Chapter

DCLK1 and DNA Damage Response

Janani Panneerselvam, Dongfeng Qu, Courtney Houchen, Michael Bronze and Parthasarathy Chandrakesan

Abstract

Genome integrity is constantly monitored by sophisticated cellular networks, collectively termed as the DNA damage response (DDR). The DDR is a signaling network that includes cell cycle checkpoints and DNA repair and damage tolerance pathways. Failure of the DDR or associated events causes various diseases, including cancer. DDR is primarily mediated by phosphatidylinositol-3-kinase-like protein kinase (PIKKs) family members ataxia-telangiectasia mutated (ATM) and ataxia telangiectasia and Rad3-related protein (ATR). However, one of the many unanswered questions regarding these signal-transduction pathways is: how does the cell turn the DDR signals on? There was no conclusive demonstration of the involvement of a specific sensory kinase in DDR signals until our recent research on the DCLK1 role in regulating ATM after genotoxic injury. Currently, various studies are demonstrating the importance of DCLK1 in DNA damage response. Here, we discuss the novel insights into the role of DCLK1 in DNA damage response.

Keywords: DNA damage, DDR, ATM, ATR, DCLK1

1. Introduction

DNA damage exists in all cellular organisms, and DNA, the genetic material in each living cell is the fundamental unit of life and its integrity and stability are essential to life [1]. However, DNA is not passive; rather, it is a chemical unit subject to be attacked from a range of endogenous and environmental damaging agents. The endogenous damages are the damage caused by reactive oxygen species or metabolic byproducts, and DNA metabolization; exogenous damages are caused by external agents, like radiations, toxins, chemicals, and microorganisms [2]. In response to the DNA damage, cells rapidly recruit a sophisticated network which is called DNA damage-response (DDR) systems. DDR systems include DNA repair mechanisms, damage tolerance processes, and cell-cycle checkpoint pathways [3]. Failure of DDR causes genomic instability which results in various diseases including immune deficiency, neurological degeneration, premature aging, and severe cancer susceptibility [2, 4]. Indeed, great progress has been made towards understanding the mechanisms of DDR in homeostasis, carcinogenesis and cancer advancement but much remains to delineate how the DDR network systems are regulated. Furthermore, how the DDR network is formed and how it is fine-tuned by upstream and downstream mediators or signaling pathways that support the homeostasis or disease progression required to understand. While the rapid activation of DDR against the

DNA damage is expected, it is unclear how and who activates or gives the instruction to DDR network systems? Gaining knowledge about DDR and its regulators will not only enhance our understanding of DDR functions but will undoubtedly giving us opportunities to better manage human diseases. Although, very few studies reported that protein kinases and DNA adaptor molecules or DNA regulators may influence or send signals to DDR after DNA gets damaged [5, 6]. DCLK1 is a member of the protein kinase superfamily and the doublecortin family, that belongs to the group of microtubule-associated proteins [7]. Our novel findings that DCLK1 regulate DNA damage response and cell survival following genotoxic injury opens many windows of how DDR is regulated [8]. In this chapter we will highlight the functional role of DCLK1 in injury, DDR and cell survival, which will lead us to a better understanding of DCLK1 expression in helping genomic stability in normal and neoplastic cells.

