Accepted Manuscript

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PII: S1383-5742(18)30013-9
DOI: https://doi.org/10.1016/j.mrrev.2018.06.001
Reference: MUTREV 8242

To appear in: *Mutation Research*

Received date: 22-2-2018
Accepted date: 13-6-2018

Please cite this article as: George VC, Ansari S, Chelakkot VS, Chelakkot AL, Chelakkot C, Menon V, Ramadan W, Ethiraj KR, El-Awady R, Mantso T, Mitsiogianni M, Panayiotidis MI, Dellaire G, Rupasinghe HPV, DNA-dependent protein kinase: Epigenetic alterations and the role in genomic stability of cancer, *Mutation Research-Reviews in Mutation Research* (2018), https://doi.org/10.1016/j.mrrev.2018.06.001

Review

DNA-dependent protein kinase: Epigenetic alterations and the role in genomic stability of cancer

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Abstract

DNA-dependent protein kinase (DNA-PK), a member of phosphatidylinositol-kinase family, is a key protein in mammalian DNA double-strand break (DSB) repair that helps to maintain genomic integrity. DNA-PK also plays a central role in the immune cell development and protects telomerase during cellular aging. Epigenetic alterations due to endogenous and exogenous factors may affect the normal function of DNA-PK, which in turn can impair DNA and contribute to genomic instability. Recent studies implicate a role for epigenetics in the regulation of DNA-PK expression in normal and cancer cells, which may impact cancer progression and metastasis as well as provide opportunities for treatment and use of DNA-PK levels as a novel cancer biomarker. In addition, several small molecules and biological agents have been identified recently that can inhibit DNA-PK function or expression, and thus hold promise for cancer treatments. This review discusses the impact of epigenetic alterations and the expression of DNA-PK in relation to the DNA repair mechanisms with a focus on its differential levels in normal and cancer cells.

Abbreviations

53BP1; P53-Binding Protein 1

Ac; Acetylation

ALC1; Amplified in Liver Cancer 1

ATM; Ataxia Telangiectasia Mutated

ATR; Ataxia Telangiectasia and Rad3-Related

CK2; Casein Kinase II

CTD; C-Terminal Domain

DNA-PK; DNA-Dependent Protein Kinase

DRF; Dose Reduction Factor

Dsbs; Double Strand Breaks

FA; Fanconi Anemia

FAT; FKBP12-Rampamycin-Associated Protein

FATC; C Terminal of FAT Domain

HIF-1; Hypoxia Inducible Factor

HR; Homologous Recombination

LRR; Leucine Rich Region

Me; Methylation;

MMEJ; Microhomology-Mediated End Joining

Mmps; Matrix Metalloproteinase

Mtor; Mammalian Target of Rapamycin

NHEJ; Non-Homologous End Joining

NLS; Nuclear Localization Signal

NSCLC; Non-Small Cell Lung Cancers

P; Phosphorylation

PARP; Poly (ADP-Ribose) Protein

SCID; Severe Combined Immunodeficiency

TRRAP; Transactivation/Transformation-Domain-Associated Protein

Ub; Ubiquitylation

Vwa; Von Willebrand-Like Domain

Keywords: DNA-PK, Genomic stability, DNA repair, DNA damage, Epigenetic alternations, Cancer

1. Introduction

DNA replication and cell division are biological processes inherent in all prokaryotic and eukaryotic cells. In metazoans, errors in DNA replication caused by endogenous and exogenous factors, are common and result in thousands of DNA lesions each day [1]. In addition, normal cellular metabolism generates metabolic intermediates and by-products such as reactive oxygen species (ROS) and reactive nitrogen compounds that can induce DNA breaks. For the cells, these processes often "collide" when DNA replication machinery encounters ROS-damaged DNA bases or single-strand DNA breaks, which can be converted to DNA double-strand breaks (DSBs) during replication fork collapse [2]. Cellular processes such as meiotic recombination [3] or cleavage of genes during immunoglobulin gene rearrangement can also give rise to DSBs [4]. Exposure to environmental DNA damaging agents such as ultraviolet radiations (UVR) and other chemical or genotoxic agents are also an important cause of DNA lesions, and DNA DSBs are perhaps the most lethal kind of damage that a cell could undergo. DNA DSBs when not repaired or managed properly threatens genomic stability and results in the development of cancers and

other syndromes such as ataxia telangiectasia (AT), Nijmegen breakage syndrome or the Lig4 syndrome [5–7]. The cells have developed an array of mechanisms to combat the threats posed by different kinds of DNA damage. These mechanisms collectively called the DNA-damage response (DDR), detects DNA lesions, signal their presence and promote the repair genes [8,9]. In this review, we will primarily focus on the DDR as it pertains to DNA DSB repair.

When DNA DSBs trigger the DDR, a series of cellular responses converge on a fundamental binary decision: a) the activation of cell cycle checkpoints to facilitate DNA repair or b) activation of apoptosis when the degree of DNA damage passes a threshold from which the cell cannot recover and/or for which loss of the cell can be tolerated by cell replacement [7]. The cells are equipped with three distinct DNA repair pathways to combat the DSBs: homologous recombination (HR), non-homologous end joining (NHEJ), or microhomology-mediated end joining (MMEJ; also referred to as Alternative-NHEJ or Alt-NHEJ), and [10–12]. HR is an error-free process and uses sister chromatids as templates for DNA repair and is mediated by RAD51. This is the predominant repair pathway during development, S and G2 phase of the cell cycle and has the longest sequence homology requirement [13]. As the name suggests, MMEJ requires only a 5-25 bp microhomologous sequence to align the broken strands before joining the ends, and although active throughout the cell cycle is most prominent during S/G2 [14]. The requirement for the small stretch of microhomology results in deletions and induces chromosomal abnormalities and rearrangements [15]. NHEJ is responsible for the repair of the majority of the DSBs in G1 and G_0 phase of the cell cycle. Unlike HR and MMEJ, NHEJ does not require any homologous sequence for DNA repair and is highly error prone [16]. The NHEJ pathway is mediated by an enzyme complex called DNA-dependent protein kinase (DNA-PK) [17,18].

2. DNA-PK Structure and Function

Identified as individual components during the early 1980's DNA-PK is a nuclear serine /threonine kinase, consisting of a catalytic subunit called DNA-PKcs and a regulatory heterodimer Ku (Ku70/Ku80). The initially recognized roles for DNA-PKcs (originally termed p350) involved phosphorylation and transcriptional activation of SP-1, p53, and hsp90. The Ku subunits were known to bind double strand DNA, but their function remained unknown. Isolation of these two factors together led to the discovery of DNA-PK holoenzyme and its function in DNA repair pathways [11,19]. With the nuclear polypeptides reaching up to 4127 amino acids, DNA-PKcs is considered as the largest kinase subunit, which depends entirely on DNA binding for its activity [19,20]. Studies on the amino acid sequence of DNA-PKcs has identified them to be a member of phosphatidyl inositol-3 kinase (PI3K) like kinase (PIKKs), but other than protein kinase activity, no lipid kinase activity has ever been reported for DNA-PKcs [21,22]. Regulatory subunit of DNA-PK called Ku is a heterodimeric protein with two tightly associated subunits Ku70 and Ku80, which forms a ring like structure through which DNA can pass. The abundant expression of DNA-PK in the nucleus allows it to identify and bind to any DNA DSBs, which constantly occurs in the body and thereby initiating its repair mechanisms [23].

Structurally, DNA-PKcs consists of a DNA binding domain, a Ku binding domain, a Leucine Rich Region (LRR), FKBP12-rapamycin-associated protein (FAT), Ataxia Telangiectasia Mutated (ATM), transactivation/transformation-domain-associated protein (TRRAP), C terminal of FAT binding domain (FATC) and two phosphorylation clusters; PQR and ABCDE. Ku heterodimer consists of a conserved von Willebrand-like domain (vWA), DNA

heterodimerization core domain, SAP domain, nuclear localization signal (NLS) and a widely conserved C-terminal domain (CTD). Ku subunits have a high affinity for DNA fragments (higher affinity for DSBs than for single strand breaks) and DNA-PKcs affinity to DNA increases to ~100 folds in the presence of Ku subunits. For an efficient binding and subsequent activation of DNA-PKcs, an interaction between the C-terminal 12 residues of Ku80 with DNA-PKcs is necessary [23,24]. Once activated DNA-PKcs initiate a series of phosphorylation/auto phosphorylation events that are required primarily for cell cycle checkpoint signaling and DNA repair [25]. However, studies have shown that DNA-PKcs can also phosphorylate peptide substrates that are not bound to the DNA, suggesting that the DNA itself can induce a conformational change in the DNA-PKcs to activate its phosphorylation activity.

