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Radiosensitization Strategies Through Modification of DNA Double-Strand Break Repair

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<http://dx.doi.org/10.5772/54684>

1. Introduction

DNA double-strand break (DSB) is considered most critical type of DNA damage. In eukaryote, DSB is repaired mainly through non-homologous end-joining (NHEJ) and homologous recombination (HR). Our understanding on the molecular mechanisms of these DNA repair mechanisms has been greatly deepened in the last two decades.

In NHEJ, DSB is first recognized by Ku protein (Fig.1 (1)), heterodimer consisting of Ku70 and Ku86 (also known as Ku80), which in turn recruits DNA-PK catalytic subunit (DNA-PKcs) (Fig.1 (2)). The complex consisting of Ku70, Ku86 and DNA-PKcs is termed DNA-dependent protein kinase (DNA-PK). When the DSB are not readily ligatable, processing takes place prior to ligation (Fig.1 (3)). Processing might involve a number of enzymes depending on the shape of each DNA end and compatibility of two ends to be ligated: Artemis nuclease, DNA polymerase μ/λ , polynucleotide kinase/phosphatase (PNKP), Aprataxin (APTX) and Aprataxin and PNKP-like factor (APLF, also known as PALF, C2orf13 or Xip1). DSBs are finally joined by DNA ligase IV, which is in tight association with XRCC4 (Fig.1 (4)). XRCC4-like factor (XLF, also known as Cernunnos), is essential at this step, especially when two ends are not compatible.

In HR, a complex consisting of Mre11, Rad50 and Nbs1, termed MRN complex, is thought to play two important roles in the initial stage (Fig.1 (1')): recruitment of ATM (Fig.1 (2')) and resection of one of the strands (Fig.1 (2')). ATM is a protein kinase structurally similar to DNA-PKcs. Although ATM is thought to phosphorylate a great number of proteins as revealed by

phosphoproteomic analyses, the phosphorylation of histone H2AX at Ser139 is thought one of the most important events, triggering signal transduction cascade involving mediator protein like MDC1 and ubiquitin ligases like RNF8 and RNF168. As Mre11 bears 5'-3' exonuclease activity, MRN resects one of the DNA strands to generate single-stranded DNA (ssDNA), which serves as a probe for the search for homology. Replication protein A (RPA) binds to ssDNA (Fig.1 (2')) and facilitate the formation of Rad51 filament in cooperation with BRCA2, PALB2, Rad52 and Rad51 paralogues (Fig.1 (3')). RPA also recruits ATRIP, which in turn recruits ATR, another protein kinase structurally related to DNA-PK and ATM (Fig.1 (3'')). ATR phosphorylate checkpoint kinase Chk1 to initiate signal transduction pathway leading to cell cycle checkpoints. Rad51 promotes strand exchange between homologous sequences (Fig.1 (4')). Template-dependent strand synthesis is proceeded by replication machinery including PCNA and DNA polymerase δ and ϵ (Fig.1 (5')). Finally, the junction of two DNA molecules (Holliday's junction) are resolved by nucleases Mus81-Eme1, ERCC1-XPF or SLX1-SLX4 (Fig.1 (6')). Alternatively, synthesized strand anneals with opposite end of DSB, detaches from the template strand, followed by synthesis and ligation of complementary strand (synthesis-dependent strand annealing; SDSA, not shown here).

Here, we will overview approaches to radiosensitization through the modification of DSB repair enzymes.