2. DNA damage, DNA damage response, and DNA repair

DNA is the source of genetic information in all living cells, its integrity and fidelity are essential to life. Because DNA is not passive, it is a chemical entity subject to be assaulted from various reactive agents, causing DNA damage [9]. DNA damage can be subdivided into two types: (1) endogenous damage caused by reactive oxygen species (ROS) that are derived from metabolic byproducts and (2) exogenous damage caused by radiation (UV, X-ray, gamma), hydrolysis, plant toxins, and viruses, chemical toxins [9, 10]. Most of the DNA damage can be repaired by the host systems called the DNA damage response (DDR) and DNA repair systems. Such systems also face failure and not 100% efficient, which resulted in either cell death or cell survival with un-repaired DNA causing mutation and eventually cancer [11]. In some cases, the un-repaired DNA damage accumulates in non-replicating cells, such as neurons or myocytes of adult mammals, and can cause aging [12]. The DDR is a sophisticated cellular network, which constantly monitors the integrity of the genome, in response to DNA damage [13]. Once the DDR gets activated it rapidly recruit downstream protein sensors and adaptors establishing the sensing, activating repair, tolerating damage and apoptosis (**Figure 1**). DDR is primarily mediated by phosphatidylinositol-3-kinase-like protein kinase (PIKKs) family members, ataxia-telangiectasia mutated (ATM) and ataxia telangiectasia and Rad3-related protein (ATR) and DNA-dependent protein kinase catalytic subunit (DNA-PKcs) [13, 14]. The ATM pathway for homologous recombination (HR) repair is activated after a double-stranded break. The ATR pathway for nonhomologous end-joining (NHEJ) is associated with single-stranded DNA and stalled DNA replication forks. ATM pathway is a higher-fidelity repair pathway than the ATR. For lesions repaired by the HR, double-strand breaks (DSBs) are detected and processed by the MRE11–RAD50–NBS1 (MRN) complex [15, 16]. For lesions repaired by the NHEJ, DNA breaks are detected and the process by the ATR interacting protein (ATRIP) complex. ATM and ATR transduce the most upstream DDR signal by phosphorylating the checkpoint kinases CHK1/CHK2 and the tumor suppressor protein p53, which resulted in cell cycle arrest to allow time for DNA repair. The main function of DNA-PK activated under the ATM/ATR pathway is to induce cell cycle arrest and DNA repair [17]. DNA repair is a vital cellular process required for the maintenance of genomic integrity and fidelity [18]. Living cells employ several DNA repair pathways for distinct types of DNA damage. There are five major DNA repair pathways: (1) mismatch repair (MMR), (2) nucleotide excision repair (NER), (3) base excision repair (BER), (4) homologous recombinational repair (HR), and (5) non-homologous end joining (NHEJ) [19, 20]. MMR's primary

