Role of DNA-dependent protein kinase catalytic subunit in cancer development and treatment

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Abstract

DNA-dependent protein kinase catalytic subunit (DNA-PKcs), a key component of the non-homologous end-joining (NHEJ) pathway, is involved in DNA double-strand break repair, immunocompetence, genomic integrity, and epidermal growth factor receptor signaling. Clinical studies indicate that expression and activity of DNA-PKcs is correlated with cancer progression and response to treatment. Various anti-DNA-PKcs strategies have been developed and tested in preclinical studies to exploit the benefit of DNA-PKcs inhibition in sensitization of radiotherapy and in combined modality therapy with other antitumor agents. In this article, we review the association between DNA-PKcs and cancer development and discuss current approaches and mechanisms for inhibition of DNA-PKcs. The future challenges are to understand how DNA-PKcs activity is correlated with cancer susceptibility and to identify those patients who would most benefit from DNA-PKcs inhibition.

Keywords

DNA damage; carcinogenesis; anti-DNA-PKcs strategies

Introduction

The catalytic subunit of DNA-dependent protein kinase (DNA-PKcs) is the key component of the non-homologous end-joining (NHEJ) pathway for DNA double-strand break (DSB) repair and is required for cellular resistance to ionizing radiation (IR) (1,2). DNA-PKcs and the NHEJ pathway are also essential for V(D)J recombination during T and B cell lymphocyte development. Thus, deficiency in DNA-PKcs activity results in a severe combined immunodeficiency (SCID) phenotype in mammals (3-5). In response to DSB formation, DNA-PKcs is recruited to DSBs by the DNA end-binding Ku70/80 heterodimer and is rapidly phosphorylated at multiple serine and threonine residues (1,2). *In vivo*, DNA-PKcs phosphorylation has been examined at the Thr2609 cluster region and Ser2056. DNA-PKcs phosphorylation at the Thr2609 cluster region is particularly significant since it is

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critical for DSB repair and radioresistance (6-9). This phosphorylation is regulated by the ataxia telangiectasia mutated (ATM) and the Rad3-related (ATR) kinases in response to various genotoxic stresses, making it a critical regulatory element of DNA-PKcs (6,10). DNA-PKcs phosphorylation at Ser2056, on the other hand, is primarily mediated by autophosphorylation, making it an excellent marker to monitor DNA-PKcs activation *in vivo* (11).

In addition to its critical function in DSB repair, DNA-PKcs activity has been implicated in cell cycle control; it appears to be involved in mitosis, microtubule dynamics, and proper chromosomal segregation (12). DNA-PKcs activation, as monitored by DNA-PKcs phosphorylation, is physically and functionally associated with mitotic spindle formation. Inhibition of DNA-PKcs activity via a small interfering RNA or a kinase inhibitor results in mitosis delay, abnormal spindle formation, and chromosome misalignment. Furthermore, DNA-PKcs is required to prevent mitotic catastrophe in response to DNA damage (13). These studies unveiled a novel function of DNA-PKcs in safeguarding the genome integrity and cancer suppression as chromosomal instability (CIN) plays an important role in cancer development and is a hallmark of cancer cells (14).

Carcinogenesis is a multi-step process wherein abrogation of multiple cancer susceptibility genes leads to cancer development. Genes that suppress carcinogenesis have been classified as gatekeepers that regulate cellular proliferation and cell death and as caretakers that are primarily encode DNA repair proteins required for the maintenance of genome integrity (15). A direct link between DNA DSBs, genomic instability, and cancer is evidenced the fact that many cancer-predisposition syndromes in humans characterized by genomic instability are caused by mutations in DSB-responsive genes (16,17). In this review, we will discuss our current understanding of the role DNA-PKcs in cancer development and the implications of anti-DNA-PKcs strategies in advanced cancer therapeutics.