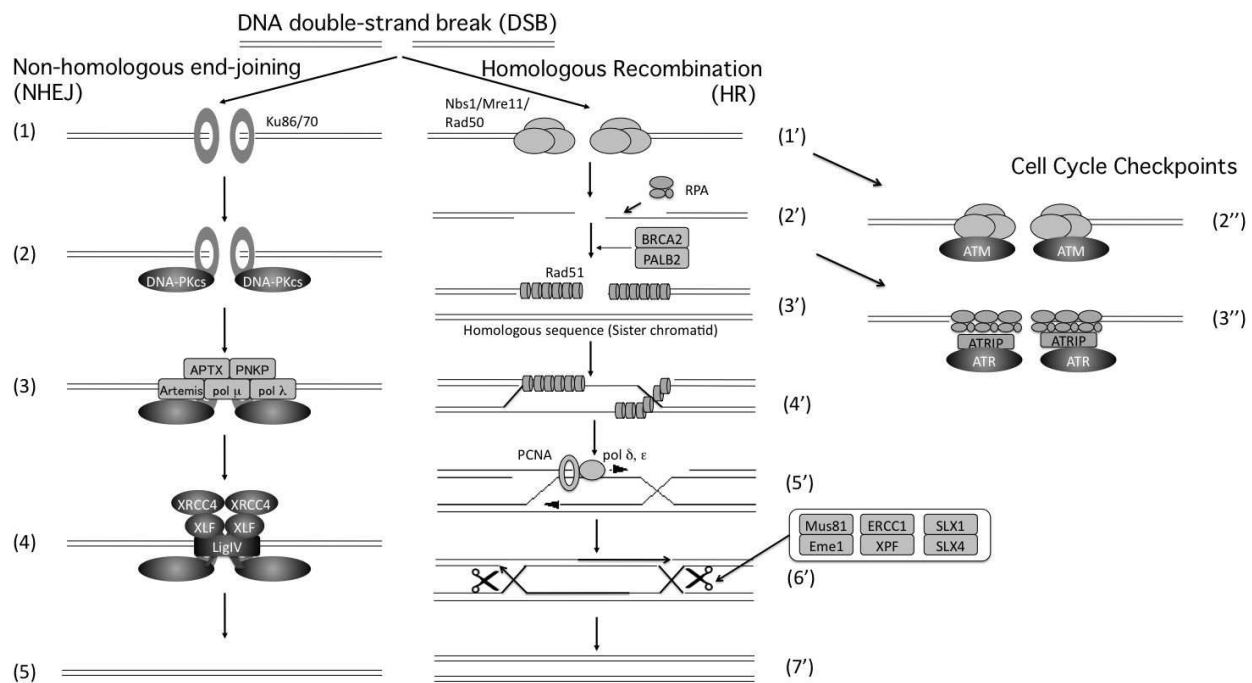


Figure 1. DNA double-strand break repair mechanisms.

2. DNA-PK, ATM and ATR kinases as targets for radiosensitizer

2.1. DNA-PK

DNA-PK was initially found in the extracts of HeLa cell, rabbit reticulocyte, *Xenopus* egg and sea urchin egg (Walker et al., 1985) and was purified from HeLa cell nuclei as a 300-350 kDa protein, which is now called DNA-PKcs (Carter et al., 1990; Lees-Miller et al., 1990). Later it was found that Ku is an essential component of DNA-PK (Dvir et al., 1992, 1993; Gottlieb and Jackson, 1993). Furthermore, it was also shown that DNA-PK requires binding of DNA-PKcs to DNA ends via Ku to be activated, suggesting its possible role in sensing DSBs (Gottlieb and Jackson, 1993). Ku86 was shown to be equivalent to XRCC5 (X-ray repair cross complementing) gene product, which is missing in X-ray sensitive rodent cell lines including *xrs-5*, *-6*, XR-V9B and XR-V15B (Taccioli et al., 1994; Smider et al., 1994). Subsequently, DNA-PKcs was found to correspond to XRCC7, which is deficient in *scid* mouse as well as several radiosensitive cultured human and rodent cell lines (Kirchgessner et al., 1995; Blunt et al., 1995; Peterson et al., 1995; Lees-Miller et al., 1995). DNA-PK is abundant in human cells and its activity can be measured using synthetic peptides derived from p53 (Lees-Miller et al., 1992), enabling extensive studies on its biochemical properties even before molecular cloning of DNA-PKcs.

First reported selective inhibitor of DNA-PK is OK-1035, 3-cyano-5-(4-pyridyl)-6-hydrazonomethyl-2-pyridone (Fig.2 A), which was found by screening of more than 10,000 microbial extracts and synthetic compounds (Take et al., 1995). IC₅₀ (50% inhibitory concentration) on DNA-PK was 8 μM, which was more than 50-fold lower than that on other seven kinases examined, although it was reported to be much higher, *i.e.*, 100 μM, in others' study (Stockley et al., 2001). OK-1035 was shown to suppress adriamycin-induced p21 expression in cultured human carcinoma cell at concentrations 500 - 2000 μM (Take et al., 1996) and also to retard the repair of DSB measured by neutral single cell gel electrophoresis (comet) assay (Kruszewski et al., 1998).