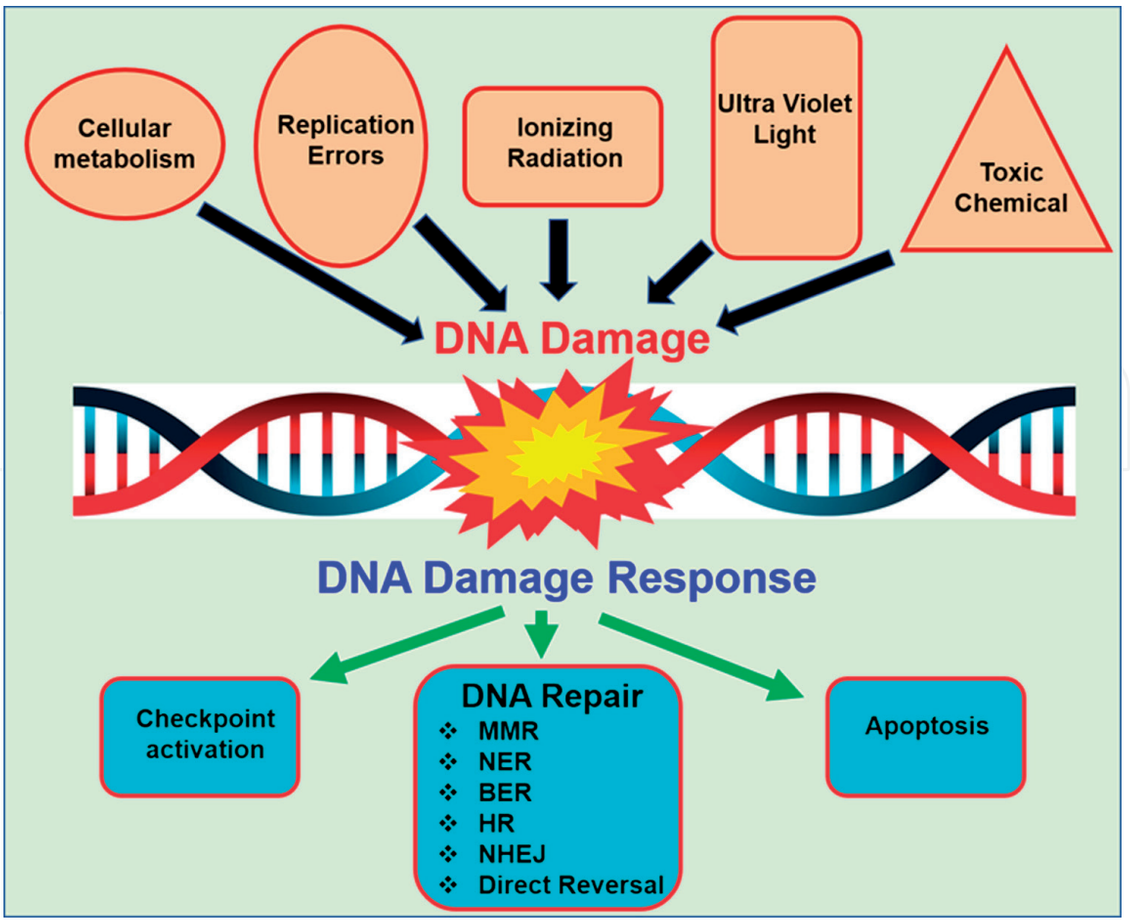

Figure 1.
DNA damage, DNA damage response, and repair. Graphical illustration demonstrating the DNA damage caused by different sources and the cellular response to DNA damage.

function is to remove base mismatches and small insertion and deletion loops which is introduced during replication. The NER pathway is a multistep process that serves to repair a variety of DNA damage, including DNA lesions caused by UV radiation, toxic chemicals, or chemotherapeutic drugs that form huge DNA adducts. BER primarily repairs non-bulky lesions produced by alkylation, oxidation or deamination of bases. The BER pathway deals with base damage, the most common insult to cellular DNA. DSBs can be repaired by either HR or NHEJ. HR uses a homologous DNA template and is highly accurate, whereas NHEJ rejoins the broken ends without using a template and is often accompanied by loss of some nucleotides. Direct reversal of DNA damage is one repair mechanism used to restore damaged DNA without using excision, resynthesis, and ligation [21, 22].

3. DCLK1

The human doublecortin (DCX) gene family comprises members that share the tubulin-binding domain and known to have limited functions in microtubule-associated regulation and neuronal-regulation [23]. One of the best known and most interesting members of this DCX family is doublecortin-like kinase 1 (DCLK1 also known as DCAMKL1), a gene encoding for a protein that is 70% identical to doublecortin in the microtubule-binding N-terminal domain. However, unlike doublecortin, the DCKL1 gene also encodes for a serine–threonine kinase C-terminal domain that is similar to Ca^{2+}/calmodulin-dependent protein kinase II but lacks a canonical calmodulin-binding site [24, 25]. DCLK1 gene also encodes

