2-15-2022

DNA-PKcs: A Targetable Protumorigenic Protein Kinase.

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ABSTRACT

DNA-dependent protein kinase catalytic subunit (DNA-PKcs) is a pleiotropic protein kinase that plays critical roles in cellular processes fundamental to cancer. DNA-PKcs expression and activity are frequently deregulated in multiple hematologic and solid tumors and have been tightly linked to poor outcome. Given the potentially influential role of DNA-PKcs in cancer development and progression, therapeutic targeting of this kinase is being tested in preclinical and clinical settings. This review summarizes the latest advances in the field, providing a comprehensive discussion of DNA-PKcs functions in cancer and an update on the clinical assessment of DNA-PK inhibitors in cancer therapy.

Introduction

DNA-dependent protein kinase catalytic subunit (DNA-PKcs) is a multifunctional serine—threonine protein kinase that plays pleiotropic roles in cancer. Since its first identification as a component of a transcriptional complex, the role of DNA-PKcs has been extensively studied in DNA double-strand damage repair via nonhomologous end joining (NHEJ), transcriptional regulation, genomic instability, and innate immunity, whereas other functions are yet to be fully elucidated.

DNA-PKcs dysregulation has been commonly reported in multiple solid and hematologic tumors, including chronic lymphomas, colon, prostate, breast, cervical, and brain cancers (1–3). Cumulative evidence suggests that DNA-PKcs overexpression and increased phosphorylation (activation) in human malignancies are predictive of poor prognosis (2, 4), and targeting DNA-PKcs sensitizes cells radiotherapy and DNA-damaging agents (5–7). Thus, DNA-PKcs has been proposed as a potential therapeutic target in cancers that overexpress DNA-PKcs. Perturbation of DNA-PKcs function via genetic and pharmacologic tools decreases malignant cell survival (8, 9), thus supporting the use of DNA-PKcs–targeted small-molecule drugs as potential therapeutic agents alone or in combination with standard-of-care treatments. Although first-proposed inhibitors of DNA-PKcs lacked specificity and exhibited a poor pharmacokinetic profile (10), a newer generation of more specific and efficacious DNA-PKcs inhibitors has provided encouraging preclinical data and are currently being evaluated in clinical trials in advanced malignancies. Developing a comprehensive understanding of DNA-PKcs function and identification of tumor subtypes that may be most responsive to DNA-PKcs inhibitors is critical for clinical translation. Although gaps remain regarding the overall means through which DNA-PKcs promotes protumorigenic phenotypes, this review will summarize relevant advances in understanding of protein kinase regulation and function, and detail the current state of clinical research.

DNA-PKcs Regulation

DNA-PKcs is encoded by the *PRKDC* gene, which localizes on chromosome 8q11 (11). As a ∼469-KDa protein composed of 4,128 amino acids, DNA-PKcs is one of the largest and most abundant kinases in higher eukaryotes. Based on structure homology, DNA-PKcs is a member of the phosphatidylinositol 3-kinases (PI3K) superfamily, but due to lack of lipid kinase activity is further classified in the phosphatidylinositol 3-kinases-related kinase (PIKK) family (12, 13). DNA-PKcs structure consists of the N- terminus region arranged in HEAT (Huntingtin, Elongation Factor 3, PP2A and TOR1) repeats followed by a leucine-rich domain that can be involved in protein–protein interactions and innate DNA affinity (14, 15), a noncanonical bromo domain important in DNA repair (16), a uniquely conserved DNA-PKcs domain NUC194 (17), phosphorylation clusters JK, ABCDE, and QPR that modulate its activity, and the C-terminus region that contains PI3K-like domains FAT [named due the region's homology in FRAP, ataxia-telangiectasia mutated (ATM), and transcription domain-associated protein TRRAP], FRB (FKBP12-rapamycin-binding), PRD (PIKK-regulatory domain), FATC (FAT at the C-terminus), and the kinase domain (**Fig. 1A**; refs. 18–20). DNA-PKcs kinase activity is important for DNA-PKcs function in cancer and beyond, as it has been linked to regulation of numerous cellular processes.

The activity of the protein kinase is regulated at multiple levels including (i) protein–protein interaction, (ii) phosphorylation, and (iii) various other posttranslational modifications (PTM). Regulation through protein–protein association is important for modulation of DNA-PKcs activity and downstream functions. For example, the best studied protein–protein interaction of DNA-PKcs with other proteins is the binding to Ku70/80, which promotes DNA-PKcs conformational changes leading to activation/deactivation of DNA-PKcs, thus affecting DNA repair. Other factors including epidermal growth factor receptor (EGFR) directly bind with DNA-PKcs (21) and affect DNA-PKcs' role in DNA repair. The EGFR–DNA-PKcs protein–protein interaction increases upon damage and promotes DNA-PKcs activity in response to double-strand break (DSB) induction (22). Inhibition of EGFR, by gefitinib and cetuximab, impairs the EGFR–DNA-PKcs protein–protein interaction and inhibits DNA repair of IR-induced DSBs (23, 24). Similarly, casein kinase II (CK2) binds with DNA-PKcs, and this interaction is enhanced after DNA damage (25, 26). Down-regulation of CK2 leads to decreased DNA-PKcs phosphorylation,