2.1. Role of DNA-PK in DNA repair mechanisms

The core protein complexes of NHEJ are the Ku subunits (Ku70/Ku80), DNA-PKcs, DNA ligase IV (Lig4), its cofactor the X-ray cross complementation group 4 protein (XRCC4) and the nuclease Artemis [26]. The process of NHEJ starts with the recognition and binding of the broken DNA ends by the ring-shaped Ku70 and Ku80 subunits [27,28]. This recruits monomeric DNA-PKcs through its interactions with Ku and DNA on both sides. Together with the Ku subunits, DNA-PKcs form the heterodimer DNA-PK. Following this, the DNA-PKcs dimerizes and interacts across the DNA termini and forms a synaptic complex [29]. DNA-PKcs recruitment facilitates the translocation of the Ku heterodimer into the DNA duplex and allows DNA-PKcs to serve as a tether for broken DNA ends [30]. It is also proposed that DNA-PKcs protect the DNA from exonucleolytic degradation and aligns the broken ends of DNA. In this regard, DNA-PKcs act as a scaffold protein and aids in the localization of repair proteins to the

site of DNA damage. DNA binding activates the kinase activity of DNA-PKcs and phosphorylates and alters the activity of other proteins that mediate NHEJ, including Ku70, Ku80, Artemis, XRCC4, and Lig4 [27]. Ligation of DNA ends is mediated by Lig4 in addition with XRCC4. An additional factor, Cernunnos/XRCC4-like factor (XLF), has also been identified as a binding partner of the Lig4-XRCC4 complex and is necessary for efficient ligation by NHEJ [31]. Activated DNA-PKcs also phosphorylates Ser139 on histone variant H2AX (γ-H2AX), which is a well-known marker for DNA DSBs and recruits the repair factors to the site and coordinates the signaling cascades required for efficient repair [32,33]. DNA-PK activation and its activity are modulated by the DNA to which it binds. The 5' end of the DNA activates the kinase activity while the 3' end anneals the DNA termini across the break [34]. Mutation studies on the Ku subunits and DNA-PKcs have shown that the Ku80/DNA-PKcs interactions are necessary for DNA-PK activity and are not specific to any structural region of the Ku80 C-terminus. Moreover, each structural region within the Ku80 C-terminus is necessary for the activation of the kinase activity. It was also observed that the structural features of the substrate like DNA length, DNA overhangs, orientation and sequence of the overhangs, influence Ku80/DNA-PKcs interaction and DNA-PK activation [35].

<**Fig. 1.**>

DNA-PK's kinase activity is requisite for its role in NHEJ [36]. Although a significant number of DNA-PK target proteins have been identified (including Ku70, Ku80, Artemis, XRCC4, XLF and DNA Lig4), it is now considered that the phosphorylation of these sites by DNA-PK is not required for successful NHEJ [37–39]. A recent study has shown that DNA-PK mediated phosphorylation facilitates DNA polymerase λ (pol λ)-mediated gap filling DNA synthesis during NHEJ [40]. The most important target site of DNA-PK phosphorylation is the

auto phosphorylation of the catalytic subunit itself [41–43]. DNA-PK autophosphorylation is essential for regulation of end processing, enzyme inactivation, and complex dissociation. The autophosphorylation of two clusters of residues, ABCDE (residues 2609-2647) and PQR (residues 2023-2056) regulates DNA end access for subsequent processing and ligation [44–46]. Mutational studies have shown that phosphorylation within ABCDE opens the ends for processing, while phosphorylation within the PQR cluster was shown to have an inhibitory effect on end processing [41,47]. These phosphorylation events point towards a mechanism by which DNA-PK protects the DNA ends and allows processing only when it is needed. DNA-PKcs autophosphorylation also results in the loss of kinase activity leading to the dissociation of DNA-PKcs from the Ku-DNA complex. Both the ABCDE and PQR regions seem to be necessary for DNA-PKcs dissociation [47].

Despite NHEJ being the prevalent mechanism of DNA repair in G1, NHEJ and HR are in direct competition in S/G2 of the cell cycle, as evidenced by continued expression of NHEJ factors throughout the cell cycle [48]. This suggests that there exists a mechanism that facilitates HR even if DNA-PK is recruited to the DSB first. One suggestion is that NHEJ and HR may be regulated in part by autophosphorylation of DNA-PK. DNA-PKcs autophosphorylation at the T, J, and K (JK cluster, Thr946, and Ser1004), does not affect end processing, and protect certain DSBs from NHEJ and promotes HR [49]. However, what mediates the autophosphorylation in the JK cluster is not known yet. Both the abundance of DNA-PKcs/Ku throughout the cell cycle and the higher rate of Ku recruitment to DSB sites over RAD51 recruitment, may also explain in part how NHEJ is preferred over HR [50,51] and it has been suggested that NHEJ is the default pathway for DSB repair and HR may be triggered only when NHEJ fails [52]. However, this is a highly over-simplified and inaccurate picture of the complex mechanisms controlling DNA

repair pathway choice. These mechanisms are reviewed in detail elsewhere [53,54] and include cell cycle control of HR via CDK activity and the Cullin ligases [55–59], topics beyond the scope of this review.

With regard to alternative pathways of end joining, Ku is known to repress MMEJ [60], and DNA-PK activity is required for this suppression [37,61]. In addition, the poly-ADP ribose polymerase 1 (PARP1) can directly compete with DNA-PK and the Ku heterodimer for DNA end-binding to promote MMEJ [62]. Although not discussed here in detail, DNA-PK can also play a role in none-DSB repair pathways including repair of single-strand breaks and base excision repair (BER) of oxidized DNA bases [63–66].

2.2. Role of DNA-PK in telomere maintenance and immunity

The functions of DNA-PK in the cells are not limited to DNA repair mechanism but include telomere maintenance, transcriptional and translational regulation of various genes, innate immunity, etc. [11,19,67,68]. DNA-PKcs plays a crucial role in the protection of the telomeres and telomere capping. Ample expression of DNA-PKcs and Ku subunits in the telomere region coincides with this notion. It is, therefore, speculated that the presence of DNA-PKcs at the telomere serves to protect the chromosome ends from nuclease activity. In agreement to this, studies conducted in mice deficient in both telomerase and DNA-PKcs showed a significantly higher rate of telomere shortening in comparison to telomerase knockout mice suggesting that DNA-PKcs also prevent shortening of the telomeres and hence can also play a critical role in aging [10,19,69]. Being the core component of NHEJ pathway, DNA-PK plays a major role in the generation of B-cells and T-cells by V(D)J recombination, where the non-specificity of the pathway results in the production of wide range of immunological cells. The

process is essential for the normal immunological functions of the body, and any alternations could result in Severe Combined Immunodeficiency (SCID) phenotype or other immune diseases. Recent studies have shown the involvement of DNA-PK in viral infection-mediated innate immunity, where DNA-PK acts as a nucleic acid sensor, binding to cytoplasmic DNA's and activating Interferon Regulatory Factor-3 (IRF-3)-mediated transcriptional activation of various cytokines and chemokines [67,68].

Because of the importance of DNA-PK in the development of immunological cells, any mutation to DNA-PKcs mostly presents with a SCID phenotype or radiosensitive SCID (RS-SCID) phenotype, especially if there is a defect in other components of the NHEJ pathway as well. SCID mice with a mutation in DNA-PKcs showed defects in V(D)J recombination, developed thymic T-cell lymphoma and also showed telomere fusions or shorter telomeres, but were viable and lived beyond one year of age [10]. However, spontaneous DNA-PKc mutations in specific strains of horses and dogs could not survive more than a few months of age and died due to infections [70,71]. Therefore, it could be speculated that DNA-PKcs is highly expressed in humans compared to mice and rodents. Even though SCID patients with a mutant DNA-PKc were not reported until recently, mutations in other components of NHEJ pathway have been reported and showed similar phenotypes as in the mice models. In 2009, van der Berg and colleagues identified the first human RS-SCID patient, with an L3062R missense mutation in DNA-PKcs FAT domain. The mutation led to deficient Artemis activation and resulted in reduced B and T cells in peripheral blood, but did not affect the kinase activity or its auto phosphorylation. Mouse models with mutations in Ku subunits also result in overlapping phenotypes including RS-SCID, growth defects, etc., but no spontaneous mutations or cases have been reported for the same [71,72].