The role of DNA-PKcs activity in cancer development

The expression of DNA-PKcs in clinical tumor samples has been investigated to elucidate the impact of DNA-PKcs activity on cancer development. Results generated from such studies not only provide crucial information to predict radio- or chemo-sensitivity of tumor and normal tissues but also help to optimize the treatment plan for each cancer patient. Elevation of DNA-PKcs protein levels has been found in various tumor types by several research groups (Table 1). Significant increases of DNA-PKcs expression levels (protein and mRNA) and kinase activity have been found in colorectal cancers, and these increases are correlated to elevated Sp1 protein levels and poor survival (18,19). High DNA-PKcs protein levels were found in esophageal tumor tissues with an intratumoral heterogeneity pattern compared to the adjacent normal mucosa tissues, where DNA-PKcs expression can be detected in the basal cell layers but not the luminal cells (20). Investigation of nasopharyngeal carcinoma revealed that DNA-PKcs overexpression was found in 36.8% of tumor specimens and had remarkable correlations to advanced clinical stages and poor survival (21). A separate study by Lee *et al.*, however, reported no association between DNA-PKcs overexpression and the clinical outcome of nasopharyngeal carcinoma (22). The difference might be due to the smaller patient volume of the Lee *et al.* study.

Elevated mRNA and protein levels of DNA-PKcs as well as several related DSB repair molecules in non small cell lung carcinomas (NSCLCs) have been reported by independent laboratories (23,24). Furthermore, expression profile analysis revealed that patients with higher T/N ratios (tumor samples verse normal tissues) of either DNA-PKcs or ATM have poorer prognosis and increased risk of death; hence, more aggressive therapy is needed (24). This is in agreement with the report by Yu *et al.* that the high levels of expression of DNA-

PKcs protein is associated with tumor grading and expression of p53 and BCL-2, and it might be one of the important causes of radioresistance in NSCLCs (25). There are also reports suggesting no association between DNA-PKcs expression and clinical characteristics or outcome in patients with NSCLC (23,26). Finally, levels of DNA-PKcs activity in glioma specimens coincided with the advanced tumor grading (27). In glioma, DNA-PKcs activity is associated with radioresistance and the epidermal growth factor receptor (EGFR) III variant (VIII) (28), which is among the most mutated genes in glioblastoma multiforme (29,30).

Contrary to the elevated DNA-PKcs activity found in many tumor tissues, the loss of DNA-PKcs expression has been linked to the development of gastric tumors (Table 1). Lee *et al.* reported that 23% of gastric tumors are negative of DNA-PKcs expression. Lack of DNA-PKcs expression coincides with significant lymphatic invasion, lymph node metastasis, and poor patient survival (31). Further analysis revealed that the loss of DNA-PKcs in advanced gastric tumors is also correlated with intratumoral neutrophils, microsatellite instability, and frame-shift mutations of $(A)_{10}$ repeats in the *DNA-PKcs* gene (associated with a higher risk of lymph node metastasis) (32). It is interesting to note that DNA-PKcs is not expressed in epithelium of normal gastric mucosa but is observed in most *Helicobacter pylori-associated* gastritis, intestinal metaplasia, and gastric adenoma tissues (32). It is possible that the induction of DNA-PKcs is due to hyperproliferation or inflammatory responses triggered by *H. pylori*. Further studies are needed to clarify the role of DNA-PKcs in *H. pylori*-induced gastric carcinogenesis (33). Attenuated expression of DNA-PKcs has also been found in ovarian cancer tissue, whereas DNA-PKcs is expressed in 100% of the normal ovaries, in 95% of benign ovary tumors, in 90% in of borderline ovarian neoplasms, and in 60% of malignant serous ovarian neoplasms (34).

Expression of DNA-PKcs and kinase activity in peripheral blood lymphocytes (PBLs) has been examined to correlate individual DNA-PKcs activity to cancer development (Table 1). Studies of PBL samples from cancer patients demonstrated that there is an inverse correlation of DNA-PKcs activity with cancer risk and disease prognosis (35-37). Someya *et al.* reported that DNA-PK activity is generally attenuated in cancer patients compared to healthy volunteers with statistical significance in breast and cervix cancers (36). Decreased DNA-PKcs activity in PBLs is also found in advanced cancer and is associated with aggressive cancer phenotypes (37). Analyses of advanced cancer patients who have been treated with radiotherapy unveiled a strong correlation between DNA-PKcs activity and the outcome of radiotherapy: Lower DNA-PKcs activity in PBLs was correlated with poorer prognosis including decreased disease-free survival and higher frequency of distant metastasis (37). Additionally, decreased DNA-PKcs activity has also been correlated to chromosomal instability and an increase in chromosomal aberrations (breaks and gaps) in PBLs (36,38). Auckley *et al.* reported that reduced DNA-PKcs activity in peripheral mononuclear cells (a subset of cells that include PBLs) coincides with lung cancer development (35). Although the authors measured comparable DNA-PKcs activities in PBLs and bronchial epithelial cells (one progenitor cell for lung cancer) of the same individuals, it is unclear whether the coincidence with lung cancer development is due to the reduced DNA-PKcs activity in bronchial epithelial cells or in the immune cells. Nonetheless, these results demonstrate that DNA-PKcs activity in PBLs can be an indicator of aggressiveness of cancer phenotype and patient prognosis.