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Cancer Res 2022;82:523–33

doi: 10.1158/0008-5472.CAN-21-1756

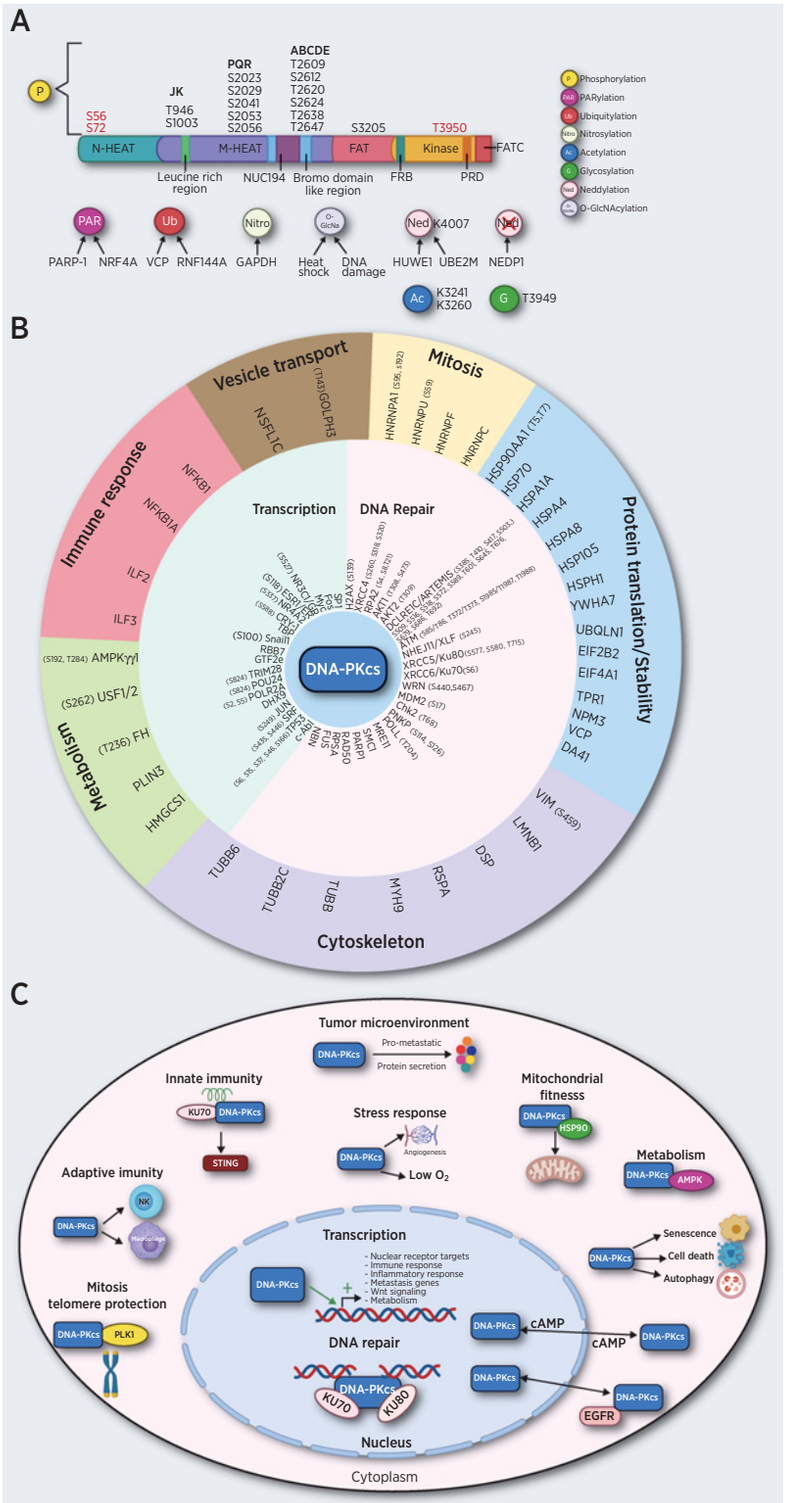

Figure 1.

DNA-PKcs regulation and substrates are important for a variety of cellular functions. **A,** DNA-PKcs structure highlighting DNA-PKcs domains and DNA-PKcs regulation through posttranslational modifications. **B,** DNA-PKcs substrates identified *in vitro* and *in vivo* categorized by function. Known validated phosphorylation sites are shown next to each substrate. **C,** Summary of DNA-PKcs nuclear and cytoplasmic functions. Figures were generated using BioRender.

persistent DNA damage, and sensitization to radiotherapy through destabilization of the DNA-PKcs–Ku80 complex (25, 26). On balance, direct protein–protein interactions are important for modulation of DNA-PKcs activity and downstream biological processes.

