed. These 2-fold symmetric enzymes cleave a pair of opposing phosphodiester bonds

four base pairs apart. Topoisomerase 2 inhibitors promote the formation of DNA lesions by increasing the steady state level of the cleavage complex.

Topoisomerase 2 inhibitors promote the formation of DNA lesions by increasing the steady state level of the cleavage complex.

Podophyllotoxins (etoposide and teniposide) arrest cells in G2/M phase. They may cause DNA single and double strand breaks.

Quinoxalines may be active in multi-drug resistant cancers.

Other Topoisomerase 2 inhibitors include asulacrine isethionate, razoxane, elliptinium, amonafide, and batracyclin.[7]

Antimetabolites

Folic acid (folacin, vitamin B9) was discovered and synthesized by Lucy Wills in 1937. Previously, nutritional research in the early part of the twentieth century had identified a factor present in green leafy vegetables that was important for bone marrow function. It turned out to be folic acid, a vitamin crucial for DNA metabolism. However, the same compound administered to children with acute lymphoblastic leukemia (ALL) seemed to exacerbate their cancer.[25] Sidney Farber (1903–1973) in Boston recognized that folic acid stimulated the proliferation of leukemia cells. In one of the first examples of rational anti-cancer drug design, he collaborated with Harriett Kilte and Lederle Laboratories of the American Cyanamid Company to devise folate analogs. In November 1947, when a sufficient amount of aminopterin became available, Farber administered it to 16 children with acute leukemia and achieved remissions in ten of them. He demonstrated that aminopterin blocked a critical chemical reaction needed for DNA reduplication. Aminopterin was the predecessor of methotrexate (developed by Seeger and colleagues at Lederle Laboratories in 1948 and called amethopterin), which in 1956 became the first compound cure of metastatic cancer, when it was used by Roy Hertz (1909–2002) and Min Chiu Li (-1980) to treat two cases of choriocarcinoma. The principal architect of the treatment, using methotrexate in an unusual way for the time, was Min Chiu Li. As a sign of the times, after the first two patients went into remission, they were presented at National Cancer Institute Grand Rounds under the title "the spontaneous regression of cancer" with the speaker being Gordon Zubrod (Li's superior and at that time a detractor of chemotherapy). Li was told if he persisted in using his radical treatment, he would have to forfeit his position at the newly opened clinical center. He continued and was asked to leave. After his initial success with methotrexate, Li went on to develop the first effective combination chemotherapy programs for metastatic testicular

cancer. In 1972, when the Lasker Prize was awarded to investigators who had contributed to studies of the cure of gestational choriocarcinoma, Li had to share his part of the prize with the person who had discharged him.

Anti-metabolites have affinity to the enzymes of nucleic acid biosynthesis. They may be incorporated into cellular polymers and act as false building blocks. Alternatively, they may compete with the normal monomeric components of nucleic acids for essential synthetic enzymes and inhibit them. Because this group of drugs compromises the synthesis of DNA and RNA in healthy and transformed cells alike, they are more effectively used in localized therapy than in systemic therapy. However, only a few types of cancer are accessibly for this treatment modality.

Anti-metabolites may act as false building blocks for nucleic acids.

Anti-metabolites may inhibit essential synthetic enzymes for nucleic acids.

Due to their mechanism of action, anti-metabolites inhibit cell proliferation in S phase.

Antifolate

Folic acid (pteroylglutamic acid) dependent oxidation or reduction reactions of single carbons are important in biosynthetic pathways leading to the production of DNA, RNA, and membrane lipids. Dietary folates must be chemically reduced to their tetrahydro-forms, bearing four hydrogens on the pteridine ring, to be active. The enzyme responsible for this reduction is Dihydrofolate Reductase (DHFR). Folic acid in its fully reduced form serves as a carbon carrier for transfer reactions that are required in purine and thymidylate synthesis, and consecutively in the formation of DNA and cell division. Hence, folate is an important biosynthetic component for proliferating cells.

Old generation anti-folates comprise Dihydrofolate Reductase inhibitors and Thymidylate Synthase inhibitors.

New generation anti-folates include Glycinamide Ribonucleotide Formyl Transferase inhibitors and Dihydropteroate Synthase inhibitors.

Within cells, anti-folates are converted to polyglutamates, which typically are more efficient enzyme inhibitors.

Agents are available to enhance the efficacy and ameliorate the toxicity of anti-folates (folinic acid and uridine).