Comparisons have also been made of expression levels of DNA-PKcs in tumor tissues prior to therapeutic intervention and in the residual tumors after radio- or chemotherapies. High expression of DNA-PKcs was reported to be a predictor for better response to radiation therapy in esophageal cancer and early breast cancer but not in nasopharyngeal cancer (22,39,40). Glioma patients with higher DNA-PKcs activity measured from tumor

specimens failed to response to cisplatin-based chemotherapy (27). Conversely, DNA-PKcs and Ku proteins were found to be upregulated after radiation treatment with increased frequency of positive cells in the residual tumors (41,42). The induction of DNA-PKcs and Ku by radiation treatment could be recapitulated in cultured tumor cells suggesting that upregulation of DNA-PKcs and Ku is due to a selective survival advantage against radiation and is associated with radioresistance in the recurrent tumors (41). Thus small molecule inhibitors of DNA-PKcs should be able to sensitize tumors to radiotherapy and facilitate eradication of the radioresistant tumors.

DNA-PKcs activity in lymphoid malignancies

Measurement of DNA-PK activity in PBLs revealed that DNA-PK activity is generally attenuated in cancer patients. The one exception is lymphoma patients who display slightly higher DNA-PKcs activity than the healthy control group (36). Since DNA-PKcs and its downstream NHEJ-dependent DSB repair is essential for V(D)J recombination and lymphocyte development (43,44), it is reasonable to speculate that the increase of DNA-PKcs activity is associated with lymphoid malignancies. Holgersson *et al.* examined the expression levels of DNA-PK components in different lymphoid malignancies and found that elevated DNA-PKcs and Ku80 protein levels coincide with maturation and high-grade lymphoid malignancies. An increased frequency of DNA-PKcs positive cells was also found in lymph node samples of higher-grade lymphoma patients suggesting that DNA-PKcs is associated with proliferation rate (45). Similar observations were made in patients with B cell chronic lymphocytic leukemia (CLL). Higher levels of DNA-PKcs were found in patients with aggressive types of B cell CLLs (deletions at 17p or 11q) and were correlated to short survival and chemoresistance (46-48). Treatment with DNA-PKcs kinase inhibitor NU7441 sensitizes CLL cells to fludarabine, chlorambucil, and mitoxantrone (47,48). Additionally, increased DNA-PKcs activity was linked to cancer cell survival in acute myeloid leukemia (AML) patients and chemoresistance against various antitumor agents including topoisomerase II (Topo-II) inhibitors (doxorubicin and etoposide) and anti-microtubule vincristine (49). Taken together, these studies demonstrate that DNA-PKcs is an indicator of poor patient prognosis in lymphoid malignancies and may contribute to disease progression. In addition, DNA-PKcs is involved in repair of DNA lesions other than DSBs.

Mutations in the *DNA-PKcs* gene and cancer development

In addition to the changes in DNA-PKcs activity or expression level, mutations and polymorphisms in *DNA-PKcs* gene (also referred to as *Prkdc* or *XRCC7*) have been examined to explore the possible connection to cancer susceptibility. Several somatic mutations in the coding region of *DNA-PKcs* have been identified in patients with breast and pancreatic cancers; one missense mutation (c.7825A>C) occurred at the critical Thr2609 phosphorylation cluster of DNA-PKcs (50). DNA-PKcs phosphorylation at the Thr2609 cluster region plays a key role in regulating DNA-PKcs activity and is essential for NHEJ-mediated DSB repair (6-9,51). It is conceivable that the resulting Thr2609Pro mutant DNA-PKcs will interfere with DSB repair, accelerate the accumulation of mutations, and promote genome instability. This notion is further supported by our recent findings that ablation of mouse Thr2605 (human Thr2609) phosphorylation cluster led to multiple tissue stem cells defects and increase of chromosomal aberrations (52). Recently, the first germline mutation of human DNA-PKcs Leu3062Arg was identified from a radiosensitive T-B-SCID patient who also had attenuated Artemis activation and an impaired NHEJ pathway (53). It remains to be determined whether the Leu3062Arg mutation will lead to a tumorigenic phenotype in the patient.