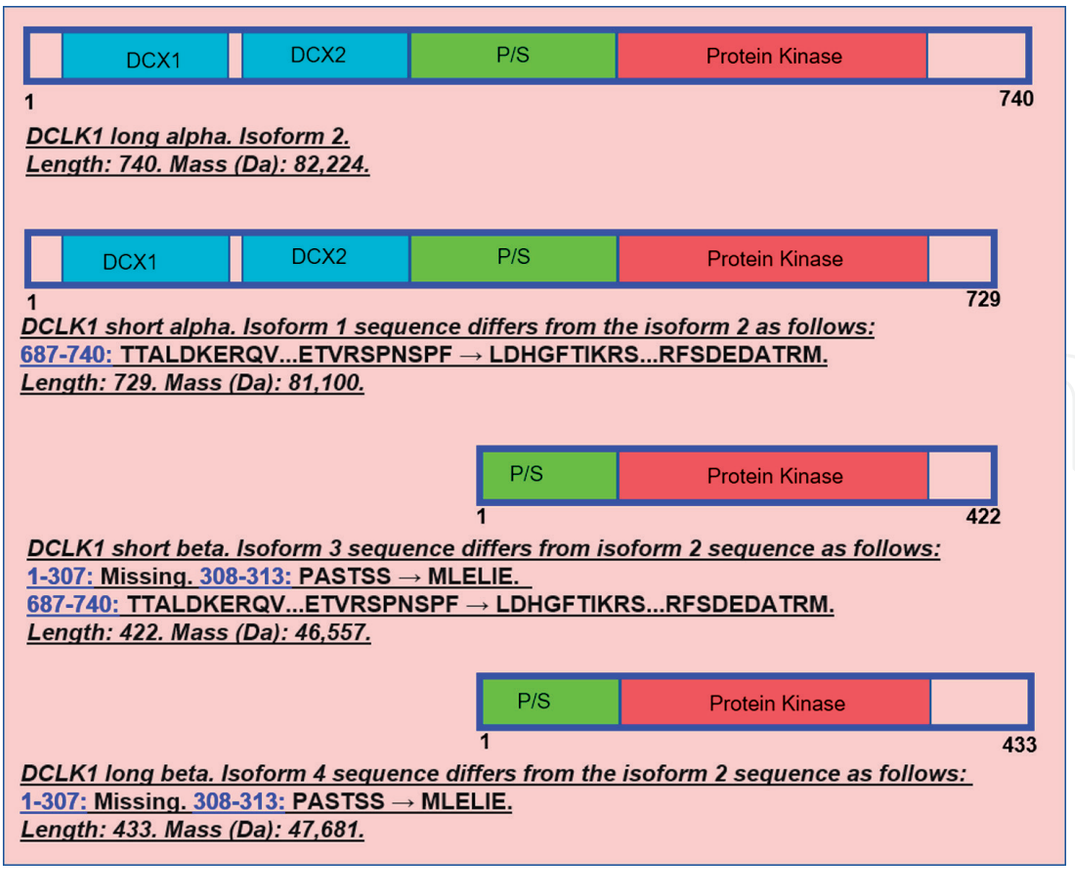

Figure 2.
Human DCLK1-isoforms. Graphical illustration demonstrating the length of each isoform and shared protein kinase domain between DCLK1 isoforms referenced in UniProt; www.uniprot.org/uniprot/O15075. DCX1 = Doublecortin1; DCX2 = Doublecortin2; and P/S = pro/Ser rich domain and a protein kinase domain.

for a serine/proline-rich domain in between the doublecortin and the protein kinase domains, which mediates multiple protein–protein interactions. In humans, DCLK1 consists of four primary isoforms with a shared kinase domain-driven from two promoter regions termed α and β (**Figure 2**) [26–28]. The α-promoter drives the expression of isoforms termed α-long (isoform 2) and α-short (isoform 1) which contain an N-terminal microtubule-binding region with high homology to DCX. Importantly, the α-promoter isoforms are specifically expressed in the DCLK1+ tuft cells that eventually give rise to tumor stem cells following relevant mutagenesis in the colon and pancreatic cancer [29–31]. The β-promoter drives the expression of two isoforms termed β-long (isoform 4) and β-short (isoform 3) that can be used to predict survival in colon cancer [32]. Although these isoforms likely play a significant role in tumorigenesis through their kinase activity, there is no evidence that they are functionally involved in the regulation of DDR, until our first report to demonstrate its direct interaction with ATM.

4. DCLK1 and DDR following injury and inflammation

Cell survival after severe injury requires highly coordinated complex interplay between the diverse molecular signaling responses to repair the injury [15, 33]. We discussed three fundamental standards about the critical role of DCLK1 in intestinal epithelial cell survival after severe genotoxic injury: (1) how intestinal epithelial cells respond to severe DNA damage because intestinal epithelial cells are the most affected cells after bone marrow during radiotherapy or accidental or incidental radiation exposure [34] and (2) how DCLK1 a kinase protein expression

play key role in injury response, because DCLK1 expressing cells survive high dose radiation and DSS-induced inflammation [29]. It is reported that the deletion of DCLK1 (Villin^{Cre};DCLK1$^{f/f}$ mice) in the intestinal epithelial cells does not confer a significant deleterious phenotype in adult mice, compared with their wild-type littermates [35]. However, after 24 h of 12 Gy total body irradiation (TBI), none of the intestinal epithelial-specific DCLK1 knockout mice survived longer than 5 days [35]. The best-known primary defense mechanism against the genotoxic injury-induced DNA damage is the DDR, which repairs the damaged DNA and increased the survival of intestinal epithelial cells [36]. Indeed studies demonstrated that deficient DDR has been suggested to increase intestinal epithelial death and loss of survival [37]. During the early event of DNA damage, the ATM-H2AX axis gets activated, generating gamma-H2AX and other adaptors, providing a stage for efficient homologous recombinant repair [38]. Recently, ATM knockout or loss of Rad50 and Mre11 was reported to increase intestinal injury and lethality [39, 40]. But how these DDR signaling pathways were regulated following radiation injury is not well known. Chandrakesan et al. reported that the absence of DCLK1 expression in the intestinal epithelial cells abrogated the activation and expression of ATM, gamma-H2AX, and downstream adopter proteins BRCA1, Rad50, and MRE11 in the intestinal epithelial cells 24 h post-TBI [8]. Furthermore, it is reported that this reduction persisted up to 3.5 days post-TBI. It is suggested that there is a profound defect in intestinal DDR in DCLK1 knock-out mice, which