Initiators of the DDR

The DDR is a crucial signaling pathway that serves to coordinate the necessary series of biochemical and cellular events in response to both exogenous and endogenous induced DNA damage. The complexity of the DDR is in part a function of the requirement to detect and respond to a wide variety of DNA damage events and to regulate the numerous potential outcomes of the genetic insult. The clinical use of DNA damage-inducing therapies remains a mainstay in the treatment of cancer. Targeting the rapidly dividing cancer cells with genotoxic agents has demonstrated clinical utility and more recently, it has become apparent that the DDR impacts the response to these therapies both in terms of anti-cancer activity and toxicity to non-cancer cells. Thus, to begin to understand why different cancer types respond to various DNA damage therapies, the detailed mechanisms involved in the initiation of the DDR in response to these therapies is essential and recently has begun to be addressed.

Phosphatidylinositol-3 kinase- like protein kinases (PIKKs)

The PIKK family of protein kinases includes Ataxia-Telangiectasia Mutated (ATM), ATM and Rad3 Related (ATR) and the DNA-Dependent Protein Kinase Catalytic Subunit (DNA-PKcs) (Table 1). These kinases are relatively large in size and show a target preference for serine or threonine residues that are followed by glutamines. As discussed below, however, some important targets have recently been identified in non-consensus sequences. Despite the fact that the PIKKs are involved in different repair pathways, their respective activation involves some common themes. They are all initial responders to DNA damage and as far as we know the first kinases to initiate the DDR signaling cascade. In addition they are all activated at the site of DNA damage but cannot bind DNA, damaged or undamaged, without the assistance of DNA scaffolding proteins. While some early work suggested that ATM might bind directly to DNA, no new studies have confirmed or supported this. Even DNA-PKcs which has clearly defined DNA binding domains, does not bind DNA by itself under physiological salt concentrations.[28] Evidence suggests that the scaffolding proteins Ku80, Nbs1 and ATRIP not only recruit the kinases to the sites of DNA damage but also play a major role in activation of DNA-PKcs, ATM and ATR respectively. Interestingly these scaffolding proteins also share significant sequence similarity at their extreme C-termini, a feature essential for complex formation and DDR signaling.[29] The scaffolding proteins themselves must also be in complexes in order to efficiently activate the signaling kinase. Ku80 must be in complex with Ku70 while Nbs1 interacts with Mre11 and Rad50. ATRIP

interacts with the RPA bound ssDNA complex and TOPBP1.[30] While similarities certainly exist within the PIKKs family, their differences become apparent upon examination.

ATM

Ataxia-telangiectasia mutated (ATM) is a 315kDa protein that plays a major role in initiating the DDR. ATM remains a homodimer while inactive, but upon activation undergoes trans-autophosphorylation at serine 1981, leading to disruption of the dimer and allowing monomeric ATM to be recruited to dsDNA via an interaction with the MRN complex. While this phosphorylation event may be necessary for disruption of the dimmer, data suggest that it is not sufficient (See next paragraph). How this initial autophosphorylation event is stimulated is not well understood but may rely on chromatin relaxation. The nuclease activity of the MRN complex results in 3′ssDNA which along with its interaction with the C-terminus of Nbs1 stimulates ATM kinase activity and ultimately promotes homologous recombination (HR).[32] In an independent activation pathway, ATM has been shown to be activated by ATMIN under hypotonic stress which is independent of Nbs1 interactions. Interestingly, HR is restricted to S and G2 phases of the cell cycle, yet ATM is activated following DSBs regardless of cell cycle stage.[34] Some data suggests that DNA resection is a major component of whether ATM activation promotes HR or NHEJ. ATM activation following damage occurring in G1 leads to a minute amount of DNA resection due to low levels of cyclin dependent kinases and promotes NHEJ. ATM activation in S or G2, when cyclin dependent kinase levels are high, promotes DNA resection by MRN leading to HR promotion via ATR signaling chapman. Regardless S checkpoint cell cycle arrest is a hallmark of ATM activation.[35] Upon recruitment of ATM to DSBs via the MRN complex, monomeric ATM undergoes autophosphorylation at additional sites including the recently identified Serine 367 and Serine 2996. Importantly, when these sites were mutated to phosphor-ablating alanines ATM was unable to arrest the cell cycle at the S checkpoint, suggesting these phosphorylation events are essential in the DDR.