Sequence of DNA-PKcs revealed its similarity to phosphatidylinositol 3-kinase (PI3K) (Hartley et al., 1995). This study also that fungal metabolite wortmannin (Fig.2 B), which had been known as an inhibitor of PI3K, could inhibit DNA-PK (Hartley et al., 1995). IC₅₀ of wortmannin is reported to be 0.016 μM and 0.12 μM (Sarkaria et al., 1998; Izzard et al., 1999). It was also shown that wortmannin binds covalently to DNA-PKcs and functions as non-competitive, irreversible inhibitor of DNA-PK (Sarkaria et al., 1998; Izzard et al., 1999). Expectedly, a number of studies have demonstrated radiosensitizing effects of wortmannin but there is a concern whether the observed radiosensitization was really due to inhibition of DNA-PK. In this regard, some studies showed that radiosensitization by these compounds could be observed even in DNA-PKcs-deficient cells (Rosenzweig, et al., 1997; Hosoi et al., 1998), indicating that radiosensitization by these compounds was not solely due to inhibition of DNA-PK. In addition to ATM discussed next, PI3K-Akt pathway, which might be even more sensitive to wortmannin, might be important to sustain cell survival after irradiation. On the other hand, there are also studies showing that radiosensitization was not observed in DNA-PKcs deficient cells (Chernikova, et al., 1999; Hashimoto, et al., 2003). These studies argue that, even if

wortmannin affect PI3K or other kinase more potently than DNA-PK, the radiosensitizing effect might be mainly due to inhibition of DNA-PK.