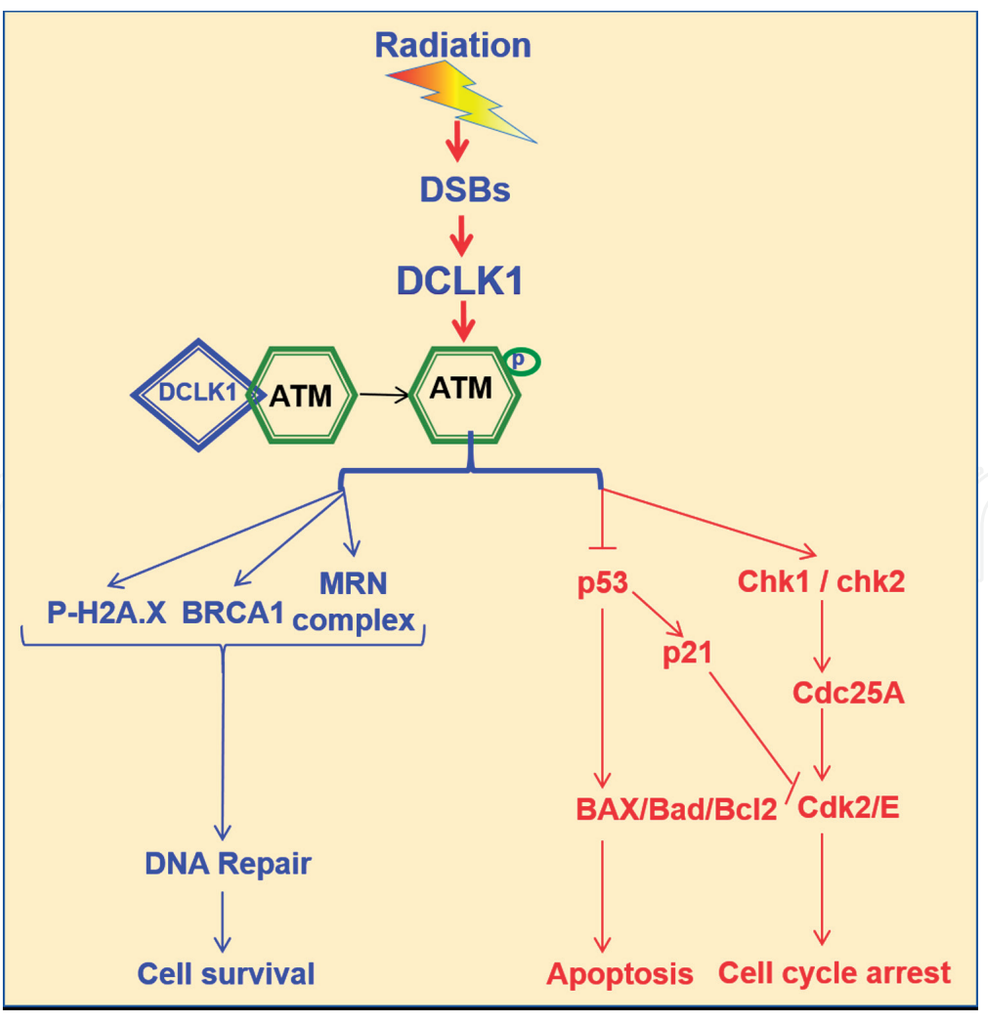

Figure 3.
DCLK1 and DDR. Graphical illustration demonstrating the regulatory role of DCLK1 in DDR following radiation injury.

might contribute to defective epithelial survival and overall survival. Interestingly they established that phosphorylation of ATM which is critical for its activation is reduced in the intestinal epithelial cells of DCLK1 knock-out mice, under physiological conditions, and discovered that DCLK1 can directly interact with ATM for its activation. ATM activation during and or after radiation injury directly depends on the ratio of DCLK1-ATM interaction [8]. Furthermore, DCLK1 knockdown and overexpression experiments with the YAMC cell line in vitro established that DCLK1 interaction is important for ATM activation. It is the first study to establish a direct link between DCLK1 and ATM mediated DDR, for the survival of cell in response to severe genotoxic injury (**Figure 3**).