Unlike ATR and DNA-PK, ATM seems to be activated independently of DNA damage through direct exposure to reactive oxygen species (ROSs). Guo and colleagues have demonstrated and defined a distinct mechanism for activation that is independent of DNA or MRN. In this pathway, oxidized ATM becomes activated and retains autophosphorylation at 1981 but remains a dimer. Indeed dimerization via intermolecular disulfide bonds involving Cystine 2991, which is near the kinase domain of ATM, is essential for this mechanism of

activation. Interestingly, oxidatively-activated ATM and DNA damage dependent activated ATM share some, but not all, downstream targets. For example both pathways lead to phosphorylation of p53 at Ser15 and Chk2 at Thr68 but oxidatively activated ATM does not phosphorylate H2AX or Kap1. Guo et al. suggest that the specificity of targets stems from their stable association with DNA: i.e., H2AX and Kap1 phosphorylation is restricted to ATM activated by DNA damage. This seems logical in that DNA damage dependent activation of ATM involves a close association of ATM with DNA through MRN interactions. While the distinction between the activation pathways is apparent both lead to the DDR as evidenced by the fact that major downstream signaling factors including Chk2 and p53 are induced. This point is apparent in the context of cancer therapies which can produce a tremendous amount of reactive oxygen species (ROSs) that, in turn, may cause DNA damage in vivo, leading to both ATM activation pathways and the DDR.[8,9]

ATR

The last member to be identified in the PIKKs is ATR. ATR has been demonstrated to respond to DNA replication stress and signal to CHK1 via phosphorylation of ser345 in an RPA:ATRIP dependent process. Consistent with the model ATR activation is restricted to S and G2 phases of the cell cycle. Current models involve RPA detecting and binding the single-stranded DNA generated as a function of disrupted DNA replication. What distinguishes normal DNA replication-associated RPA from RPA associated with stalled replication forks is a combination of the unique gapped DNA structures associated with stalled replication and the proteins bound to these structures. The association of ATRIP and ultimate activation of ATR required a combination of DNA- protein and protein-protein interactions. DNA damage-dependent ATM phosphorylation of TOPBP1, mediated in part by CtIP and the MRN complex, stimulates association of TOPBP1 with the ATR-ATRIP complex. This association has been demonstrated to activate ATR leading to downstream target phosphorylation. The various protein complexes formed by ATR and ATRIP are further evidenced by the finding that both ATR and ATRIP exist as oligomers. This oligomeric state is not changed as a function of DNA damage and ATR oligomers are not dependent on ATRIP. Similarly ATRIP oligomers are not dependent on ATR. This is very different from the ATM dimer which when disrupted by phosphorylation leads to ATM activation. Part of the difficulty in assessing these differences in activation lies in the indirect measures used to measure ATR activation. To date the best measure of ATR activation is measurement of downstream target phosphorylation though many of the targets are substrates

for other kinases, thus complicating the analyses. More recently, a putative ATR autophosphorylation, site Thr1989, was characterized that could potentially be a useful marker for ATR activation, perhaps enabling the mechanism of ATR activation to be more completely elucidated.

DNA-PK

The largest member of the PIKKs is the DNA-dependent protein kinase catalytic subunit (DNA-PKcs). Indeed this protein with the staggering size of 469kDa is believed to be the largest single subunit protein in mammalian cells. It also seems to be the most abundant member of the kinase family with approximately 500,000 molecules per nucleus in human cells (Meek personal communication). Unlike ATM and ATR, DNA-PKcs plays a major, direct role in DNA repair and also initiates the DDR. Similar to ATM, DNA-PKcs forms homodimers; however the nature and role of these dimerization events are completely different. Dissimilar to the inactive ATM dimer and active monomer form, DNA-PKcs exists as a monomer in the cell when inactive. Following a DSB, the Ku70/80 protein binds to both termini of the break and recruits monomeric DNA-PKcs to both sides. Together, Ku70/80 and DNA-PKcs form the heterotrimeric DNA-PK. The DNA-PKcs molecules dimerize and interact across the DNA termini forming the synaptic complex. As part of these interactions, DNA-PK undergoes trans-autophosphorylation at over 40 sites. A substantial amount of data suggests that Ku70/80 is required for DNA-PK formation and activation. Several groups including our own have presented evidence for a direct protein/protein interaction between the carboxy-terminus of Ku80 and DNA-PKcs. While some early work suggested that these interactions are necessary for kinase activity, more recent work has questioned this, concluding that the c-terminus is dispensable for activation. Bridging the divide in the contrasting conclusions of previous studies, work from our group shows that the influence of the c-terminus of Ku80 on DNA-PK activation varies depending on the structure of the DNA cofactor to which DNA-PK is bound (data not published). Keeping with this theme SAXS structural studies have shown that the nature of DNA-PK dimerization across the synapse is different depending on the structure of the dsDNA termini to which the complex is bound. Thus, it seems that the structure of the DNA termini induce different protein/protein and protein/DNA interactions. Further, structural studies have revealed an extensive interface between the Ku and DNA-PKcs that does not involve the C-terminus of Ku80. It is possible that these interactions contribute to Ku80 C-terminus independent DNA-PK activation; however, it is also possible that the binding of Ku to the DNA changes the conformation of