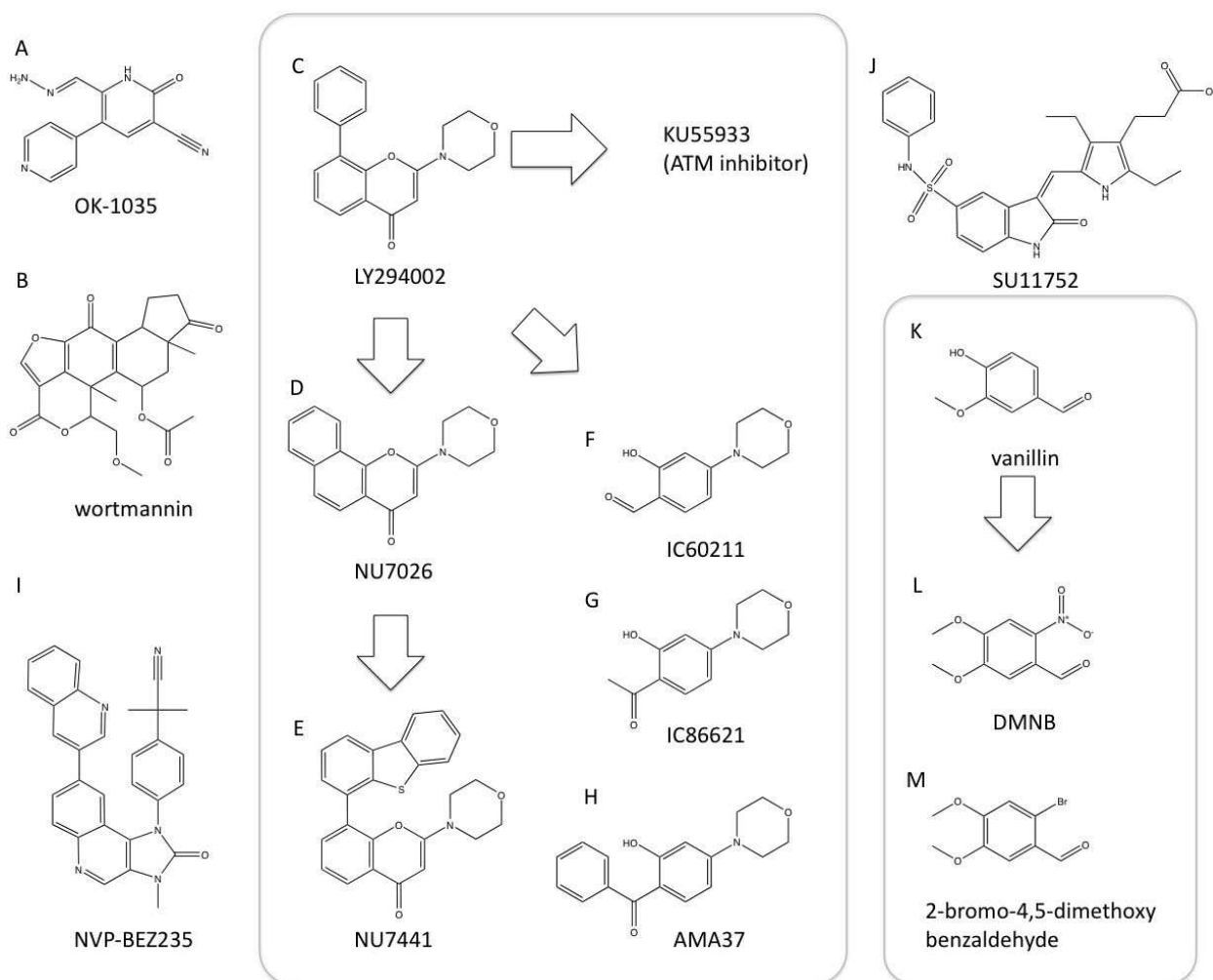


Figure 2. Structure of DNA-PK inhibitors.

Another PI3K inhibitor LY294002, 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one (Fig.2 C) was also shown to inhibit DNA-PK. In contrast to wortmannin, LY294002 competes with ATP (Izzard et al., 1999). IC_{50} of LY294002 is reported to be 6 μ M (Izzard et al., 1999). LY294002 was used as a leading compound to explore more potent and selective inhibitors of DNA-PK. NU7026, 2-(morpholin-4-yl)-benzo[h]chromen-4-one (Fig.2 D), was found as selective inhibitor of DNA-PK (Veuger et al., 2003). IC_{50} of NU7026 was 0.23 μ M for DNA-PK, 13 μ M for PI3K and >100 μ M for ATM and ATR (Veuger et al., 2003). NU7026 sensitized cultured cells toward radiation in a manner dependent on DNA-PK (Veuger et al., 2003). Synthesis and screening of chromen-4-one library resulted in identification of NU7441 (Fig.2 E), 8-dibenzothiophen-4-yl-2-morpholin-4-yl-chromen-4-one (Leahy et al., 2004; Hardcastle et al., 2005). IC_{50} of NU7441 was 0.014 μ M for DNA-PK, 5.0 μ M for PI3K and >100 μ M for ATM and ATR (Leahy et al., 2004). NU7441 sensitized cultured cells toward radiation and etoposide in a manner dependent

on DNA-PK at 0.5 μM (Zhao et al., 2006). Screening of the derivatives of LY294002 also led to the identification of other selective inhibitors of DNA-PK; IC60211 (Fig.2 F, 2-Hydroxy-4-morpholin-4-yl-benzaldehyde, IC_{50} : 0.43 μM), IC86621 (Fig.2 G, 1-(2-Hydroxy-4-morpholin-4-yl-phenyl)-ethanone, IC_{50} : 0.12 - 0.17 μM), AMA37 (Fig.2 H, 1-(2-Hydroxy-4-morpholin-4-yl-phenyl)-phenyl-methanone, IC_{50} : 0.27 μM) (Kashishian et al., 2003; Knight et al., 2004).

It was recently reported that NVP-BEZ235 (Fig.2 I, 2-methyl-2-(4-(3-methyl-2-oxo-8-(quinolin-3-yl)-2,3-dihydro-1H-imidazo[4,5-c]quinolin-1-yl)phenyl)propanenitrile), which had been initially identified as a dual inhibitor for PI3K and mammalian target of rapamycin (mTOR) (Maira et al., 2008), inhibited DNA-PK, ATM and ATR and sensitizes cells to ionizing radiation (Toledo et al., 2011; Mukherjee et al., 2012). NVP-BEZ235 sensitized the cultured cells to radiation and inhibited DSB repair, as shown by persistence of 53BP1 foci, to a greater extent than NU7026 and KU55933 (Mukherjee et al., 2012). NVP-BEZ235 sensitized ATM-deficient cells, i.e., fibroblast from ataxia telangiectasia patient, and also DNA-PKcs-deficient human glioma cell M059J (Mukherjee et al., 2012), which could be due to dual inhibition of DNA-PK and ATM. Moreover, inhibition of *in cellulo* phosphorylation mediated by DNA-PK and ATM was achieved at low concentration, i.e., 0.1 - 0.5 μM , while the similar extent of inhibition was achieved at 10 μM (Mukherjee et al., 2012).