In parallel, DNA-PKcs is modulated by phosphorylation as induced by DNA-PKcs itself, as well as other cancer relevant kinases. Studies have identified approximately 60 DNA-PKcs serine/threonine autophosphorylated sites, with mutagenesis showing that at least 16 sites

alter DNA-PKcs (**Fig 1A**; refs. 27–29). Alterations at only three sites (S56, S72, T3950 shown in **Fig 1A**, red) lead to an enzymatically inactive kinase that does not disrupt the DNA-PK complex but affects the role of DNA-PKcs in DNA repair and sensitization to therapy (30, 31). Moreover, DNA-PKcs has been shown to autophosphorylate the PQR/2056 and ABCDE/2609 phosphorylation clusters in response to DSB induction (32–34). Additional studies have shown that the PQR cluster autophosphorylation limits DNA end processing during NHEJ, whereas the ABCDE cluster promotes DNA end processing during NHEJ (33, 35). Although primarily autophosphorylated, the ABCDE cluster can also be phosphorylated by ATM and ATR (ATM and RAD-3 related protein) kinases (36, 37), thus suggesting that other proteins contribute to DNA-PK phosphorylation and activity. Interestingly, recent studies have reported up to 88 serine, 34 threonine, and 21 tyrosine residues can be modified by phosphorylation on DNA-PKcs (29, 38). Although much remains to be understood functionally about these sites and their effectors, several proteins have been shown to regulate DNA-PKcs activity and function via binding and/or phosphorylation. For example, AKT (also known as protein kinase B) has been shown to bind to DNA-PKcs post-irradiation, promote DNA-PKcs autophosphorylation and protein kinase activity to support DNA-damage repair (39, 40). Similarly, ATR binds and phosphorylates DNA-PKcs in response to UV irradiation (41), whereas ATM phosphorylates DNA-PKcs in response to ionizing radiation (36), both promoting DNA-PK activity and subsequent repair. On the contrary, the proto-oncogene c-Abl (42, 43) and Lyn tyrosine kinase (44) binding and (*in vitro*) phosphorylation of DNA-PKcs inhibit DNA-PKcs and lead to its dissociation from the DNA-PK complex. Furthermore, DNA-PK activity is attenuated by inhibition of ERK and MEK kinases in response to etoposide-induced damage, thus inhibiting DNA repair via NHEJ (45). Taken together, the current state of knowledge indicates that although DNA-PKcs autophosphorylation plays a key role in DNA-PKcs regulation and downstream functions in DNA repair, additional kinases alter DNA-PKcs activity.

In addition to phosphorylation, DNA-PKcs is directly modified by several other PTMs including PAR-ylation, acetylation, ubiquitylation, neddylation, nitrosylation, and glycosylation. DNA-PKcs was reported to be subject of PAR-ylation by poly(ADP-ribose) polymerase 1 (PARP-1; refs. 46, 47) and by orphan nuclear receptor NR4A (48), upon damage, impacting DNA-PKcs activity in a context specific manner. As PARP family proteins exert differential effects on DNA-PKcs activity, it will be important to delineate the interplay between PAR-ylation and DNA-PKcs activity in cancer, especially with the onset of approvals for PARP inhibitors in the clinical setting. Although preclinical data show that inhibition of PAR-ylation and DNA-PKcs activity synergistically inhibits cancer cell survival (47), further studies are needed to better inform the use of PARP and DNA-PKcs inhibitors in combination therapies.

Although gaps in knowledge remain, DNA-PKcs is also posttranslationally modified by lysine-targeted PTMs. Recent studies found 16 lysine residues marked for acetylation, with two of these residues (K3241 and K3260) confirmed to have direct role in DNA-PKcs–dependent DSBs repair, genomic integrity, and radiation resistance in *in vivo* studies (49, 50). Furthermore, DNA-PKcs was reported to be ubiquitylated indirectly through valosin-containing protein in a proteasome-dependent manner (51), as well as directly by the ring finger protein 144A (RNF144A; ref. 52), which mark DNA-PKcs for degradation, leading to sensitization of glioblastoma cells to radiation, and promotes apoptosis, respectively. DNA-PKcs is also marked by neddylation via neddylation E-2-conjugating enzyme UBE2M and E-3 ligase HUWE1 in its kinase domain, which promote DNA-PKcs S2056 autophosphorylation and NHEJ (53), whereas NEDP1 is responsible for DNA-PKcs deneddylation (53). The consequence of these PTMs remains to be fully elucidated; however, these data highlight the complexity of DNA-PKcs regulation and the need to understand the cross-talk between the PTMs and the resulting impact on cancer processes.

DNA-PKcs Substrates

Although understanding of DNA-PKcs regulation is emergent, gaps in the identification of DNA-PKcs substrates remain with studies largely focused on the components of DNA repair. The most well-known DNA-PKcs substrate is DNA-PKcs itself, followed by other mainly *in vitro* substrates that are phosphorylated on the consensus sequence of serine and threonine sites followed by glutamine (SQ/TQ; ref. 54). Some of these substrates include DNA-PKcs (55), Hsp90 (56, 57), p53 (54, 58), and Artemis (59). Nevertheless, many substrates have been identified *in vitro* where DNA-PKcs does not utilize the canonical phosphorylation sequence but rather phosphorylates non-SQ/TQ sites such as serine and threonine residues followed leucine or tyrosine. For example, proteins such as XRCC4 (60), WRN (61), Artemis (59), XLF (62), Ku70/80 (63), and DNA-PKcs (55) have been identified as non-SQ/TQ DNA-PKcs substrates. Furthermore, DNA-PKcs has been shown to have substrates that do not contain any consensus sequence such as in the case of the C-terminal domain (CTD) of RNA polymerase II (64, 65). As such, challenges exist in the prediction of DNA-PKcs substrates and therefore in discerning the mechanisms by which the protein kinase elicits protumorigenic functions.