3. Differential expression of DNA-PK in normal and cancer cells

DNA-PK is widely expressed in all mammalian cells, with primates showing up to 50 fold more expression compared to other mammals. Cultured human cells also express DNA-PK abundantly, and there exist conflicting reports on DNA-PK being differentially expressed in different human tissues [20,73]. A study led by Moll et.al, in various normal human tissues, reported a higher expression of DNA-PK in meiotic/actively dividing cells (neural cells and reproductive tissues), while epithelial cells from different tissues (colon, kidney, pancreas, endometrium, prostate, testis, brain, nerve ganglia and skin) showed a moderate expression profile. Fewer tissues like resting breast and liver showed very less to no expression at all [73]. However, a similar study by Sakata and group reported the expression of DNA-PKcs and Ku in the liver and resting breast tissues as well and attributed these differences to the different antibody used and a number of samples tested [74]. Transcription level expression of components of DNA-PKcs did not show any drastic difference between the tissues analyzed, other than the expression of Ku subunits being 2-4 fold higher than DNA-PKcs [73]. Terminally differentiated cells do not replicate their DNA and therefore are less likely to undergo any damages due to replication. They still undergo transcription and need to maintain their genetic integrity. Interestingly, these cells undergo damage response by other repairing pathways such as Nucleotide Excision Repair (NER), or Transcription Coupled Repair (TCR) and do not undergo NHEJ, in which DNA-PK is essential [75]. While the earlier belief was that DNA DSB repair is down regulated in certain differentiated cells. Recent studies on differentiated adipocytes and astrocytes showed an up regulation of DNA DSB repair with an increased expression of DNA-PK components [76,77].

<Fig. 2.>

Genomic instability caused by DNA damage and exacerbated by defects in the DDR is a ubiquitous feature of cancer cells and how cells respond to DNA damage is a major driver of cancer progression as well as a determinant of a tumor's response to therapy [1,78–80]. Differential expression of DNA-PK in clinical samples of tumors strongly implicates dysregulation of DNA-PK levels in cancer development. Elevated expression of DNA-PK is observed in esophageal cancers and colorectal cancers compared to the normal mucosal cells surrounding the tumor [81]. Clinicopathological studies have identified that DNA-PKcs elevated expression in colorectal cancers, which correlated with the clinical stage of the disease, lymphatic invasion, and distant metastasis, making it a potential biomarker for clinical assessment of pathogenesis and prognosis of colorectal carcinoma [82]. DNA-PKcs over expression is also observed in nasopharyngeal carcinoma and was associated with poor overall survival rate compared to patients with lower expression of DNA-PKcs. However, a couple of studies on Korean population showed no significant correlation between DNA-PKcs expression and clinical outcome of nasopharyngeal carcinoma [83]. Another study has reported a loss of DNA-PKcs expression of around 22.5% (63 out of 279) in gastric cancers, especially in stage I of gastric cancers [84]. Intra-tumoral heterogeneity of DNA-PK expression has made its quantification difficult in cancer cells. However, these studies implicate a crucial role for DNA-PKcs in the cancers of the gastrointestinal system. Non-small cell lung cancers (NSCLC) also exhibit a significant up regulation of DNA-PK expression and is also correlated with the differentiation degree of the disease, but was not associated with metastasis [85,86]. In glioma patients, the median survival rate of patients with high DNA-PK level was longer than that of patients with low DNA-PKcs. Recently it was reported that DNA-PK is involved in melanoma

tumor progression and metastasis by regulating tumor angiogenesis, migration, and invasion. Secretomic analysis revealed that DNA-PK regulates the secretion of several metastases associated proteins involved in tumor microenvironment modification, further indicating its crucial pro-metastatic role [87].

<Table 1>

Interestingly, in lymphoblastic cell lines, in spite of the higher mRNA transcript level, DNA-PKcs level is reduced compared to normal cells, indicating a post-transcriptional, proteasome-dependent regulation of DNA-PKcs [88]. Despite the elevated DNA-PK level observed in many tumors, the attenuated DNA-PK level has also been reported in several studies. In peripheral blood lymphocytes of cancer patients, there was an inverse correlation between the DNA-PK activity and disease progression [89]. Attenuated and reduced level of DNA-PK is also observed in certain breast, cervical and lung cancers [90]. Somatic mutation in DNA-PK is also closely associated with tumor pathogenesis. A mutation in the critical threonine residue (Thr2609) is essential for the catalytic activity of DNA-PK, as observed in breast and pancreatic cancers. Single nucleotide polymorphism analysis has identified a mutation in a non-coding intron (6721 G to T) of DNA-PK, to be associated with bladder cancer and hepatocellular carcinoma [91]. These findings suggest a complex and intricate regulation of DNA-PK during tumor progression and its dual role in DNA damage and pro-tumorigenic survival pathways.

4. Epigenetic alternations and genomic instability

DNA in eukaryotic cells is packaged into chromatin through histone and non-histone proteins which complicate the DNA damage repair mechanisms. The access of DNA repair proteins to damaged DNA lesions is essential for the efficient repair process. To overcome this

physical barrier, major alterations including post-transitional histone modifications and ATP-dependent chromatin remodeling factors are required in order to facilitate the entrance of repair proteins to the damage lesions [92–94].

With the term ‘epigenetic alterations’ we refer to reversible and heritable changes in gene function which are not caused by modifications in the underlying DNA sequence. These involve DNA methylation and multiple types of histone modifications such as various acetylations/deacetylations, methylations, etc. Moreover, extensive studies on microRNAs (miRNAs) have revealed their ability to induce post-transcriptional modifications on their target genes, thus having an impact on gene expression [95–97]. Although the implication of these alterations in a plethora of cellular processes (e.g. cell differentiation, gene expression, imprinting, X chromosome inactivation, etc.) is fundamental for maintaining normal function, there is accumulating evidence that these changes are also associated with the pathophysiology underlining various human diseases including cancer [98–100]. Several studies have demonstrated the interaction between DNA-PK and epigenetic alterations during DNA repair mechanisms [33,101]. DNA DSBs initiate the phosphorylation of histone H2AX protein at the conserved serine residue (Ser139) in C terminus to generate γ-H2AX. This phosphorylation event is important for stable association of repair factors at DNA damage sites and is essential for maintaining genomic stability [102,103]. Moreover, the phosphorylation of H2AX by DNA-PK is stimulated by histone acetyltransferase (HAT), which act mainly on the N-terminal tails of H3 and H4, by inducing conformational changes in nucleosome [104]. Interestingly, data from a study revealed the existence of a bromodomain (BRD)-like module in DNA-PKcs which is able to identify H2AX acetyl-lysine 5 (K5ac) as well as to promote the phosphorylation of H2AX at Ser139. Radioresistant tumor cells appeared to show increased levels of DNA-PKcs activation

while binding of JQ1, a Kac antagonist, to the bromodomain module led to re-sensitizing them to radiation [105]. Furthermore, DNA-PK may indirectly modulate the levels of γ-H2AX after genotoxic damage through activation of Akt that in turn inhibits GSK3β, as inhibition of GSK3β signaling appears to inhibit the dephosphorylation of γ-H2AX to a similar extent to chemical inhibition of PP2A [33]; a known γ-H2AX phosphatise [106].

DNA-PK is also implicated in the epigenetic regulation of DNA repair. For example, DNA-PK can affect the activity of histone acetyl transferase (HAT) hGCN5 during DNA repair. A study was done by Barlev et al. reported that the DNA-PK repress the activity of hGCN5, which has a conserved domain called bromodomain (BrD) at several levels. At the first level, Ku70/80 may sequester hGCN5 in non-functional complexes through binding to its BrD. Later, DNA-PKcs interacts with Ku and phosphorylates hGNC5, resulting in inhibition of HAT activity. However, more studies are required to investigate the role of DNA-PK in modulating hGCN5 activity [101]. MOF is another HAT protein that specifically acetylates histone H4 at lysine 16 (H4K16ac) position. Depletion of MOF resulted in a reduced level of H4K16ac which correlate with the defective DDR process. This results in delayed accumulation of DNA-PK in post-irradiation and decreased the association of MOF with DNA by preventing chromatin alterations that are essential for efficient DNA repair [94]. Tip60 is a HAT protein which has a crucial role in activation of DNA-PKcs kinase activity. This has been proved through silencing Tip60 expression which blocks the autophosphorylation of DNA-PKcs. Furthermore, the association of DNA-PKcs with HAT increases the activity by 5-fold in response to bleomycin treatment [107]. ATP-dependent chromatin remodeling factors are another type of alterations that affect the function of DNA-PK. One study showed that the chromatin remodeling factor, ALC1 (Amplified in Liver Cancer 1) binds to DNA-PK and catalyzes nucleosome sliding through its

interaction with poly (ADP-ribose) protein [108]. In addition, SIRT6, another chromatin regulatory factor, was found to play a critical role in the global deacetylation of Histone H3 Lysine 9 and is capable of stabilizing DNA-PKcs to chromatin at DNA DSB sites [109].