Screening of a three-substituted indoline-2-one library led to identification of SU11752 (Fig. 2 J) as selective DNA-PK inhibitor (IC_{50} : 0.13 μM) (Ismail, et al., 2004). Vanillin, 4-hydroxy-3-methoxybenzaldehyde (Fig.2 K), was found to inhibit DNA-PK albeit at a relatively high concentration, i.e., IC_{50} 1500 μM (Durant and Karran, 2003). Screening of library of vanillin derivatives led to finding of more potent inhibitors, 4,5-dimethoxy-2-nitrobenzaldehyde (DMNB, Fig.2 L) and 2-bromo-4,5-dimethoxybenzaldehyde (Fig.2 M), whose IC_{50} were 15 μM and 30 μM , respectively (Durant and Karran, 2003).

DNA-PK can be inhibited by homopolymeric phosphorythioate oligonucleotides, suramin and heparin (Hosoi et al., 2002). Inhibitory activities of homopolymeric phosphorothioate oligonucleotides on DNA-PK were independent of base composition but were dependent on length. IC_{50} decreased as length increased: 0.975 μM for 12 mer and 0.013 μM for 36 mer (Hosoi et al., 2002). IC_{50} of suramin and heparin were 1.7 μM and 0.27 $\mu\text{g ml}^{-1}$, respectively (Hosoi et al., 2002). Suramin sensitized cultured human cancer cell toward ionizing radiation but not to ultraviolet radiation (Hosoi et al., 2004). Furthermore, suramin did not affect the radiation sensitivity of *scid* cells, which are deficient in DNA-PK, indicating that radiosensitizing effects of suramin were mediated through inhibition of DNA-PK (Hosoi et al., 2004).

Single chain antibody variable fragment (scFv) is another approach to achieve specific inhibition of DNA-PK. ScFv was initially generated from existing murine monoclonal antibody 18-2, expressed in *E. coli* and introduced into the cell by microinjection (Li et al., 2003). The epitope of scFv 18-2 was mapped within 2001-2025 region, which is outside of kinase domain and thus ScFv 18-2 inhibited DNA-PK activity only modestly (Li et al., 2003). Nevertheless, microinjection of scFv 18-2 resulted in the inhibition of NHEJ, indicated by persistence of γ -H2AX foci and sensitized cells toward ionizing radiation (Li et al., 2003). However, the use of scFv as clinical radiosensitization might be difficult without a method to deliver it efficiently into the cell nucleus. ScFv 18-2 conjugated with nuclear localization signal was developed

(Xiong et al., 2009). In more recent study, scFv was conjugated with folate and introduced into the cell nucleus via folate receptor-mediated endocytosis and exhibited radiosensitization in terms of clonogenic survival (Xiong et al., 2012). Another study screened a phage-displayed library of humanized scFv and identified a new antibody against DNA-PKcs, anti-DPK3-scFv (Du et al., 2010). Transfection of cDNA of anti-DPK3-scFv into human cancer cells resulted in increased radiosensitivity with decreased repair capability (Du et al., 2010). It also sensitized transplanted tumor on mice toward radiation (Du et al., 2010).

2.2. ATM

ATM, ataxia-telangiectasia mutated, was identified as the gene responsible for the genetic disorder ataxia telangiectasia, showing similarity to PI3K (Savitsky et al., 1995). Subsequently similarity between ATM and DNA-PKcs, suggesting ATM might also be a protein kinase rather than a lipid kinase (Hartley et al., 1995). ATM was shown to be a protein kinase, which is activated by DNA damage and phosphorylates p53 at Ser15 (Bainin et al., 1998; Canman et al., 1998).