5. DCLK1 in the regulation of DDR in cancers

A faulty DDR system can initiate cancer development [41]. Cancer cells with a DDR deficiency are profoundly dependent on remaining DDR [42, 43], for example in the case of ATM deficiency cancer cell relies on the ATR pathway. Therefore, DDR inhibition in cancers exploits these defects by inhibiting the remaining DDR system, and which in turn causes cancer cell death. Indeed the healthy cells are not vulnerable to DDR targeted therapies because normal cells can have higher expression of DDR only if they exposed to injury [44, 45]. Most cancer cells depend on their enhanced DDR activation for their survival, mainly activation of ATM and ATR pathways, and associated, CHK2, histone H2AX, and p53 [46, 47].

The present conventional radiotherapy and chemotherapies including platinum-based therapies are used to kill cancer cells by inducing DNA damage. A huge problem that arises when using conventional therapies is the development of resistance by these cancer cells whose DDR repair the genomic instability, which causes conventional therapies to fail [48, 49]. Cancer cells as a short-term solution can bypass the DNA damage caused by chemotherapeutic agents by a mechanism known as translesion synthesis [50, 51]. Cancer cells with high DNA damage tolerance allow DNA replication to proceed in the presence of DNA damage include the convergence of adjacent replicons, re-priming of DNA synthesis downstream of lesions on the leading strand and discontinuous synthesis of Okazaki fragments on the lagging DNA strand [52, 53]. Given the fundamental role of DDR in the gain of chemo-resistance, the novel strategies of combination therapies including DDR targeted therapies will be effective [41]. Recent regulatory approval of olaparib (Lynparza), a poly (ADP ribose) polymerase (PARP) inhibitor, which inhibits PARP enzyme activity and forms severe DSBs [54]. In cancers, PARP inhibitor increases genomic instability that results in tumor cell death [55, 56]. Although, the pharmacological inhibitors of PARP have shown promising results in preclinical studies and in clinical trials, the gain of resistance in cancer cells to PARP inhibitors, is inevitable [57]. However, the combination of PARP inhibitors with other DDR agents including ATR inhibitors, CHK1 inhibitors, ATM inhibitors, and DNA-PKs inhibitors, or with chemotherapeutic agents are novel strategies currently investigated to overcome resistance to PARP inhibitors [57] (**Table 1**—[58]). However, while the DDR targeted therapies are expected to cause DNA damage in tumor cells, it is unclear how these DDR networks are regulated in cancer cells? DDR regulators in cancers are reported recently, (1) MORC2 (MORC Family CW-Type Zinc Finger 2) is required for DNA damage-induced PAR production and PAR-dependent DNA repair signaling cascades and stimulates chromatin remodeling [59, 60]. Inhibition of MORC2 in breast cancer cells impaired DDR and sensitize cancer cells to PARP inhibitors. (2) MYB is an oncogene that plays an important role in regulating DDR in ER+ breast cancers and inhibition of MYB induces DNA