<Fig. 3.>

Histone methylation is another abundant post-translational modification that implicated in DDR process. The indirect interplay between DNA-PK and histone methylation in response to DNA damage was demonstrated by Jiang et al [110]. DNA-PK phosphorylates a metabolic enzyme fumarase, at Thr236 during ionizing radiation. The phospho-fumarase interacts with H2A.Z, a H2A variant, at DSB regions and result in generation of fumarate which inhibits KDM2B histone demethylase activity that are responsible for H3K9me3 demethylation. This inhibition promotes the accumulation of DNA-PK at DSB for NHEJ-DNA repair by enhancing demethylation of H3 at Lys 36 position. However, Young et al. showed that DNA-PK is not required for the recruitment of KDM4B to DNA damage region induced by a laser micro-irradiation [111]. The decrease in the level of H3K9 methylation is important for DNA repair by inducing chromatin relaxation [112]. Furthermore, a recent study showed that inhibition of DNA-PK resulted in elevated histone methyltransferase activity of EZH2 thereby suggesting that its phosphorylation by DNA-PK is responsible for the decreased enzyme's methyltransferase activity [113]. Moreover, in another report, heterochromatin protein 1β (HP1β) was shown to interact with DNA-PKcs with the resulting binding being dependent on the methylation status of three specific lysine residues namely Lys1150, Lys2746 and Lys3248. Finally, replacement of lysine with arginine caused the improper function of DNA-PKcs, in DDR, and consequently led to higher levels of sensitivity to radiation [114]. DNA-PK was also found to be involved in histone ubiquitination which regulates the DDR process especially the ubiquitination of H2AX

and H2A that are essential for further recruitment of repair proteins such as ATM, 53BP1 and BRCA1. DNA-PK has shown to promote H2AX and H2A monoubiquitination in response to DSBs induced by camptothecin, which causes transcription-blocking Top1cc, in WI38 fibroblast cells [115].

DNA methylation is another epigenetic marker which affects chromatin structure and genome stability through the methylation of cytosine residues by DNA methyltransferase (DNMT). Despite of the interplay between DNA-PK and different histone modifications, the interaction between DNA-PK and DNA methylation is still not well understood. Indeed, DNMT1 was found to be involved in modulating DDR in DNA-methylation-independent manner by its recruitment to DSBs [116]. Ha et al. reported that DNA-PK is not involved in the recruitment of DNMT1 and it was primarily dependent on its interaction with ATR effector kinase CHK1 [117]. Furthermore, another group has shown that glioblastoma and lung carcinoma cells treated with DNMT inhibitors were more sensitive to radiation due to impairment of DDR [118]. In particular, DNA-PK-deficient glioblastoma cells were preferentially more sensitive to Zebularine (a DNMTs inhibitor) thus implying its potential interaction with epigenetic mechanisms [119].

Apart from DNA methylation and histone modifications, miRNAs have also been shown to act as an epigenetic mechanism capable of regulating gene expression. In general, miRNAs are small, single-stranded RNAs which are firstly transcribed to their primary form (pri-miRNA), then are processed to a precursor form in the nucleus (pre-miRNA) and finally are exported to the cytoplasm where they are cleaved, by RNAse III endonuclease Dicer, to mature miRNAs [120,121]. There is evidence that a single miRNA may have more than one mRNA targets while an individual mRNA may be targeted by multiple miRNAs [122]. Findings from a recent report

demonstrated that mi-RNA-488-3p was capable of sensitizing malignant melanoma cells to cisplatin treatment by targeting *PRKDC* (the gene encoding DNA-PKcs) thus leading to a decline in its protein expression levels [123]. In addition, miR-21 was shown to provoke an increase in the activity of DNA-PKcs by targeting GSK3B, thus stimulating an increase in DSBs repair leading to radioresistance observed in various tumor cell lines [124]. On another note, overexpression of miR-101 in lung and brain cancer cell lines was found to reduce the protein levels of DNA-PKcs, while increasing their sensitivity to radiation [125]. In another study, miR-101 sensitized pancreatic tumor cells to the effect(s) of gemcitabine while also promoted apoptosis by down-regulating DNA-PKcs [126]. Reduced protein levels of DNA-PK were also observed in lung cancer cell lines following transfection with miR-101, hence causing an elevation in their radiosensitization [127]. Furthermore, miR-136 overexpression was associated with a decrease in the expression levels of DNA-PK in ovarian tumor cells [128]. On the contrary, overexpression of miR-1323 was found to increase the protein levels of DNA-PKcs in primary lung cell lines, whereas silencing of miR-1323 in radioresistant lung tumor cells was followed by a decline in the protein content of DNA-PK [129].

In addition, histone deacetylase (HDAC) inhibitors have been recorded as novel anticancer drugs and were found to cause an accumulation of DNA damage by affecting the expression of DNA repairing genes including DNA-PKcs [130]. Suberoylanilide hydroxamic acid (SAHA), a HDAC class I and II inhibitor, has been reported to downregulate the expression DNA-PK in human prostate carcinoma and glioma cells [131]. The treatment with Trichostatin A (TSA) radiosensitizes NSCLC cells by decreasing the expression level of Ku70, Ku80, and DNA-PKcs, leading to the inhibition of DNA repair capability [132]. Moreover, HAT inhibitors have been reported to sensitize the cancer cells towards radiotherapy and chemotherapy [133].

CBP and P200 are HAT proteins that were recruited to DSBs and cause acetylation of specific lysine within histone H3 and H4. The inhibition of CBP and P200 in lung cancer cells using inhibitors or small interfering RNA leads to the suppression of NHEJ by preventing the histone acetylation at damage sites and thereby suppressing the recruitment of Ku70 and Ku80 to DSBs [134]. These examples further indicated the role of epigenetic alterations in the function and regulation of DNA-PK during DNA repair mechanism and the effect of DNA-PK on the proteins that are involved in these regulations.

5. Transcriptional regulation by DNA-PK

Altered expression of DNA-PK contributes to cancer development, progression and metastasis by regulating a plethora of canonical pro-survival signaling pathways. Apart from the critical role of DNA-PK in DDR, it can regulate specific pro-tumorigenic pathways including genomic stability, hypoxia, metabolism and inflammatory responses. A major function of DNA PK in transcriptional regulation of several genes (c-Myc, c-Jun, and p53) is through direct involvement in tumor cell survival and proliferation. Several mechanisms by which DNA-PK regulate these cellular events have been identified in recent years. One study reported the interaction of DNA-PKcs with Akt which induces autophosphorylation of DNA-PKcs and promotes its kinase activity and recruitment at the broken DNA ends [135]. Positive regulation by survival factors may affect the genomic rearrangement as it is reported that increased survival may alter the genomic stability [136]. DNA-PKcs was also shown to be regulated by casein kinase II (CK2), a kinase associated with enhanced cell cycle progression. Inhibition of CK2 in human glioblastoma cell lines (M059K and T98G) shown decreased phosphorylation of Akt kinases that were earlier reported to associate with DNA-PKcs [137]. Recently, DNA-PKcs has

been shown to interact with the transcription factor SNAI1 (also referred to as snail), in response to DNA damage and promote cancer cell migration. The snail is a zinc finger protein belonging to the family of transcription factors that repress E-cadherin and thereby regulates epithelial to mesenchymal transition. Ionizing radiation (IR) activated DNA-PKcs was shown to phosphorylate Snail at Ser100 residue leading to Snail stabilization [138]. Phosphorylation of Snail at this residue negatively regulates DNA-PKcs kinase activity leading to inhibition of DNA damage repair resulting in genomic rearrangement and instability. Snail overexpression also contributes to survival after DNA damage, a phenomenon not seen in cells lacking DNA-PK [139]. Findings from a recent report also outlined the role of DNA-PKcs as a transcriptional modulator by stimulating tumor progression and metastasis in prostate carcinoma [140]. Furthermore, DNA-PK is activated by mild hypoxic conditions by auto phosphorylation at Ser 2056 by a mechanism independent of DNA repair pathway and positively regulates hypoxia inducible factor (HIF-1) thereby activating several pro-tumorigenic genes [141]. RPA70, another protein involved in hypoxic response and DNA repair in cancer cells is also indirectly regulated by DNA-PK. The interaction between RPA70 and TP53 under normal conditions is disrupted by hypoxia induced DNA-PK by phosphorylating TP53, resulting in the release of RPA70, which mediates apoptotic resistance in cancer [142–144]. The interaction between DNA-PK and TP53 following cellular stress is complicated with conflicting results generated from in-vitro and in vivo studies [145].