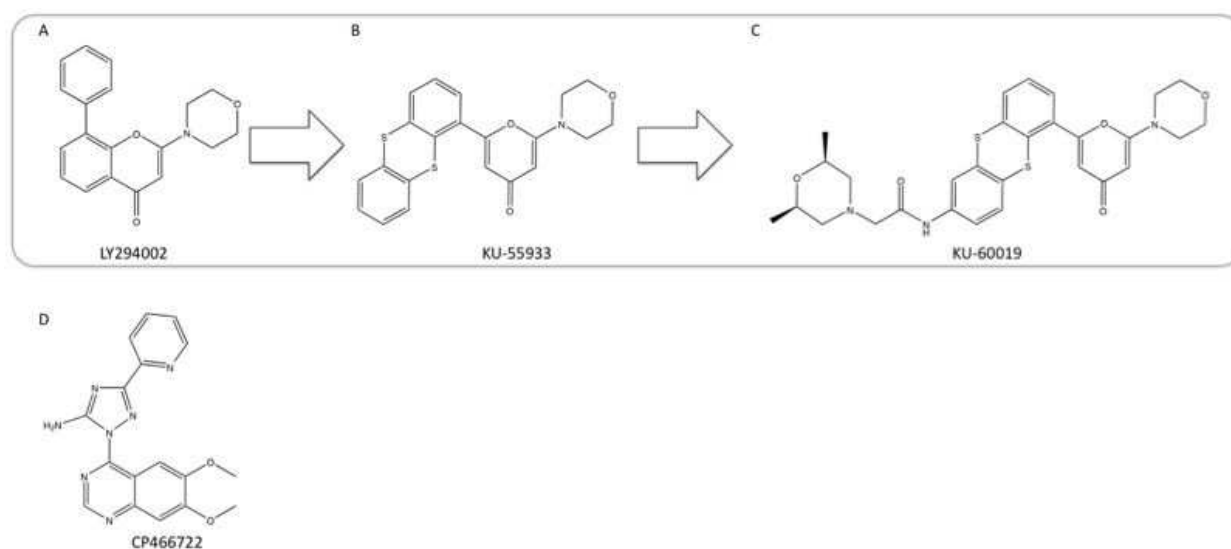


Figure 3. Structure of ATM inhibitors.

Like DNA-PK, ATM was also shown to be inhibited by wortmannin with IC_{50} of $0.15 \mu\text{M}$ (Sarkaria et al., 1998). It was also shown that administration of wortmannin to cultured cell phenocopies the defect of ataxia telangiectasia cell, *e.g.*, defective accumulation of p53 (Price and Youmell, 1996) and radioresistant DNA synthesis, which is thought to reflect defective G1/S- or S-phase checkpoint (Hosoi et al., 1998; Sarkaria et al., 1998). Caffeine, which was known to abrogate cell cycle checkpoint, was shown to inhibit ATM and ATR (Sarkaria et al., 1999; Hall-Jakson et al., 1999). IC_{50} for ATM and ATR was $200 \mu\text{M}$ and $1,100 \mu\text{M}$, respectively (Sarkaria et al., 1999).

Selective inhibitors were found from the small molecule library of LY294002 derivatives (Hickson, et al., 2004; Hollick et al., 2007). Among them KU-55933, 2-morpholin-4-yl-6-

thialanthren-1-yl-pyran-4-one (Fig.3 B) showed inhibition of ATM with IC_{50} of 0.013 μM (Hickson, et al., 2004). IC_{50} values for other PI3K-related kinases were greater than 1.8 μM , which is approximately 200-fold higher than that for ATM (Hickson, et al., 2004). As in the case of DNA-PK inhibitors, morpholine group is important for inhibitory activity, as KU-58050, in which morpholine group was replaced by piperidine group was much less effective: IC_{50} was 300 μM (Hickson, et al., 2004). KU-55933 inhibited *in cellulo* phosphorylation of ATM substrates, *e.g.*, p53 at Ser15 and histone H2AX at Ser139, 10 μM induced by ionizing radiation, but not that induced by ultraviolet irradiation (Hickson, et al., 2004). Even at lower concentration, *i.e.*, 0.3 μM , the inhibition of p53 phosphorylation was significant, although there was trace amount of residual phosphorylation (Hickson, et al., 2004). It was also shown that KU-55933 sensitized cultured cell to ionizing radiation and to radiomimetic compounds, *e.g.*, etoposide and doxorubicin but did not alter the sensitivity of fibroblast from ataxia telangiectasia patients to ionizing radiation (Hickson, et al., 2004). Furthermore, KU-55933 was found to suppress HIV infection (Lau et al., 2005).