the DNA which subsequently promotes DNA-PK activation. Distinguishing between these two possibilities has proven difficult.

Similar to ATM and ATR regulation, DNA-PKcs activity seems tunable. Two important components of the control of this activity are the proteins with which DNA-PK interacts and the structure of the DNA to which DNA-PKcs is bound. A probable result of the DNA substrate specific tuning is its regulation of DNA termini processing and facilitating DNA repair. Our group was the first to show that DNA-PKcs is activated by the 5′ end of the DNA terminus while the 3′ end is involved in mediating microhomology pairing across the synapse. Additionally, DNA-PK autophosphorylation at the ABCDE cluster promotes DNA processing, while phosphorylation at the PQR cluster limits processing. More recently, related work has shown that DNA-PK autophosphorylation at the JK cluster and threonine 3950 promotes DNA double strand break repair through homologous recombination (HR) and inhibits NHEJ. This same study identified a novel phosphorylation site at the N-terminus of DNA-PKcs which seems to ablate DNA-PK activity. Whether the phosphorylation status of this site is regulated by ATM, ATR, DNA-PKcs itself or some combination therein is yet to be seen.[10]

CONCLUSION

DNA damaging agents have been and will continue to be a mainstay in numerous cancer therapies. These drugs induce a variety of different DNA lesions which the cell must recognize and counter in order to survive. The pathways utilized in this endeavor converge on the DDR. Originally thought to be three separate pathways, we are just beginning to elucidate the network of interactions and regulations that impact the outcome following DNA damage. The dysregulation of DNA repair and damage response a contributing factor in carcinogenesis, can be exploited for cancer therapy.

REFERENCES

1. Malkin D, et al. Cancer Genet Cytogenet, 1993; 66: 83–92. [PubMed] [Google Scholar]
2. Martín-López JV, et al. Fam Cancer, 2013; 12: 159–168. [PMC free article] [PubMed] [Google Scholar]
3. Fischhaber, P.L., Gall, A.S., Duncan, J.A., and Hopkins, P.B. Direct demonstration in synthetic oligonucleotides that N,N’-bis(2-chloroethyl)-nitrosourea cross links N1 of deoxyguanosine to N3 of deoxycytidine on opposite strands of duplex DNA. Cancer Res., 1999; 59: 4363–4368.

4. Rosenberg, B., Vancamp, L., and Krigas, T. Inhibition of Cell Division in Escherichia Coli by Electrolysis Products from a Platinum Electrode. Nature, 1965; 205: 698–699.
5. DeVita VT Jr, Chu E. A history of cancer chemotherapy. Cancer Res., 2008; 68: 8643–8653. PubMedGoogle Scholar
6. Meek K, Dang V, Lees-Miller SP. DNA-PK: the means to justify the ends? Adv Immunol, 2008; 99: 33–58. doi: 10.1016/S0065-2776(08)00602-0. [PubMed]
7. Falck J, Coates J, Jackson SP. Conserved modes of recruitment of ATM, ATR and DNA-PKcs to sites of DNA damage. Nature, 2005; 434: 605–11. doi: 10.1038/nature03442. [PubMed]
8. Bakkenist CJ, Kastan MB. DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. Nature, 2003; 421: 499–506. doi: 10.1038/nature01368. [PubMed]
9. Chapman JR, Taylor MR, Boulton SJ. Playing the end game: DNA double-strand break repair pathway choice. Mol Cell., 2012; 47: 497–510. doi: 10.1016/j.molcel.2012.07.029. [PubMed]
10. Kozlov SV, Graham ME, Jakob B, Tobias F, Kijas AW, Tanuji M, et al. Autophosphorylation and ATM activation: additional sites add to the complexity. J Biol Chem., 2011; 286: 9107–19. doi: 10.1074/jbc.M110.204065. [PMC free article] [PubMed].