Known DNA-PKcs substrates are linked to numerous cellular and cancer processes, summarized in **Fig. 1B**. Not surprisingly, given the functional focus on DNA repair–related activities, the majority of known targets are associated with this category. It is important to mention that although most of these DNA repair substrates have been identified *in vitro*, relatively few are found *in vivo*. Importantly, phosphorylation events identified *in vivo* have been shown to have biological impact, for example, on DNA repair such as in the case of WRN (Ser440/Ser466, Werner syndrome ATP-dependent helicase; refs. 66, 67) and H2AX (Ser139, histone H2AX; refs. 68–70), regulation of transcription by POLR2A (Ser2/Ser5/CTD heptad repeats, DNA directed RNA polymerase II subunit RPB; refs. 64, 71), mitosis by RPA2 (Ser 4/Ser 8/ Thre21, replication protein A, 32 kDa subunit; refs. 72, 73), cell survival in response to damage by GOLPH3 (Thr143, Golgi phosphoprotein 3; ref. 74), and cell migration by VIM (Ser459, vimentin; ref. 75). Nevertheless, many of the phosphorylation events identified *in vitro* remain to be validated *in vivo* and their biological impact is yet to be discovered. These data present an important avenue of research that highlights the need to discern the biological outcome of DNA-PKcs phosphorylation on its substrates. The second most prevalent grouping are proteins with functions in DNA replication and transcriptional regulation, consistent with newly appreciated functions of DNA-PKcs in processes including the immune response, protein translation and stability, vesicle transport, metabolism, and cytoskeleton organization. The impact of DNA-PK on these substrates and associated function as related to cancer is discussed herein. As will be described, although DNA-PKcs was primarily studied as related to nuclear functions, it also serves critical nonnuclear roles associated with malignancy (**Fig. 1C**).

DNA-PKcs Nuclear Functions

DNA-PKcs studies have focused mainly on its roles in the nucleus and especially DNA repair through NHEJ. Nevertheless, DNA-PKcs is involved in DNA repair beyond NHEJ including in V(D)J recombination, homologous recombination (HR), and single-strand break repair (SSB). More recently appreciated is the role of DNA-PKcs in driving the transcriptional regulation of numerous cancer relevant pathways.

DNA damage repair

DNA-PKcs is a key player in multiple DNA damage repair (DDR) pathways, with NHEJ being the most well studied. In brief, DNA-PKcs acts as a sensor of DNA damage and phosphorylates γH2AX (76). The DNA repair factors Ku70/80 bind, encircle, and align the DNA ends and recruit DNA-PKcs at the site of damage where interaction of DNA-PKcs/Ku70/80 with the damaged DNA leads to conformational changes that activate the complex (28, 77). DNA-PKcs activation is thought to require synapses of two DNA-PK complexes, where these trans protein–protein interactions lead to DNA-PKcs autophosphorylation on the ABCDE cluster (78). Moreover, conformational changes facilitate DNA end processing, with DNA-PKcs acting as a scaffold to recruit repair machinery components and phosphorylate NHEJ components to complete repair. Upon completion of DNA repair, DNA-PKcs undergoes further autophosphorylation events (in both ABCDE and PQR), which induces a conformational change prompting dissociation of DNA-PKcs from the DNA-PK complex (79, 80). In cancer models with elevated DNA-PKcs expression and activity, NHEJ activity is elevated, thus allowing for more DNA repair and cancer cell proliferation upon intrinsic and extrinsic damage. DNA-PKcs ablation through genetic or biochemical perturbations decreases NHEJ activity, sensitizes cells to DNA damaging agents, and reduces cellular proliferation in multiple human cancer models (4). Targeting DNA-PKcs' role in DNA repair through inhibition of NHEJ activity presents an opportunity to potentiate sensitization of tumors to DNA damaging agents and reduce proliferation. Combined, it is clear that DNA-PKcs is a critical component of the NHEJ DDR pathway and a candidate therapeutic target.

Given the ability to affect DNA repair, DNA-PKcs exerts NHEJ functions to facilitate V(D)J and class switch recombination in lymphocytes (81). Defects in any of the DNA-PK components lead to the well-described severe combined immunodeficiency phenotype (SCID), marked by absence of T and B cells, increased radiosensitivity, developmental defects, and susceptibility to tumor development (82–84). The implication of these DNA-PKcs functions in the context of cancer has yet to be assessed. Although well studied in NHEJ, DNA-PKcs has been implicated in regulation of HR and SSB repair. For example, phosphorylation of DNA-PKcs in the JK cluster (T946 and S1004) and T3950 redirects repair from NHEJ to HR (30). Similarly, interaction of the transcriptional comodulator TIP60 with DNA-PKcs diminishes DNA-PKcs activity and promotes HR; conversely, mutations in TIP60 enhance DNA-PKcs phosphorylation and NHEJ, inhibit HR, and render cancer cells more sensitive to IR and PARP inhibitors (85). DNA-PKcs has also been suggested to promote HR in response to replication stress and IR-induced DSBs by phosphorylation of RPA32 (72, 73, 86, 87), and in response to replication inhibitor hydroxyurea by cooperating with PARP-1 (88). Additionally, DNA-PKcs (along with other PIKKs) is involved in cell-cycle checkpoint regulation, where it plays a role in S and G_2–M phase and DNA repair pathway decision-making. Given that mutations in checkpoint proteins promote genomic instability, these observations point to a role of DNA-PKcs in governing this process. Moreover, checkpoint proteins have been described as therapeutic targets (89), highlighting the importance of delineating the mechanisms of checkpoint protein regulation by DNA-PKcs. Thus, it is clear that DNA-PKcs plays key roles in DDR beyond NHEJ, yet more studies are needed to assess the implications of these functions in cancer development and/or progression.