Modification of KU-55933 lead to identification of KU-60019 (Fig.3 C), 2-((2R, 6S)-2, 6-Dimethyl-morpholin-4-yl)-N-[5-(6-morpholin-4-yl-4-oxo-4H-pyran-2-yl)-9H-thioxanthen-2-yl]-acetamide as a more potent inhibitor of ATM (Golding et al., 2009). IC_{50} of KU-60019 for ATM was 0.0063 μM , whereas IC_{50} values for DNA-PKcs and ATR were 1.7 μM and >10 μM , respectively (Golding et al., 2009). KU-60019 mostly abolished ionizing radiation-induced phosphorylation of p53 at Ser15 and Chk2 at Thr68 at 1 to 3 μM , whereas > 10 μM concentration of KU-55933 was required to obtain similar extent of inhibition (Golding et al., 2009). KU-60019 at 1 μM showed similar extent of radiosensitization to KU-55933 at 10 μM (Golding et al., 2009).

Independent screening of chemical library lead to identification of CP466722, 2-(6,7-dimethoxyquinazolin-4-yl)-5-(pyridin-2-yl)-2H-1,2,4-triazole-3-amine, as a novel inhibitor of ATM (Rainey, et al., 2008). CPP466722 inhibited *in cellulo* phosphorylation of ATM at Ser1981, SMC1 at Ser957 and Chk2 at Thr68 but not affected the phosphorylation events, which are thought to be mediated through other PI3K-related kinases (Rainey, et al., 2008). CPP466722 sensitized cultured cells to radiation to a similar extent to KU-55933 (Rainey, et al., 2008).

CGK733 was reported to be a dual inhibitor of ATM and ATR, but the report was retracted thereafter because of fabrication. Even after the retraction, CGK733 was marketed as an inhibitor of ATM and ATR and several studies used CGK733 to show the involvement of ATM and/or ATR in response to DNA damage caused by a variety of agents. On the other hand, however, there is a report that this compound did not affect ATM and ATR kinase as shown, respectively, by ionizing radiation-induced phosphorylation of ATM at Ser1981 and Chk2 at Thr 68 and by ultraviolet radiation-induced phosphorylation of Chk1 at Ser317 (Choi et al., 2011, and references therein).

2.3. ATR

ATR was initially identified as a molecule structurally related to human ATM and yeast Rad3 (Cimprich et al, 1996; Keegan et al., 1996). ATR was then shown to be a protein kinase, which is capable of phosphorylating itself and p53 at Ser15 (Canman et al., 1998). ATR is thought to

be a sensor of single-stranded DNA (ssDNA), binding to RPA (Replication Protein A) via ATRIP (ATR-interacting protein) (Zou and Elledge, 2003).

Despite of its structural similarity to DNA-PKcs, ATM and PI3K, ATR appeared refractory to wortmannin inhibition: IC_{50} of wortmannin for ATR was 1.8 μ M, which was 10- to 100-fold higher than that for DNA-PKcs and ATM (Sarkaria et al., 1998). Selective inhibitors of ATR emerged recently.