Single nucleotide polymorphism (SNP) analysis revealed that the 6721G to T (rs7003908) variant located in intron 8 of the *DNA-PKcs* gene is associated with bladder cancer and hepatocellular carcinoma (HCC). Wang *et al.* reported a dose-dependent and significant increase of bladder cancer risk associated with the 6721T alleles; the risk was profoundly increased in subgroup of 6721TT carriers older than 65 who had smoked (OR=3.24, 95% CI=1.35-7.78) compared with those carrying the 6721 (GG + GT) genotypes (54). Contrary to these findings, Long *et al.* reported that individuals carrying the homozygote 6721G alleles (GG or GT) have a higher risk of HCC (OR=5.04, 95% CI=3.28-7.76 and OR=3.45, 95% CI=2.40-4.94, respectively) than the 6721TT carriers (55). Additionally, two SNPs in non-coding regions of the DNA-PKcs gene, rs12334811 (G to A, intron 21) and rs8178085 (A to C, intron 29), were associated with decreased risk of lung cancer in a dose-dependent manner (OR=0.53, 95% CI= 0.35-0.80) and significantly in nonsmokers (56). Although the mechanism remains to be determined, these SNPs located in non-coding regions of the DNA-PKcs gene may alter the production levels and/or expression patterns of DNA-PKcs.

Effect of DNA-PKcs inhibitors on cancer cell radiosensitization *in vitro*

DNA-PKcs and the underlying NHEJ pathway are essential for DSB repair and radioresistance (1,2). As discussed above, elevation of DNA-PKcs activity is associated with tumor radioresistance in some advanced stage tumors. Different anti-DNA-PKcs strategies have been developed to enhance radiotherapy-based control of local tumors or for use in combined modality treatment with other antitumor agents. A number of small-molecule ATP-competitive inhibitors that target DNA-PKcs have been developed including vanillin, SU11752, IC87361, NU7026, and NU7441 (57-61). Among these, NU7441 is the most potent and selective molecule with a half maximal inhibitory concentration (IC_{50}) of 14 nM against DNA-PKcs in a cell free system and no significant activity against the closely related ATM and ATR kinases (60). All these DNA-PKcs kinase inhibitors have strong radiosensitization effects without causing significant cellular toxicity in various human and murine tissue culture models (57,58,61-65). The presence of a DNA-PKcs inhibitor within the first two hours after radiation exposure, when the majority of DSBs are being repaired, effectively blocks the re-ligation of chromosomal DSBs in a dose-dependent manner; the radiation enhancement ratio of lethal dose to 90% of cells (LD_{90}) ranges from 1.5 to 4.2 in various human cancer cell lines (62). Prolonged incubation with a DNA-PKcs inhibitor further enhanced radiosensitization effects proportionally to exposure time up to 24 hours (63).

DNA-PKcs inhibitors promote IR-induced cell killing primarily through the inhibition of DSB repair and the induction of apoptotic programmed cell death (61,66). Additional mechanisms including acceleration of senescence, induction of mitotic catastrophe, and autophagy have also been implicated in radiosensitization associated with DNA-PKcs kinase inhibition (13,67,68). Azad *et al.* reported that inhibition of DNA-PKcs induces G2/M checkpoint arrest and an accelerated senescence phenotype in irradiated human NSCLC cells (67). The response of senescence-associated β-galactosidase activity coincides with the induction of p53/p21 signaling pathway and is observed in both tissue culture and tumor xenografts after IR. Recent evidence demonstrates that DNA-PKcs plays a critical role in mitotic spindle formation, proper chromosome segregation, and prevention of mitotic catastrophe in response to DNA damage (12,13). Shang *et al.* further suggested that DNA-PKcs activity is required for checkpoint kinase 2 (Chk2) activation during the G2/M transition as inactivation of DNA-PKcs results in a prolonged G2/M cell cycle arrest, polyploidy outcome, and higher incidence of multipolar spindles after IR (13). The involvement of DNA-PKcs in autophagy regulation was reported by Zhuang *et al.* that knockdown of DNA-PKcs radiosensitizes glioma-initiating cells due to IR-induced autophagy responses; expressions of autophagy markers microtubule associated protein light