Apparently, DNA-PK specifically activates TP53 by phosphorylating its active sites. However, how this regulates TP53-mediated signaling that links to DNA damage response and cell cycle arrest/apoptosis, needs further in depth analysis [1,143]. $p21^{WAF1/CIP1}$ also known as cyclin dependent kinase inhibitor 1 or CDK-interacting protein 1, is a key factor in p53-mediated

cell fate after DNA damage. Following DNA damage, DNA-PKcs is recruited to the p21 promoter where it forms a complex with p53 protein and suppresses p21 gene transcription leading to cell death. Inhibition of DNA-PKcs with its pharmacological inhibitor, NU-7026 blocked its interaction with p53 and restored p21 transcription equivalent to undamaged levels and significantly reduced cell death following the pro-death stimulation. No such effects were observed on inhibiting ATM or ATM and Rad3-related (ATR) proteins, the other members of the PI3KK family, suggesting that DNA-PKcs negatively regulates p21 gene expression by modulating p53 binding at CDKN1A promoter.

It has been shown that radiosensitive mice have a reduced expression of DNA-PKcs and in-efficient DNA damage induced repair response [146]. Residual tumor cells in cervical cancers, which were resistant to radiation treatment, had a higher expression of DNA-PKcs showing a positive correlation between radioresistance and elevated DNA-PK level [147]. Down regulation of DNA-PKcs is also positively correlated with chemosensitization in human cervical carcinoma and radiosensitive phenotype in lymphoblastic cell lines [88,148]. Similarly, prostate cancer patients with elevated expression of DNA-PKcs in tumors, respond less to standard radiation therapy [149]. DNA-PK is also implicated in cetuximab (EGFR specific antibody) induced radiosensitization in lung and breast cancer cell lines, by immobilizing the complex of EGFR- DNA-PK in the cytoplasm and blocking EGFR transport into the nucleus [150]. A recent study, however, showed that patients with high levels of CD44 and DNA-PK had better overall survival rate and sensitized mesenchymal subtypes of glioblastoma to radiotherapy and temozolomide.

Differential secretomic studies have revealed that DNA-PKcs is also directly involved in regulating tumor microenvironment by controlling the secretion of several proteins involved in

tumor microenvironment modulation, like matrix metalloproteinase (MMPs) and at least 44 metastasis associated genes. In tumors where DNA-PK was inhibited, there was a delay in tumor proliferation, mainly due to inhibition of MMPs. Furthermore, DNA-PK is also involved in regulating neo-angiogenesis in primary tumors. A low level of DNA-PK is associated with a delay in angiogenesis initiation, with a reduced potential to proliferate and metastasize [87]. Pre-clinical studies with dual inhibition of mammalian target of rapamycin (mTOR) kinase and DNA-PK has been shown to induce cytotoxicity and blocks cell survival pathways in chronic lymphocytic leukemia [151,152]. Although DNA-PKcs serve to repair the damages incurred due to different stress or physiological parameters and maintain the integrity of the chromosomes, its association with different transcription factors or other signaling molecules involved in cell death or cell survival, contributes to genomic alteration and instability.

6. Chemical and biological inhibitors of DNA-PK

The most successful approach to inhibit DNA-PK is by small molecules that target the ATP-binding site of the kinase domain. Various investigations have revealed that a small group of compounds (**Fig. 4**) can inhibit DNA-PK activity effectively [153,154]. The first identified inhibitor, wortmannin, obtained from the fungus *Penicillium funiculosum*, is a general competitive inhibitor of PI-3 kinase with an IC50 value of 16 nM [155]. Wortmannin exhibits its inhibitory nature by irreversible alkylation of Lysine 802 residue at the active site of DNA-PKc's that is essential for phosphate transfer reaction. Wortmannin is identified as an effective radiosensitizer in a variety of normal and cancer cells with a Dose Reduction Factor (DRF) for IR at 10% survival (between 1.4 and 3). Being a DNA-PK inhibitor, wortmannin plays a significant role in p53 phosphorylation and acetylation. Lin et al. have shown that p53

phosphorylation induced by benzo[a]pyrene on HepG2 cells suppress and accumulates p53 acetylation, which was moderately affected when treated with 20 μM wortmannin [156]. Moreover, it has a vital role in the inhibition of histone modification. In ACC-LC-91 lung cancer cells, the histone H3 acetylation and histone H3K4 methylation induced by histone deacetylase (HDAC)1 was found to be inhibited and regulated by wortmannin [157]. Further, the treatment in MCF7 cells with this inhibitor proves to be effective in preventing the formation of phosphorylated histones which gets rapidly phosphorylated during DNA damage [158]. Despite all these interesting features, lack of specificity, poor solubility, invite toxicity limits its clinical applications [10].

LY294002, a morpholine derivative of natural flavonoid quercetin, is another competitive DNA-PK inhibitor that binds irreversibly to the kinase domain of DNA-PK with an IC50 value of 1.4 μM producing a DRF at 10% survival with IR of 1.5 to 1.8. Even though LY204002 possess interesting *in vivo* results as a radiosensitizing agent, rapid metabolic clearance, high *in vivo* toxicity, lack of specificity and poor stability makes its clinical evaluation unfeasible in humans [10,159]. However, LY294002 has been proved as a productive lead molecule for a series of compounds with favorable properties. Those compounds which are synthesized using LY294002 as a template have improved specificity with regards to DNA-PK inhibition. Among these, NU7026 is considered as one of the most potent molecules with 70-fold more selectivity towards DNA-PK, compared with other PI-3Ks. NU7026 exhibited an inhibition of various targets with an IC50 value of 0.23 μM against DNA-PK, 13 μM against PI3Ks, and > 100 μM for ATM or ATR. This compound enhanced the cytotoxic nature of various drugs like idarubicin, daunorubicin, doxorubicin, etoposide, and amsacrine [159]. Rapid absorption is possible due to the mono hydroxylation of the second position of the morpholino group, resulting in an opened

ring structure. Wang et al. showed that it is efficient in inhibiting DNA-PK activation induced by cisplatin without bringing any alteration to histone H4 expression [160]**.** NU7441, another molecule based on LY294002 backbone system with improved potency and having an IC50 value of 0.3 μM for DNA-PK and seven μM for PI3K proteins [161,162].

Other compounds possessing different chemical structures found to have an inhibition property for DNA-PK that comprise OK1035 [162] and SU11752 [163]. Both compounds lack the required potency for further development studies. Another interesting molecule found to inhibit DNA-PK activity is vanillin, a phenolic aldehyde obtained from certain species of vanilla pods [164]. The structural simplicity of vanillin makes it an attractive molecule for modifications in search for better molecules. Two methoxybenzaldehyde derivatives of vanillin, 2-nitro and 3-iodo, were found to be a better inhibitor of DNA-PK than vanillin [165,166]. This may be due to the electron withdrawing nature of $–NO_2$ which increases the reactivity of aldehyde group towards the amino group of the protein, and this mechanism is not observed in 3-iodo substituted compounds [163]. Anti-cancer agent NK314 [93], is an inhibitor of both topoisomerase IIα and DNA-PK. Other compounds found to have inhibitory property against DNA-PK are PI103, PP121, KU-0060648, and CC-115 [93,151,165,167,168]. Among these, PI103 is a potent ATP-competitive DNA-PK inhibitor. PP121 inhibits DNA-PK with an IC50 value of 60 nM, while KU-0060648 is a dual inhibitor of DNA-PK, PI3Kα, PI3Kβ and PI3Kδ with an IC50 value of 8.6 nM, 4 nM, 0.5 nM and 0.1 nM, respectively. The differences in selectivity of these compounds are due to the structural differences and similarity that exists within the active sites [168]. However, there is still a dearth of knowledge to identify a potent compound that can selectively inhibit DNA-PK's expression in cancerous cells alone during therapy.