ClinicalTrials.gov identifier	Title	Phase	Drug target
NCT02797964	A Phase 1 Trial of SRA737 in Subjects with Advanced Cancer	I	CHK1 inhibitor
NCT02797977	A Phase 1 Trial of SRA737 in Combination with Gemcitabine Plus Cisplatin or Gemcitabine Alone in Subjects with Advanced Cancer	I	CHK1 inhibitor Chemotherapy
NCT03057145	Combination Study of Prexasertib and Olaparib in Patients with Advanced Solid Tumors	I	CHK1 inhibitor PARP inhibitor
NCT02516813	Phase 1 Trial of MSC2490484A, an Inhibitor of a DNA-dependent Protein Kinase, in Combination with Radiotherapy	I	DNA-PK inhibitor Radiotherapy
NCT03308942	Phase 2, Multi-Arm Study of Niraparib Administered Alone and in Combination with PD-1 Inhibitor in Patients with Non-Small Cell Lung Cancer	II	PARP Inhibitor PD-1 Inhibitor
NCT02660034	The Safety, Pharmacokinetics and Antitumor Activity of BGB-A317 in Combination with BGB-290 in Subjects with Advanced Solid Tumors	I	PARP Inhibitor PD-1 Inhibitor
NCT02264678	Ascending Doses of AZD6738 in Combination with Chemotherapy and/or Novel Anti Cancer Agents	I	ATR Inhibitor Chemotherapy PDL-1 Inhibitor
NCT01844986	Olaparib Maintenance Monotherapy in Patients with BRCA Mutated Ovarian Cancer Following First Line Platinum Based Chemotherapy. (SOLO-1)	III	PARP inhibitor
NCT02282020	Olaparib Treatment in Relapsed Germline Breast Cancer Susceptibility Gene (BRCA) Mutated Ovarian Cancer Patients Who Have Progressed at Least 6 Months After Last Platinum Treatment and Have Received at Least 2 Prior Platinum Treatments (SOLO3)	III	PARP inhibitor
NCT02446704	Study of Olaparib and Temozolomide in Patients With Recurrent Small Cell Lung Cancer Following Failure of Prior Chemotherapy	I	PARP inhibitor
NCT02789332	Assessing the Efficacy of Paclitaxel and Olaparib in Comparison to Paclitaxel/Carboplatin Followed by Epirubicin/Cyclophosphamide as Neoadjuvant Chemotherapy in Patients with HER2-negative Early Breast Cancer and Homologous Recombination Deficiency (GeparOla)	II	PARP inhibitor Chemotherapy
NCT02264678	Ascending Doses of AZD6738 in Combination With Chemotherapy and/or Novel Anti Cancer Agents Drug: administration of AZD6738 in combination with carboplatin Drug: administration of AZD6738 Drug: administration of AZD6738 in combination with olaparib Drug: administration of AZD6738 in combination with MEDI4736	I/II	PARP inhibitor Chemotherapy

ClinicalTrials.gov identifier	Title	Phase	Drug target
NCT00535353	AZD2281 and Irinotecan in Treating Patients with Locally Advanced or Metastatic Colorectal Cancer	I	PARP inhibitor Chemotherapy
NCT00678132	AZD2281 and Cisplatin Plus Gemcitabine to Treat Solid Tumor Cancers	I	PARP inhibitor Chemotherapy
NCT00515866	Study to Assess the Safety & Tolerability of a PARP Inhibitor in Combination with Gemcitabine in Pancreatic Cancer	I	PARP inhibitor Chemotherapy
NCT01460888	Radiotherapy & Olaparib in COmbination for Carcinoma of the Oesophagus (ROCOCO)	I	PARP inhibitor Radiotherapy
NCT02308072	Phase I Study of Olaparib Combined with Cisplatin-based Chemoradiotherapy to Treat Locally Advanced Head and Neck Cancer (ORCA-2)	I	PARP inhibitor Chemoradiotherapy

Selected clinical studies involving DDR inhibitors as a single agent or adjuvant therapy in combination with conventional chemotherapy or radiotherapy in cancer patients.

Table 1.
Ongoing DDR inhibitor trials.

damage and tumor cell death [61]. (3) IKKα directly activates ATM via BRAF regulates DNA damage and inhibition of IKKα induces DNA damage associated cell death in colon cancer [62]. Although these signaling molecules are involved in the regulation of DDR in cancers, their mechanism and therapeutic efficiency are yet to develop.

DCLK1, a protein kinase is overexpressed in various tumor cancers [63–65]. DCLK1 plays a critical role in injury response for repair via regulating DDR [8]. However, recently the role of DCLK1 in the regulation of DDR in cancers has established by many investigators [66–68]. In an in vitro mechanistic study, it is reported that DCLK1 caused chromatin instability, and chromatin rearrangement in colon, lung, and breast cancer cell lines, which drives the advancement of cancer cells for progression and this function is independent of its kinase activity [68]. In another study, it is reported that DCLK1 regulates the phosphorylation of CHK1 in pancreatic cancer cells. Inhibition of DCLK1 enhanced the sensitivity to gemcitabine treatment [67]. In a parallel study, it is shown that DCLK1 by regulating the phosphorylation of CHK1 enhances the sensitivity of 5-FU in colon cancer [69]. Taken together these reports suggest that DCLK1 plays a critical role in the regulation of DDR for cancer cell survival and progression. Novel therapies in the combination of targeting DCLK1 along with chemotherapeutic agents or targeting DCLK1 plus targeting an ATM or ATR with chemotherapeutic agents will be beneficial for the most effective treatment against cancers particularly the resistant cancers.