chain 3 (LC3) and Beclin1 are induced as well as the punctate staining patterns of LC3 (autophagosomes) are observed (68). DNA-PKcs-dependent autophagy responses can be blocked by autophagy inhibitor 3-methyladenine, suggesting a role of DNA-PKcs in antagonizing IR-induced autophagy activation. Further analyses are needed to clarify whether the involvement of DNA-PKcs in autophagy responses is a general phenomenon or unique to IR and whether DNA-PKcs kinase activity is required for IR-induced autophagy responses.

Effect of DNA-PKcs inhibition on tumor growth delay *in vivo*

The effect of DNA-PKcs inhibitors on human cancer cell proliferation has been examined *in vivo* using xenograft tumor models in mice. There is significant tumor growth delay when mice are treated with combined DNA-PKcs inhibition and IR or Topo-II poisoning (Table 2), and the delay in tumor growth translates into survival benefits (61,62,64,69,70). It is notable that DNA-PKcs inhibition alone does not cause significant antitumor effects and does not cause cytotoxicity. In contrast, the combination of DNA-PKcs inhibition and IR reduces levels of cell proliferation marker Ki67 and increases the signal intensity of the cleaved caspase-3, TUNEL, γH2AX (Ser139), and β-galactosidase in irradiated tumor samples (67,69). In addition to tumor cells, DNA-PKcs inhibition *in vivo* likely also impacts endothelial cells as greater regression of irradiated tumor vasculature was found in DNA-PKcs-deficient SCID mice and in C57BL6 mice pretreated with DNA-PK inhibitor than in control mice (61). Results generated from these mouse xenograft tumor models further demonstrate the benefit of DNA-PKcs inhibition in sensitizing cells to IR or Topo-II poisons. Thus, anti-DNA-PKcs strategies deserve further investigation in clinical trials.

Additional anti-DNA-PKcs strategies

Additional anti-DNA-PKcs strategies have been developed including single chain antibody variable fragments (scFV) and inhibitory RNA molecules. Anti-DNA-PKcs ScFv 18-2 was derived from an existing anti-DNA-PKcs monoclonal antibody and is able to completely inhibit DNA-PKcs-dependent DNA end joining *in vitro* (71), whereas Anti-DPK3-scFv was selected from a humanized semi-synthetic scFV library (69). These anti-DNA-PKcs scFVs antagonize DSB repair *in vivo* and sensitize cells to radiation-induced killing in tissue culture models (69,72,73). Similarly, selective inactivation of DNA-PKcs with inhibitory RNA molecules or antisense oligodeoxynucleotides blocks end-rejoining and sensitizes human cancer cell lines to radiation treatment (70,74,75). Furthermore, studies using mouse xenograft tumor models revealed that both anti-DNA-PKcs scFV (DPK3-scFv) and inhibitory RNAs were able to radiosensitize and to improve local control of tumor growth, thus confirming the antitumor potential of these anti-DNA-PKcs strategies (69,70).

The connection of DNA-PKcs to cancer development has been linked to its association with EGFR, which is frequently overexpressed in human epithelial tumors and is associated with therapy resistance. Wild-type EGFR physically interacts with DNA-PKcs and may modulate the level and activity of DNA-PKcs (76). The interaction of EGFR and DNA-PKcs is also required for IR-induced nuclear AKT phosphorylation and cell survival (77). In NSCLC cell lines expressing EGFR mutated in the tyrosine kinase domain, L858R or ΔE746-E750, there is impaired EGFR nuclear translocation, no interaction between EGFR and DNA-PKcs, and decreased DNA repair capacity after IR or cisplatin treatment (78,79). Stable exogenous expression of mutant EGFR in human bronchial epithelial cells also abrogates radiation-induced EGFR nuclear translocation and DNA-PKcs binding (78). Blockage of EGFR signaling with a monoclonal antibody or a tyrosine kinase inhibitor could further sensitize cells to radio- or chemotherapies by inhibiting DNA-PKcs activation and decreasing DNA repair capacity (80,81).