The majority of research so far has been carried out using small organic/synthetic compounds as DNA-PK inhibitors. A shift in focus to nucleotide and antibody based inhibitors have shown higher efficacy in DNA-PK inhibition. The two primary obstacles faced by small organic compounds such as poor solubility and/or short serum half-lives could be easily overcome by these inhibitors due to their biological nature [169]. One such nucleotide is GRN163L, a 13-mer oligonucleotide which inhibits the phosphorylation of DNA-PK and increases γ-H2AX phosphorylation in chronic lymphocytic leukemia (CLL) lymphocytes in response to treatment with fludarabine, a nucleotide analog [170]. A similar effect has been reported by an antibody based inhibitor, Folate-ScFv 18-2, where it also enables radiosensitization when tested against human KB oral carcinoma and NCI-H292 lung cancer cells [171]. Further, a study by Kim et al., in breast cancer cell lines NCI and MDA-MB-231 has shown how peptides can be effective in DNA-PK inhibition. A targeting peptide (HNI-38) containing c-terminus of Ku-80, inhibited the activity up to 50% by interfering with the interaction between DNA-PKcs and Ku complex thereby lowering the resistance of the cells to IR [172].

The strategy of using small interfering RNA (siRNA) oligonucleotide was found to play a remarkable role in inhibiting DNA-PK activity. A study by An et al., on HeLa cells reported that the siRNA targets the DNA-PKcs catalytic motif and could exhibit an increased efficiency to radiosensitization. It is demonstrated that DNA-PK silencing by siRNA could also lead to the downregulation of the activity and expression of the c-myc protein [173] which is an essential regulator of the progression of the cell cycle [174]. The knock down of DNA-PKcs using siRNA approach in low passage human fibroblasts also showed significant effects. The radiation-induced interphase chromosome breaks were resisted at a reduced capacity. At first in post

irradiation mitosis, there was an increase in the yield of acentric chromosome fragments in addition to an increased radio-sensitivity [175]. Research by Collis et al. also supported the role of siRNA where prostate cancer cell lines such as DU145 and PC3, which were resistant to radiation, when transiently transfected with plasmid encoding siRNA, rendered them sensitive to IR via targeting DNA-PKcs [176].

Apart from the above mentioned DNA-PK inhibitors, antisense oligonucleotides also play an active role as DNA-PK inhibitor. An antisense oligonucleotide namely 2′-O-methoxyethyl/uniform phosphorothioate chimeric antisense oligonucleotides (ASOs) modulate DNA-PK expression and increase the cell death in human glioma cell lines (M059K) after treatment with ionizing radiation, bleomycin, and etoposide [177]. Antisense oligonucleotide inhibitors not only sensitize the cells to IR but also pave the way for autophagy. Human malignant glioma M059K, U373-MG, and T98G cells, when treated with antisense oligonucleotides, inhibits DNA-PK and were sensitized to low dose IR by inducing autophagy [178]. An introduction of antisense Ku70 construct to human lung squamous cell carcinoma showed the increased sensitivity of transfected cells to cytotoxic agents such as bleomycin, methyl methanesulfonate and to IR. This study achieved a partial reduction of DNA end binding activity by partially reducing Ku 70 protein expression [179]. Moreover a histone modification wherein histone 3 lyisne 36 (H3L36) undergoes demethylation (H3L36me2) and enhance the presence of Ku70 at the damaged site and facilitates NHEJ repair process [180]**.** These examples act as a standing example to prove that biological inhibitors such as antisense oligonucleotides, siRNAs, peptides, and antibodies can also play a profound role as DNA-PK inhibitor in overcoming the hurdles faced by small organic/synthetic compounds.

7. Conclusions and future directions

Conventional cancer therapy including radiotherapy and chemotherapy depends on inducing DNA damage that is partly repaired by DNA-PK dependent pathways. Therefore, the expression of DNA-PK has a significant impact on therapy outcome in different ways. First, DNA-PK can be utilized as a biomarker for predicting prognosis and response to cancer treatments. However, the heterogeneity of DNA-PK expression in different types of tumors and within the same tumor makes it difficult to employ DNA-PK as a biomarker in clinical settings. In addition, the DNA-PK function is pleiotropic, and loss or gain of DNA-PK may impact both cell signaling pathways (e.g., Akt/G3Kb) and gene transcription via both direct interaction with transcription factors and via epigenetic mechanisms. Thus, how DNA-PK expression regulates tumor response to radiotherapy and chemotherapy is likely complex and will require further study to allow this kinase to be effectively used as a biomarker for treatment response. Secondly, chemicals and biologicals that target DNA-PK may greatly improve the outcome of cancer therapy. Despite the vast array of agents developed as DNA-PK inhibitors and the pleiotropic function of this kinase, two major problems limit the clinical approval of these compounds: lack of specificity and difficulty in targeting the inhibition specifically to cancer cells. Future studies should, therefore, aim at the development of more specific inhibitors and on finding ways to ensure the differential inhibition of DNA-PK using a broad range of cancer cells. In the new era of research, epigenetics may well address these challenges.

Conflict of interest

There is no conflict of interest to declare on this review article.

Acknowledgement

Work in HPVR laboratory is funded by Natural Sciences and Engineering Research Council (NSERC) (RGPIN 246127). GD is a Senior Scientist at the Beatrice Hunter Cancer Research Institute (BHCRI) and his laboratory's work on DNA repair is funded by a Discovery Grant from the NSERC (RGPIN 05616).

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Figure legends

Fig. 1. DNA-PK in NHEJ mechanism. The process of NHEJ starts with the recognition and binding of Ku70/80 sub-units to broken DNA ends which function as docking sites for other proteins including DNA-PKcs. DNA-PKcs recruitment to Ku70/80 complex dimerizes to form a synaptic complex which acts as scaffold proteins for localization of other repair proteins to the damaged site of DNA. Autophosphorylation of DNA-PK at ABCDE region opens the DNA ends for further processing by Artemis protein which was recruited and activated by DNA-PK. DNA-PK also recruits and mediates the phosphorylation of DNA polymerase λ for gap filling during DNA synthesis. Upon autophosphorylation of DNA-PK at PQR region along with ABCDE protein, leads to dissociation of DNA-PKcs from the Ku-DNA complex. As an end process, Lig4-XRCC4 complex mediates efficient DNA ligation with the help of cernunnos/XRCC4-like factor (XLF) and repairs the DNA DSBs successfully.

Fig. 2 The role of DNA-PK in normal cells (A) and cancer cells (B).

A. Normal cells. DNA-PK is essential for maintaining genomic stability by regulating DNA repair, chromosome segregation, and telomere capping. (i) DNA-PK is a critical component of NHEJ pathway that is required for repairing damaged DNA and for generation of B and T cells by V(D)J recombination along with other proteins including Artemis, XRCC4, and Lig4. (ii) In mitosis, phosphorylated DNA-PKcs colocalizes with polo-like kinase 1 (PLK1) at the centrosomes and kinetochores, for proper chromosome segregation with an accumulation of midbody for controlling cytokinesis. (iii) During telomere capping, heterogeneous ribonucleoprotein A1 (hnRNP-A1) gets phosphorylated by DNA-PKcs and promotes the

replication protein A (RPA) to protect telomeres 1 (POT1), by switching telomeric 3' single-strands to form a cap over newly replicated telomeres. (iv) DNA-PK also plays an important role in B/T cell generation and viral infection-mediated innate immunity. DNA-PK functions as a pattern recognition receptor to activate innate immunity. It binds to cytoplasmic DNA and activates IFN regulatory factor 3 (IRF-3)-dependent innate immune response to trigger transcription of type I interferons (IFN).