In addition to DSB repair, DNA-PKcs binds to and is activated by single-strand DNA (90), supporting a potential role of SSB repair. Congruently, DNA-PKcs interacts with multiple base excision repair proteins (XRCC1, PARP-1, APE1, and Polβ; ref. 91) and is important for repair of oxidatively induced clustered lesions in tumors (92). Furthermore, these lesions can also be repaired by nucleotide excision repair and mismatch repair (MMR), suggesting DNA-PKcs may play a role in other SSB repair pathways. Understanding roles of DNA-PKcs in different DDR pathways is essential for development of combination cancer therapies to elicit synthetic lethality and improve outcomes via targeting of multiple compensatory mechanisms. Targeting DNA-PKcs in combination with inhibitors of other DDR mediators such as PARP-1 has resulted in significant anticancer effects and is a promising therapeutic avenue for cancer treatment (6, 93). Collectively, these studies highlight the critical roles of DNA-PKcs in multiple DDR pathways, making it a promising target to enhance efficacy of cancer therapy.

Transcriptional regulation

Although DNA-PKcs is well studied in the field of DNA repair, DNA-PKcs was first discovered in complex with transcription factor SP1 (94). Studies subsequently implicated DNA-PKcs in transcriptional regulation through binding to and/or phosphorylating transcriptional mediators and impacting cancer processes. For example, DNA-PKcs promotes transcription through phosphorylating the TATA binding protein and transcription factor IIB (TFIIB; ref. 95) to alter phosphorylation of TRIM28/KAP-1 (Ser824) and activate RNA polymerase II (96). Consistent with the proposed role as a comodulator, DNA-PKcs can conversely act as a transcriptional repressor via phosphorylation of the transcription initiation complex (97), and at DSBs through DNA-PKcs–dependent WWP2 K48 polyubiquitylation of RNA Pol II (98). These data reveal a complex interplay between DNA-PKcs, the basal transcriptional, and the DNA repair machinery, which is likely impactful in cancers dependent on oncogenic transcription factors.

Finally, DNA-PKcs has been shown to interact and phosphorylate a host of cancer relevant sequence specific transcription factors. Known DNA-PKcs substrates include the stemness factors Oct-1 (99), proto-oncogenes c-Fos and Jun (100), c-Myc (101), circadian clock factor CRY1 (102), and the p53 tumor suppressor (54), all involved in driving oncogenesis. DNA-PKcs has also been implicated in transcriptional regulation of lipid metabolism genes via phosphorylation of upstream stimulatory factor (USF)1/2 heterodimer (Ser 262; ref. 103), and localization to promoters of lipogenic genes. These suggest a role for DNA-PKcs in transcriptional regulation of metabolic genes that are associated with deregulated pathways in cancer. Furthermore, components of the DNA repair machinery, including DNA-PKcs, Ku70/80, PARP-1, and Topoisomerase-IIβ, have been linked to a regulation of the estrogen receptor–responsive pS2 promoter, thus suggesting a role in transcription of nuclear receptor–regulated genes (104). DNA-PKcs has also been shown to interact and/or phosphorylate and activate transcription of various nuclear receptors such as the glucocorticoid receptor (GR; refs. 105, 106), and the estrogen receptor-

α (ERα; refs. 107, 108), progesterone receptor (PR; refs. 109, 110), and the androgen receptor (AR; refs. 111, 112). In prostate cancer, DNA-PKcs binds to and activates AR, leading to transcriptional regulation of AR target genes. Furthermore, in AR-dependent prostate cancer, DNA-PKcs affects transcription through regulation of Wnt signaling via LEF-1–mediated transcription (113), transcription of EMT, metabolism, and inflammatory genes (9), and drives transcription of Rho/Rac protumorigenic networks that lead to metastasis (8). Studies also have established the presence of positive feedback loops between DNA-PKcs-AR (111) and DNA-PK-ERα (107, 114), which are significant for prostate cancer and breast cancer , respectively. Pharmacologic suppression of DNA-PKcs decreases its transcriptional regulatory function in multiple cancer-related pathways and transcription factor/nuclear receptor driven proliferation (4, 8, 9, 113), thus supporting the use of DNA-PK inhibitors as a therapeutic target in cancers driven by DNA-PKcs–sensitive oncogenic transcriptional function.

DNA-PKcs Nonnuclear Functions

Distinct from nuclear functions, DNA-PKcs localizes in the cytoplasm (115), plasma membrane (116), cytoskeleton (75), and lipid rafts (117). DNA-PKcs cellular localization is regulated by cancer-relevant pathways such as cyclic AMP (cAMP) signaling, protein kinase A (PKA; ref. 118), and the EGFR signaling (21, 22). As such, insight into DNA-PKcs extranuclear functions is essential to understand DNA-PKcs' role in malignancy.