Implications of anti-DNA-PKcs strategies in synthetic lethality

Defect in DNA damage repair are often prerequisites to mutation accumulation, genomic instability, and cancer development; as a consequence, cancer cells become addicted to the remains of DNA repair mechanisms to thrive. The synthetic lethality approach exploits this characteristic of cancer cells and selectively targets the remaining DNA repair pathways to kill cancer cells without causing significant cytotoxicity to the normal tissues (82,83). The best example is in breast cancers with mutations in BRCA1 or 2 genes that highly sensitive to inhibitors of poly (ADP-ribose) polymerase 1 (PARP-1) (84). Synthetic lethality is also observed in combined modality therapy of DNA-PKcs inhibitors with other antitumor agents. For example, DNA-PKcs inhibitor NU7026 is able to radiosensitize PARP-1 deficient cells, whereas PARP-1 inhibitor AG14361 radiosensitizes DNA-PKcs-deficient but not PARP-1-deficient cells. NU7026 and AG14361 synergized and reduced levels of recovery of wild-type cells after IR compared to treatment with either agent alone (85,86).

DNA-PKcs inhibitors are also capable of potentiating the cytotoxicity of radiation-mimicking Topo-II poisons including etoposide (VP-16), doxorubicin, and mitoxantrone in human cancer cell lines. This combined modality approach is particularly attractive in treating hematologic (blood) cancers and is effective against most if not all of hematologic cancers including lymphocytic CLL, acute lymphoblastic leukemia, myelocytic chronic myelogenous leukemia and AML, acute promyelocytic leukemia, and adult T-cell leukemia-lymphoma (48,49,87-89). The same strategy is also effective in controlling solid tumors including those of the colon, breast, and prostate (64,65,90). These studies demonstrate that DNA-PKcs and the NHEJ pathway play a crucial role in removing Topo-II poison-induced DSBs that would otherwise lead to caspase activation and apoptosis (91,92).

Increased expression of DNA-PKcs has been linked to cisplatin-resistance in ovarian cancer cells and in glioma patients, suggesting that DNA-PKcs and/or the NHEJ pathway plays a role in DNA interstrand crosslink (ICL) repair (27,49). Consistent with this notion, combined treatment with a DNA-PKcs inhibitor and cisplatin or platinum-based drugs synergize in killing ovarian, colon, and breast cancer cells (57,90,93). In addition to the possible role of DNA-PKcs in ICL lesion repair, DNA-PKcs-mediated cisplatin resistance may occur through AKT activation, EGFR nuclear translocation, or activation/mobilization of chromatin remodeling factor structure-specific recognition protein 1 (SSRP1) from nucleolus (79,94,95). Contrary to the cisplatin resistance model, Jensen and Glazer reported that cells deficient in DNA-PKcs or Ku80 are resistance to cisplatin-induced cell killing through a gap junction-mediated cell death signaling between the neighboring cells (96). This is supported by the observation that decreases in gap junction activity via specific inhibitors or by lowering cell density attenuates cisplatin-induced cell death in DNA-PK-positive cells.

Taken together, these synthetic lethality analyses demonstrate that DNA-PKcs is involved in a variety of DNA damage repair responses in addition to its well-defined role in the NHEJ pathway. We expect that the ongoing and future studies in synthetic lethality screening will unveil other functions of the versatile DNA-PKcs. Additional examples of DNA-PKcs activity and its engagement include resistance to anti-microtubule agent vincristine, DNA intercalator cryptolepine, DNA methylation inhibitor zebularine (49,97,98).

Conclusions and future directions

Current evidence suggests that DNA-PKcs has multiple functions in carcinogenesis and the precise role of DNA-PKcs in cancer promotion or prevention remains to be clarified and may depend on circumstances. The caretaker role of DNA-PKcs in normal tissues promotes DSB repair and chromosomal stability thus preserving and safeguarding the integrity of the

genome. Diminished activity of DNA-PKcs thus facilitates accumulation of mutations and genome instability, which has been recognized as a key factor in carcinogenic transformation (99). Elevation of DNA-PKcs expression or kinase activity in the cancer cells reflects the need for greater DNA repair capacity and is likely beneficial for tumor cells given their chronically unstable genomes. Thus, increases in DNA-PKcs activity will enhance cancer cell resistance to DNA damaging and antitumor radio- or chemotherapies and result in the poor patient survival (35,46,100,101). Emerging evidence has also indicated that cancer initiating or cancer stem cells are resistant to radiotherapy and other genotoxic assaults (102,103). Future investigations are needed to determine whether DNA-PKcs plays a role in sustaining cancer stem cell renewal.