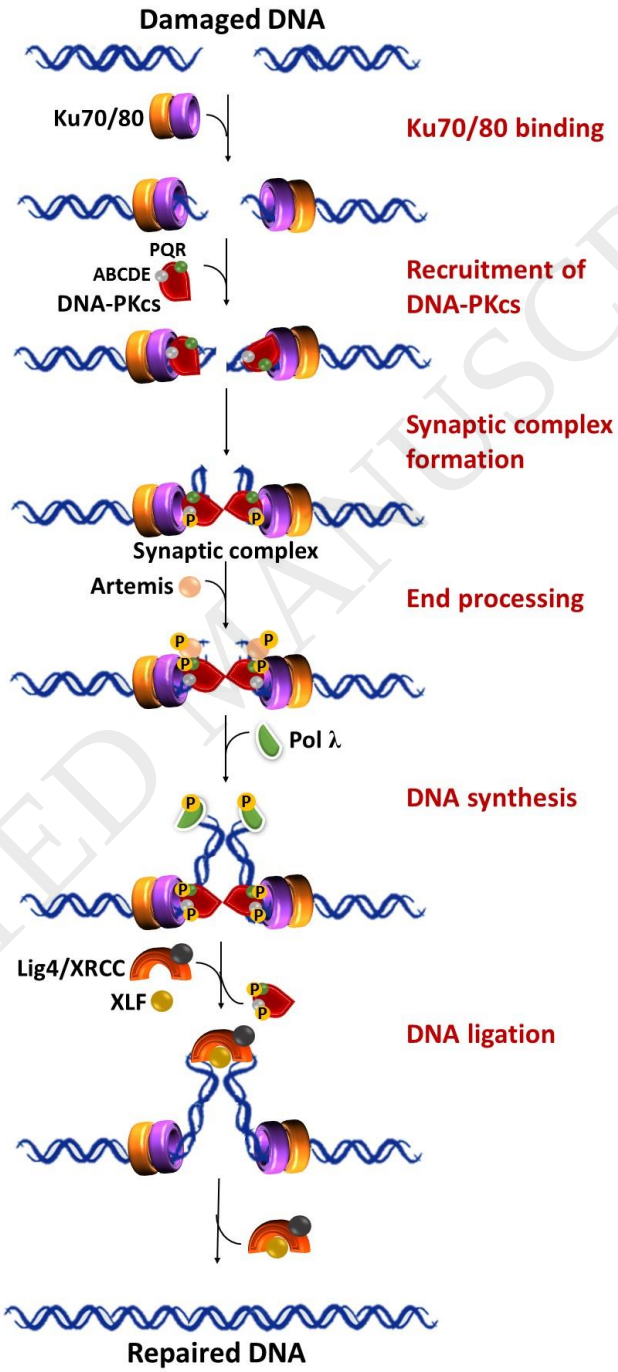
B. Cancer cells. In cancer cells, increased expression of DNA-PK regulates specific pro-tumorigenic pathways including genomic instability, hypoxia, metabolism and inflammatory responses. (i) DNA-PK is directly involved in the transcriptional regulation of c-Myc, c-Jun and p53, leading to tumor cell survival and proliferation. Under hypoxic conditions, DNA-PK is activated independent of DNA repair pathway and regulates HIFα, thereby activating various pro-tumorigenic genes. (ii) DNA-PK contributes to EMT, an essential step in tumor metastasis, by regulating zinc finger transcription factor snail. (iii) DNA-PK also maintains the tumor microenvironment by controlling the secretion of several proteins like MMP-8.-9, SERPINA3 etc. (iv) Moreover, DNA-PKcs regulates mitotic spindle organization via the Chk2–BRCA1 signaling pathway and the loss of DNA-PKcs will prevent the activation of Chk2–BRCA1 signaling pathway, leading to chromosomal instability.

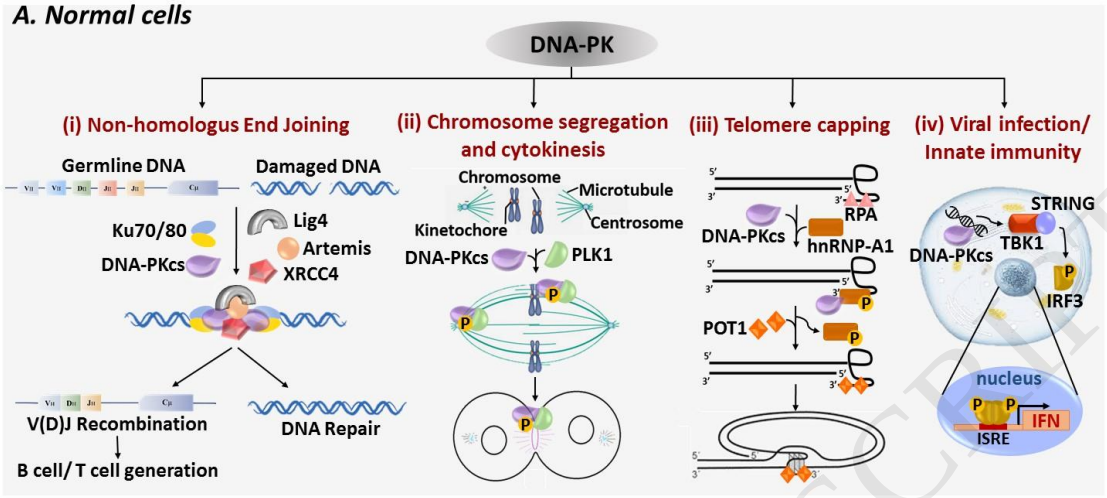
Fig. 3. Interplay between DNA-PK and epigenetic modifications. DNA DSBs caused by ionizing radiation or camptothecin initiates the phosphorylation of histone H2AX protein to generate γ-H2AX that can initiate histone modifications. DNA-PK can be affected by the function of different histone modifiers, such as acetyl transferase (HAT), MOF and Tip60 during DNA repair process. MOF specifically acetylates histone H4 at lysine 16 that are involved in

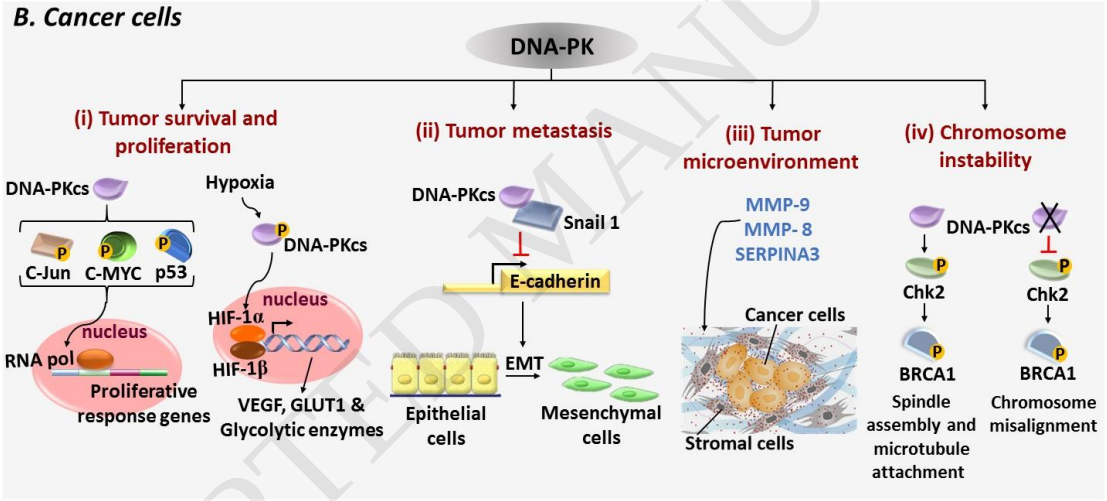
chromatin modification, to induce accumulation of DNA-PK at the damaged site, whereas Tip60 induces the activation of DNA-PKcs kinase activity. The indirect interplay between histone demethylase and DNA-PK were also found to be involved in DDR process. DNA-PK indirectly inhibits the histone demethylase activity of KDM2B by recruiting its accumulation at damaged site. This inhibition by the phosphorylated fumarase by DNA-PK, interacts with H2A.Z at DSB regions and results in local generation of fumarate to inhibit KDM2B. DNA-PK is also involved in histone ubiquitination by promoting H2AX and H2A monoubiquitination which are essential for the recruitment of ATM and 53BP1. The modulation in nucleosome packaging with DNA in response to DSBs can also be induced by DNA-PK by its interaction with ATP-dependent chromatin remodeling factors such as ALC1, to catalyze nucleosome sliding through its interaction with PARP. These major alterations including post-transitional histone modifications and ATP-dependent chromatin remodeling factors facilitate the entry of repair proteins to the damage lesions and activate NHEJ/HR repair mechanisms.