A known nonnuclear function occurs during mitosis, wherein DNA-PKcs facilitates maintenance of genomic integrity. In the M phase, phosphorylated DNA-PKcs colocalizes with PLK1 in response to DNA damage, a process that promotes effective chromosome segregation (119). Furthermore, DNA-PKcs mediates mitotic entry via phosphorylation of RPA32 (73) and Chk2–BRCA1 (120) axis by regulating mitotic spindle organization and chromosomal integrity. DNA-PKcs is also found at telomere regions where its activity is essential for telomere protection and capping (121). Depletion of DNA-PKcs leads to delayed mitotic entry, blocked mitotic exit, and increased defects in chromosome segregation and cytokinesis (122–124). These collective observations highlight an underexplored role for DNA-PKcs in maintaining DNA fidelity.

In interphase, increasing evidence supports a nonnuclear role for DNA-PKcs in metabolic regulation. For example, DNA-PKcs phosphorylation of HSP90α reduces interaction with HSP90α clients AMPK and LKB1, promoting a reduction in mitochondrial biogenesis and physical fitness decline in aging mice (125). These effects may be context specific, as studies in glioblastoma and breast cancer models identified DNA-PKcs as a positive regulator of AMPK activity via phosphorylation (126, 127). Given the function of AMPK as a critical metabolic sensor, it will be important to delineate both direct and indirect impacts of DNA-PKcs on metabolism rewiring in cancerous tissues as metabolic reprogramming is a hallmark of cancer. Complementing these observations, DNA-PKcs was found to interact with glycolytic enzyme Aldolase A (ALDOA) in response to dietary restriction (DR) in liver and cervical cancer models (128). Although the effect of this interaction on cancer metabolism was not evaluated, it was found that ALDOA promoted DNA-PKcs–mediated p53 activation, resulting in apoptotic cell death (128). DNA-PKcs also associates with the metabolic enzyme, fumarase, in response to IR (129). A positive feedback loop was discovered in between DNA-PKcs and fumarase, where DNA-PKcs phosphorylation of fumarase promotes recruitment to damaged sites and DNA repair; and in turn, DNA-PKcs accumulates to DSBs in response to fumarate-mediated chromatin remodeling (129, 130). These observations are a call to action toward understanding of DNA-PKcs on cancer metabolism.

Distinct from roles in cell division and metabolism, DNA-PKcs is linked to regulation of senescence and cell death. *In vitro* studies have shown that DNA-PKcs can promote apoptosis via p53 phosphorylation, resulting in the destabilization of p53–Mdm2 interaction (58, 131). However, recent studies have shown that *in vivo* phosphorylation of p53 (Ser15) is mainly due to ATM in response to DNA damage (132, 133). Nevertheless, DNA-PKcs inhibition or knockdown has been shown to promote apoptosis and sensitize cells in response to heat shock (134), ultrasound (135), and anticancer agents such as etoposide and doxorubicin (6, 136). DNA-PKcs has also been shown promote autophagy in cancer through regulation of AMPK in response to etoposide (137) and IR (138). Additionally, DNA-PKcs modulates senescence in response to IR in cancer (139). Although it has been thought that sensitization to radiotherapy upon DNA-PKcs inhibition is due to DNA repair blockade, recent studies suggest that other mechanisms, such as mitotic slippage, accelerated senescence, and deregulation of ATM, also contribute to sensitization (139, 140). Given that senescence is now thought to be reversible (140–143), these findings are significant for understanding mechanisms of response and resistance to DNA-PKcs inhibitors in the clinical setting.

Although the relevance to cancer remains largely understudied, DNA-PKcs was described as a DNA sensor in the cytoplasm and an activator of innate immune response. DNA-PKcs induces transcription of type I interferon (IFN), chemokines, and cytokines via stimulation of interferon genes (STING) pathway (115). Strikingly, this response is dependent on DNA-PKcs expression but not on kinase activity (115). Conversely, active DNA-PKcs drives antiviral response through a secondary pathway in humans, called STING-independent DNA-sensing pathway (SIDSP; ref. 144), where DNA-PKcs also acts as a sensor. In addition, DNA-PKcs has been shown to activate IKK and NFκB signaling directly (145) and indirectly (146) in the presence of bacterial CpG-DNA and DNA damage to activate innate immunity. In cancer, DNA-PKcs activates immunity signaling through the NFκB signaling in response to DNA-damaging agents such as N-benzyladriamycin (147) and IR (148). Complementary to the role in innate immunity, DNA-PKcs promotes adaptive immunity via activation of a proinflammatory response in natural killer (NK) cells (149) and activation of an anti-inflammatory response in macrophages (150, 151). The role of DNA-PKcs in adaptive immunity as related to the tumor microenvironment remains poorly understood but may have therapeutic implications that should be explored.