The coincidence of diminished DNA-PKcs activity in PBLs and tumor progression suggests that DNA-PKcs activity in PBLs could modulate tumor immunity and initial development of abnormal tumor cells. DNA-PKcs and its downstream NHEJ pathway are essential for V(D)J recombination and the genesis of mature T and B cell lymphocytes (4,5,104). DNA-PKcs has also been implicated in mediating CpG-DNA-dependent macrophage activation and production of IL-10 (105,106). Recent evidence further suggests that DNA-PKcs could play a critical role in the maintenance of hematopoietic stem cells (HSC) and hematogenesis (52) and that DNA-PKcs is required for natural killer (NK) cell activation and the release of pro-inflammatory cytokines (107). Taking into the account that tumor immunity plays a key role in cancer development and progression (108,109), it is possible that the reduction of DNA-PKcs activity in PBLs reflects an overall decrease in host immunity that is essential for suppression of tumor growth or tumor clearance upon radio- or chemotherapeutic interventions. For example, studies using mouse models demonstrate that ablation of NK cells leads to increases in spontaneous tumor development and the frequency of tumor metastasis (108). Future investigations are needed to clarify the function of DNA-PKcs in regulation of macrophage and NK cell activities and tumor immunity.

Acknowledgments

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Table 1

The association between DNA-PKcs activity and cancer development from clinical investigations

Tumor type	Assay	Specimen	Sample size	DNA-PKcs activity	Interpretation	Reference
Prognosis						
Nasopharyngeal cancer	IHC	tumor	66	↑ in 70% of tumor tissue	no association with locoregional control or survival	Lee 2005
Nasopharyngeal cancer	IHC	tumor	223	↑ in 37% of tumor tissue	oeverexpression associates with advanced stage and poor survival	Yan 2008
Esophageal cancer	IHC, IB, kinase activity	tumor:normal	13 paired	↑ in tumor tissue	NA	Tonotsuka 2006
Gastric cancer	IHC	tumor	279	↑ in 73% of tumor tissue	loss of expression associates with lymphatic invasion, lymph node metastasis, advanced pathological stage and poor survival	Lee 2005
Gastric cancer	IHC	tumor:normal	791	↑ in 80% of tumor tissue	loss of expression associates with intratumoral neutrophils, microsatellite instability, mutation in DNA-PKcs and poor survival	Lee 2007
Colorectal cancer	RT-PCR, IB, kinase activity	tumor:normal	12 paired	↑ in tumor tissue	NA	Hosoi 2004
Colorectal cancer	IHC, IB	tumor:normal	359 (35 paired)	↑ in 64% of tumor tissue	oeverexpression associates with clinical stage, lymphatic invasion, distant metastasis and poor survival	Lu 2008
Non-small cell lung cancer	IHC	tumor	113	↑ in 89% of tumor tissue	oeverexpression associates with tumor grade	Yu 2003
Non-small cell lung cancer	IHC	tumor	86	↑ in 87% of tumor tissue	no association with clinical characteristics or outcome	Pan 2007
Non-small cell lung cancer	RT-PCR	tumor:normal	140 paired	↑ in tumor tissue	oeverexpression associates with poor survival	Xing 2008
Non-small cell lung cancer	IHC	tumor:normal	116 (12 paired)	↑ in 75% of tumor tissue	no association with clinical characteristics or outcome	Hao 2008
Glioma	kinase activity	tumor	36	↑ in tumor tissue	hyperactivity correlates with tumor grading	Shao 2008
Ovarian cancer	IHC	tumor:nomral	100	↓ in 40% of tumor tissue	loss of expression associates with tumor progression, advanced clinical stage, and lymph node metastasis	Shao 2007
ALL, CLL, lymphoma, multiple myeloma	IHC, IB	lymhpod tissue	86	↑ during lymphoid development and in lymphoid malignancies	overexpression associates higher lymphoma grading and degree of maturation in lymhpod malignancies other than multiple myeloma	Holgersson 2004