6. DCLK1 and radiation mitigators

Radiation therapy has been used for the treatment of a wide range of malignancies, especially cancers. Radiation not only kills cancer cells, but it also kills/affects normal healthy cells. Exposure of normal tissues to a substantial amount of radiation may cause both acute and chronic damage that can result in adverse effects for intended treatment [70, 71]. For example, radiation enteritis (RE) is an

intestinal inflammatory process that occurs in response to radiotherapy [72]. It is a major health concern characterized by abdominal pain, diarrhea, and rectal bleeding [73]. It can be complicated by translocation of gut bacteria into the circulation due to the loss of intestinal epithelial cells, disruption of intraepithelial tight junctions, and loss of regenerative ability resulting in severe impairment of gut function and even death. Relatively little is known about the mechanisms underlying the intestinal epithelial injury repair, cell survival and crypt regeneration in RE. Besides the severe side effects resulted in gastrointestinal mucosal damage, ionizing radiation also impairs the bone marrow-derived hematopoietic cells and immune response, which causes a significant increase in morbidity and mortality [74]. Prevention and amelioration of radiation-induced adverse effects would improve the quality of life for patients and would help cancer curability by allowing more intense therapies [75].

There are three types of chemical/biological agents used to interfere with radiation effects. Agents used before or at the time of radiation treatment are called radioprotectors, whereas agents used post-treatment are called radiation mitigators, agents used to ameliorate established normal tissue toxicity are considered treatment [76]. Currently, Amifostine is the only radioprotector in clinical use, and a few radiation mitigators been used [76]. DCLK1 can be a novel target for radiation mitigators for action, as it is mentioned above, deletion of DCLK1 within intestinal epithelial cells results in the premature death of mice following severe radiation injury, suggesting that DCLK1 is a major mediator of the crypt epithelial survival to severe genotoxic injury via a DDR-ATM mediated mechanism [8]. Recently, single-cell analysis in the intestine has revealed that the DCLK1 expressing epithelial cells in the intestine is the primary source of Cox1 (Ptgs1) and Cox2 (Ptgs2) for PGE2 synthesis [77]. PGE2 increases the survival of murine intestinal stem cells when given before photon radiation [78, 79]. It is reported that the treatment of dimethyl-PGE2 to the intestinal epithelial cells increased the survival of the colonic epithelial cells by enhancing DCLK1 expression and reduced the DNA damage [8]. Qu et al. reported that Notch signaling in the intestinal epithelium prevents the death of epithelial cells expressing DCLK1 following radiation injury [80]. Also, dietary pectin has been demonstrated to increase intestinal crypt stem cell survival following radiation injury via a DCLK1 [81]. Kantara et al. have reported that a novel regenerative peptide TP508 can significantly increase survival and delay mortality by mitigating radiation-induced intestinal and colonic toxicity, and its mechanism of action via upregulating the expression of DCLK1 in the intestinal epithelial cells which are responsible for maintaining and regenerating intestinal crypts [82]. In summary, DCLK1 could be a potential radiation mitigator by regulating DDR to ameliorate radiation-induced adverse effects.

7. Conclusion

It is becoming clear that DCLK1 contributes to DNA damage response and repair via direct and indirect mechanisms that are distinct from its role as a stem cell marker. A long-standing question of how DDR is regulated in response to DNA damage is now getting a new clarity. Furthermore, (i) DCLK1 and ATM direct interaction for ATM activation following DSBs and (ii) radiation mitigators enhance the survival of cells following DSBs via a DCLK1 dependent mechanism, which expects that DCLK1 can be a potential target for radiation mitigators in radiotherapy. Finally, in the expanding field of DDR, it is important to consider how DCLK1 is involved in the repair of DNA in cancer and homeostatic injure conditions. This will allow clinical and non-clinical researchers and practitioners to

avoid possible issues with DCLK1 therapeutics, such as enhanced cancer survival and cancer advancement with DCLK1 dependent mitigators during radiotherapy, and, more excitingly, inhibition of DCLK1 along with DDR following chemotherapy or radiotherapy in cancers will lead the way to develop novel strategies for the effective treatment of cancer.

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Conflict of interest

The authors declare no conflict of interest.

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