Finally, DNA-PKcs utilizes a combination of nonnuclear and nuclear roles to influence the tumor microenvironment to promote proliferation and metastasis of cancer cells. DNA-PKcs contributes to maintenance of redox-homeostasis by suppressing reactive oxygen species buildup, an important factor to therapeutic response (152). Additionally, DNA-PKcs affects therapeutic response by promoting adaptive mechanisms in response to hypoxic environments in solid tumors. These mechanisms include upregulation of both hypoxia factor HIF-1α and DNA-PKcs expression (153, 154), as well as activation of DNA-PKcs in a HIF-1–dependent and –independent manner (153, 154). Activation of DNA-PKcs, in response to hypoxia in cancer, also promotes proliferation and resistance to apoptosis through various mechanisms including p53-RPA70 (replication protein A, 70 kDa subunit) complex regulation (155), Src and AMPK pathway activation (156), and association with macrophage-

stimulating protein receptor RON (157). Furthermore, DNA-PKcs has been associated with increased angiogenesis in glioblastoma in response to IR, thus utilizing the tumor microenvironment to promote tumor cell migration and invasion (158). In melanoma, DNA-PKcs has been shown to modulate the tumor microenvironment via secretion of proteins promoting a migratory phenotype (159). In summary, DNA-PKcs plays an important role in creating a tumor microenvironment conducive for tumor growth and spread, and these adverse outcomes could be mitigated through utilization of DNA-PKcs inhibitors.

Targeting DNA-PKcs in Malignancy

High DNA-PKcs expression and activity are linked to poor outcome in a number of tumor types (2, 3, 9). Elevated DNA-PKcs expression and/or activity have been shown in multiple studies to correlate with increased metastasis (e.g., prostate cancer, melanoma, and colorectal cancer), clinical stage (e.g., glioma and NSCLC), resistance to radio- and chemotherapy (e.g., NSCLC, glioma, prostate cervix, thyroid, nasopharynx cancers, and lymphoid malignancies), and poor overall prognosis (e.g., prostate, ovarian, nasopharyngeal, and hepatocellular carcinomas; ref. 1). Given the putative role of DNA-PKcs in tumor growth, disease progression, and clinical outcome, DNA-PKcs has been nominated as a therapeutic target across a multitude of advanced cancers. Although a number of DNA-PKcs inhibitors have been developed, a limited number have moved into clinical development due to poor solubility, rapid metabolic clearance, and/or high toxicity (160). DNA-PKcs inhibitors currently being investigated in clinical trials are summarized below and in **Table 1**.

Table 1. Nonspecific (gray) and specific (white) DNA-PKcs inhibitors undergoing clinical trials as monotherapy or in combination with therapeutic agents.

Targets	DNA-PKi	Combined Agent	Study Phase	Tumor type	Trial
DNA-PK + pleiotropic modulator	CC-122	Nivolumab Obinutuzumab Rituximab Cyclophosphamide Vincristine Prednosone CC-223 CC-292 Durvalumab Ibrutinib CC-220	Phase I Phase II	Advanced solid tumors, hematologic malignancies	NCT03834623 NCT02417285 NCT03283202 NCT02031419 NTC01421524 NCT02509039 NCT01421524
PI3K/mTOR/DNA-PK	LY3023414	Prexasertib Samotolisib Midazolam Fulvestrant Pemetrexed Cisplatin Abemaciclib Letrozole	Phase I Phase II	Advanced solid tumors, lymphomas	NCT04032080 NCT03213678 NCT01655225 NCT02057133 NCT03155620
DNA-PK and mTOR (mTORC1/2)	CC-115	Monotherapy Enzalutamide	Phase I Phase II	Prostate cancer (CRPC)	NCT02833883 NCT01353625 NTC02977780
DNA-PK	VX-984	Doxorubicin	Phase I	Advanced solid cancers	NCT02644278
DNA-PK	M3814	Mitoxantron Etoposide Cytarabine Radiation Doxorubicin Temozolomide Capecitabine	Phase I	Solid tumors and acute myeloid leukemia	NCT03983824 NCT04172532 NCT04071236 NCT04092270 NCT04750954 NCT04555577 NCT03770689 NCT04533750 NCT02516813 NCT04702698
DNA-PK	M3814	Avalumab ± radiation ± radium-223	Phase I`	Advanced solid tumors, hematologic malignancies	NCT03724890 NCT04071236 NCT04068194
DNA-PK	AZD7648	Monotherapy Doxorubicin laparib	Phase I	Advanced solid cancers	NCT03907969
DNA-PK and PARP binding	Dbait/ AsiDNA	Monotherapy Radiation Carboplatin Paxitaxel	Phase I	Advanced solid tumors, melanoma	NCT03579628

Broad specificity inhibitors targeting DNA-PKcs

Multiple nonspecific DNA-PKcs inhibitors have reached clinical trials including pleotropic modulator CC-122, PI3K/mTOR/DNA-PK inhibitor LY3023414, and mTOR/DNA-PK inhibitor CC-115. Although these inhibitors do not exclusively target DNA-PKcs, each suppresses kinases of cancer relevance. Importantly, phase I studies showed that oral CC-122 and CC-115 are well tolerated with no unexpected toxicities and adverse effects (161, 162). Use of CC-122 in advanced malignancies including brain cancer showed encouraging results wherein five of six patients with brain tumors did not show progression while on treatment with the drug (>6 months; ref. 161). Similarly, preliminary data from the combination of CC-115 with enzalutamide (NCT02833883; ref. 9) are under investigation in men with castration-resistant prostate cancer (CRPC), and reported data that all patients had at least a 50% PSA decline, with 60% of patients achieving a ≥90% PSA decline (163). CC-115 is also being interrogated in a phase II trial for innovative glioblastoma therapy (NTC02977780). These promising studies provide early indication that targeting DNA-PKcs may be effective in eliciting antitumor effect.