Tumor type	Assay	Specimen	Sample size	DNA-PKcs activity	Interpretation	Reference
B-cell CLL	IB, kinase activity	leukemia cells	54	↑ in del(17p) and del(11q)	overexpression associates with shorter treatment free interval	Willmore 2008
B-cell CLL	RT-PCR	leukemia cells	50	↑ in del(17p)	overexpression associates with poor survival	Elliott 2012
Cancer of breast, cervix, head & neck, esophagus and lymphoma	kinase activity	PBLs	167	↓ in advanced stage	hypoactivity associates advanced stage and distant metastsis	Someya 2011
Radiation Response						
Esophageal cancer	IHC	tumor	67	↑ in 54% of tumor tissue	overexpression predicts better response to chemoradiation	Noguchi 2002
Oral squamous cell carcinoma	IHC	tumor	42	↑ in residual tumor after RT	not preditive to radiation response	Shintani 2003
Cervical cancer	IHC	tumor	22	↑ in residual tumor after RT	no assication with clinical characteristics	Bestow 2009
Breast cancer	IHC	tumor	224	↑ in 43% of tumor tissue	overexpression predicts better locoregional control of radiation alone versus chemotherapy alone in early stage	Soderlunk Leifler 2010
Cancer Risk						
Lung cancer	Kinase activity	PBLs	cancer 41/ healthy 41	↓ in cancer patients	hypoactivity associates with cancer of lung	Auckley 2001
Breast, cervix, head & neck, esophagus and lymphoma	Kinase activity	PBLs	cancer 93/ healthy 41	↓ in cancer patients	hypoactivity associates chromosomal instability and cancer of breast and cervix	Someya 2006

Abbreviations: ALL, acute lymphocytic leukemia; CLL, chronic lymphocytic leukemia; IB, immunoblot; IHC, immunohistochemistry; PBLs, peripheral blood lymphocytes; RT-PCR, reverse transcription polymerase chain reaction; ↑ indicates increase in activity; ↓ indicates decrease in activity

Table 2

Radiosensitivation of DNA-PKcs inhibitors in mouse xenograft models

Tumor Type	Animal	Primary Treatment	DNA-PK Inhibition	Results	Reference
B16F0 Murine melanoma	C57BL6 mice	RT 3 Gy Days 0-4, 7 & 8 to total 21 Gy	IC87360 75 μg ip 1h before RT	Significant tumor growth delay	Shinohara 2005
Murine Lewis lung carcinoma	C57BL6 mice	RT 3 Gy Days 0-4, 7 & 8 to total 21 Gy	IC87360 75 μg ip 1h before RT	Significant tumor growth delay	Shinohara 2005
LNCaP Human prostate cancer	NU/NU nude mice	RT 6 Gy on Day 0	Aptamer-DNAPK-shRNA chimera 200 pM intratumoral on Days -3 & -2	Significant tumor growth delay	Ni 2011
PC3 Human prostate cancer	NU/NU nude mice	RT 6 Gy on Day 0	Aptamer-DNAPK-shRNA chimera 200 pM intratumoral on Days -3 & -2	Significant tumor growth delay	Ni 2011
HCT166 Human colorectal cancer	BALB/c nude mice	RT 150 rad on Day 1 and 3 to total 300 rad	IC88621 400 mg/Kg sc 30 min before RT followed by 3 hourly injections	4 fold increase in tumor growth delay	Kashishiran 2003
MDA-MB231 Human breast cancer	BALB/c nude mice	RT 150 rad on Day 1 and 3 to total 300 rad	IC88621 400 mg/Kg sc 30 min before RT followed by 3 hourly injections	Increase in tumor growth delay	Kashishiran 2003
Hela Human cervical cancer	BALB/c nude mice	RT 2 Gy every other day to total 10 Gy	Pretransfect cells with Anti-DNAPK scFv antibody gene	Significant tumor growth delay	Du 2010
H460 Human lung cancer	Athymic nude mice	RT 2 Gy Days 2-5 to total 8 Gy	BEZ235 40 mg/Kg oral 2 h before RT	Increased tumor growth delay	Azad 2012
SW620 Human colorectal cancer	CD-1 nude mice	Etoposide phosphate 11.5 mg/Kg Days 0-4	NU7441 10 mg/Kg immediately before RT	Significant tumor growth delay	Zhao 2006

Abbreviation: ip, intraperitoneal; RT, radiotherapy; sc, subcutaneous