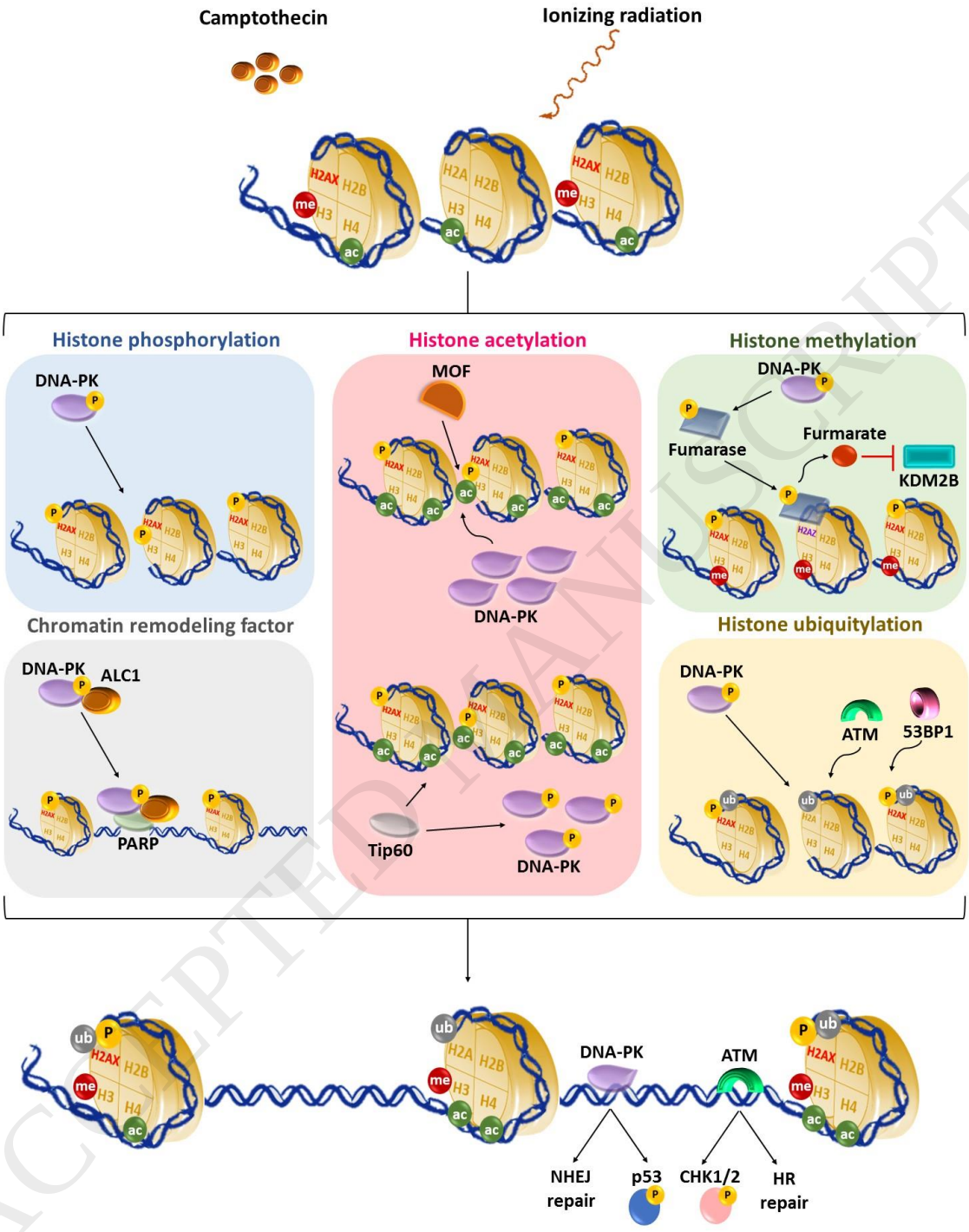
Fig. 4. Various small molecules as DNA PK inhibitors. Wortmannin (1), LY294002 (2), NU7026 (3),NU7441 (4), OK1035 (5), SU11752 (6), Vanillin (7) & derivatives; 2-nitro (8) and 3-iodo (9),NK314 (10), PI103 (11), PP121 (12), KU-0060648 (13), CC-115 (14).

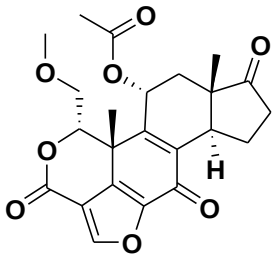
Figures:











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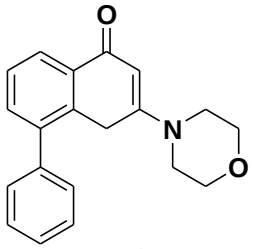

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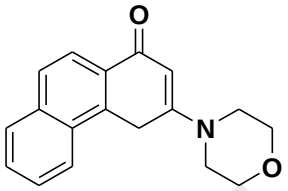

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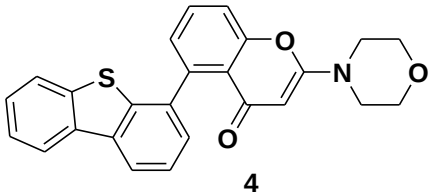

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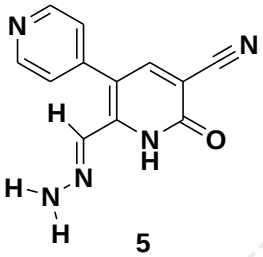

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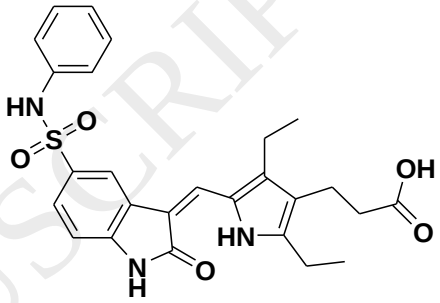

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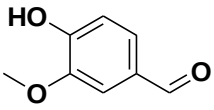

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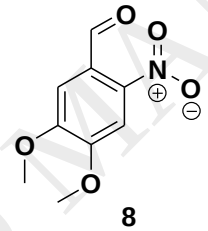

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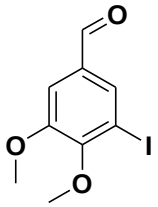

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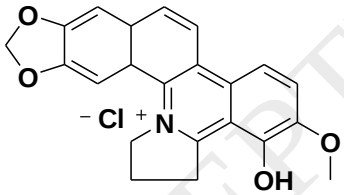

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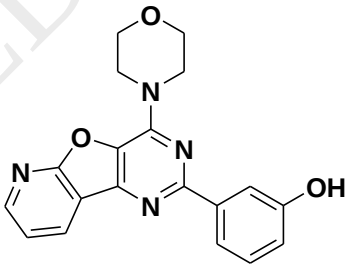

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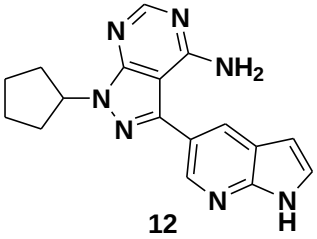

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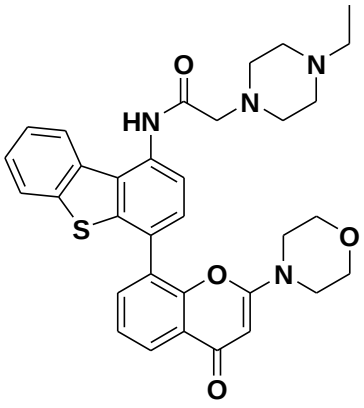

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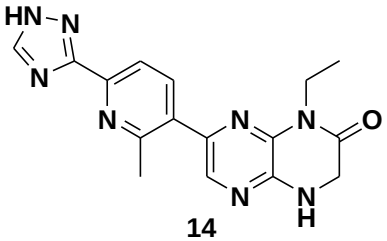

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Tables:

Table 1. Differential expression levels of DNA-PK in normal and cancer cells

Tissue/cell types	DNA-PK expression levels	Specificity	References
Normal cells			
Neural cells	High expression	Brain cortex and autonomous nervous system	[73]
Reproductive tissues	High expression Moderate expression	Testis Ovary and prostate	[73]
Epithelial cells	Moderate expression	Colon, pancreas and kidney	[73]
Breast tissues	High expression Less to no expression	Lactating breast tissues Resting breast tissues	[73]
Cancer Cells			
Esophageal cancer	Differential expression	Difficulty in prediction of radio or chemo-sensitivity of tumour	[81]
Colorectal carcinoma	High expression	Potential biomarker for clinical assessment of pathogenesis and prognosis of carcinoma	[82]
Gastric cancer	Low expression	Poor patient survival	[84]
Glioma	High expression	Better response to radiotherapy and chemotherapy	[87]

Cervical cancer	High expression	Resistant to radiation treatment	[147]
Prostate cancer	High expression	Reduced response to standard radiation therapy	[181]
Human renal cell carcinoma	Over expression	Target for renal cell carcinoma intervention	[182]
B-cell chronic lymphocytic leukemia	High expression	Short survival and chemo- resistance	[183]
Non small lung cancer	High expression	Radio-resistance	[184]
Nasopharyngeal caricnoma	High expression	Poor survival	[185]