Specific DNA-PKcs inhibitors

Although specific inhibitors such as NU7441 have not been proven clinically actionable, newly developed DNA-PKcs inhibitors with increased specificity (e.g., VX-984, M3814, AZD7648, and AsiDNA) have emerged and have entered clinical testing. VX-984 sensitizes glioblastoma cells to IR *in vivo*, and data suggest it may cross the blood–brain barrier, which may prove useful in treatment of brain cancers (164). VX-984 is currently being studied in a phase I trial in combination with pegylated liposomal doxorubicin (PLD; NCT02644278). Similarly, M3814 has been shown to sensitize cells to IR and other DNA-damaging agents (165) and is currently being tested in combination with multiple therapeutic agents including etoposide, radiation, and doxorubicin. Furthermore, recent studies have shown that targeting DNA-PKcs using NU7441 and M3814 enhanced antitumorigenic effects of immunotherapy interventions in preclinical studies (166) and the combination of M3814 with anti–PD-L1 antibody is being tested in clinical trials (NCT03724890). Another specific inhibitor, AZD7648 was shown to sensitize cells to IR, doxorubicin, and PARP-1 inhibitor olaparib (6) and is currently being evaluated as a monotherapy and in combination with PLDs and olaparib in patients with advanced cancers (NCT03907969). Moreover, combination of IR and pharmacologic derivative of Dbait (AsiDNA), a molecule that mimics DNA DSBs and is designed to bind DNA-PK and PARP-1 (167, 168), is being studied in clinical trials. AsiDNA is currently being investigated as a monotherapy and in combination with carboplatin and paxitaxel in advanced solid tumors (NCT03579628) and will soon enter the ROVOCAN trial to evaluate AsiDNA in ovarian cancer patients with acquired resistance to the PARP inhibitor niraparib. Together, these data show promise for use of DNA-PKcs–targeted therapy to treat advanced malignancies.

Summary and Future Considerations

As high DNA-PKcs expression/activity is associated with tumor phenotypes and poor prognosis, it is imperative to deepen the understanding of DNA-PKcs modes of activation and regulation driving its protumorigenic functions, to explore the role of DNA-PKcs beyond DNA repair, uncover novel DNA-PKcs substrates, and refine DNA-PKcs targeting to achieve maximum anticancer effects while minimizing toxicity. The findings reviewed herein highlight the importance of DNA-PKcs function in tumor biology and raise important questions that will shed light into mechanisms of DNA-PKcs–mediated tumor behavior and assist in development of improved clinically actionable DNA-PKcs–targeting therapeutics for human malignancies.

First, what is the relative contribution of established DNA-PK functions to cancer development and progression, including but not limited to DNA repair activity? For example, given the link between DNA-PKcs and metabolic regulation, it will be critical to investigate the role of this process in DNA-PKcs–associated poor outcomes. Relatedly, what is the mechanism(s) by which DNA-PKcs inhibitors exert antitumor phenotypes? Although targeting DNA-PKcs sensitizes cancer cells to radiation and chemotherapy, new mechanisms have been suggested to regulate these processes distinct from NHEJ (140, 169–172). Thus, much remains to be uncovered, and unveiling new functions of DNA-PKcs may help identify novel mechanisms of action that lead to protumorigenic effects, and conversely, antitumor effects of DNA-PKcs inhibitors.

Third, how does DNA-PKcs function evolve with disease progression? Determining how DNA-PKcs function and substrate specificity may change with disease progression will be a critical avenue of investigation to determine the disease stage where DNA-PKcs inhibitors may be most effective. In addition, discerning which DNA-PK substrates support its protumorigenic functions of the protein kinase will be equally impactful. Fourth, as DNA-PKcs inhibitors are being evaluated in clinical trials, is there a subset of patients who may benefit the most from DNA-PKcs inhibition? Considering the plethora of roles DNA-PKcs plays in cancer, data suggest that treatment of patients with high DNA-PKcs–expressing tumors with DNA-PKcs inhibitors would produce favorable anticancer effects. In addition, tumors with high genome instability or defects in DNA repair pathways may also benefit from use of DNA-PKcs inhibitors by leveraging the concept of synthetic lethality (6, 173).

In conclusion, continuing to discern the molecular mechanisms underlying DNA-PKcs–dependent tumor-associated phenotypes is warranted and has high potential to further enhance the impact of targeting DNA-PKcs in advance cancers.

Authors' Disclosures

K.E. Knudsen reports, although unrelated to this work, having served as consultant in or advisory roles in the past three years for CellCentric, Genentech, Celgene, Sanofi, and Janssen. No disclosures were reported by the other authors.

Acknowledgments

The authors gratefully thank all the members of the Knudsen laboratory for their intellectual support. This work was supported by NIH/NCI grants to KEK (5R01CA17640105 and 5R01CA18256905).

Received June 1, 2021; revised August 17, 2021; accepted November 10, 2021; published first December 10, 2021.

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