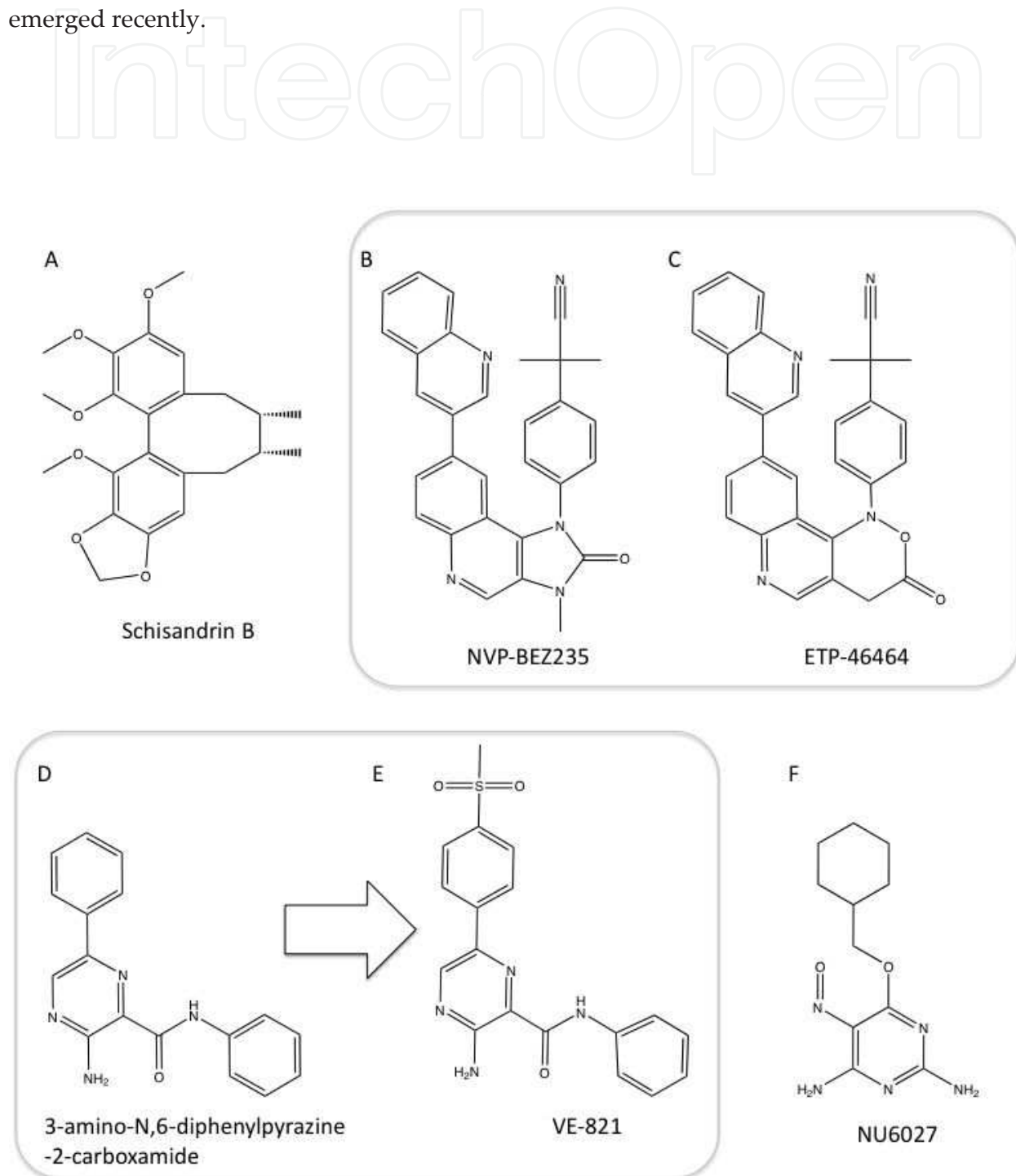


Figure 4. Structure of ATR inhibitors

Schisandrin B is an active ingredient of *Fructus schisandrae*, which has been used in traditional Chinese medicine to treat hepatitis and myocardial disorders (Fig.4 A). Schisandrin B was found to inhibit ATR (Nishida et al., 2009). IC₅₀ of Schisandrin for ATR and ATM were, respectively, 7.25 μM and 1,700 μM and DNA-PK, PI3K and mTOR were not inhibited up to ~100 μM (Nishida et al., 2009). Schisandrin B sensitized cultured human cells to ultraviolet radiation and ionizing radiation at concentrations 1 - 30 μM (Nishida et al., 2009). Sensitization was not observed in cells from Seckel patient, who harbor mutation in ATR gene (Nishida et al., 2009), showing that the sensitizing effect is mediated through ATR.

Library of 623 compounds, which had exhibited some inhibitory effects on PI3K, was screened for their effects on *in cellulo* phosphorylation of H2AX stimulated by ATR-activating domain of TopBP1 (Toledo et al., 2011). This screening identified NVP-BEZ235 (Fig.4 B) and ETP-46464 (Fig.4 C) (Toledo et al., 2011). Whereas NVP-BEZ235 also inhibited DNA-PK and ATM (see above), ETP-464 did not affect DNA-PK and ATM (Toledo et al., 2011). These compounds mostly inhibited the phosphorylation *in cellulo* of H2AX and other ATR substrates, *e.g.*, Chk1, even at 0.1 - 0.5 μM (Toledo et al., 2011).

High throughput screening of ATR by *in vitro* kinase assay identified 3-amino-N,6-diphenylpyrazine-2-carboxamide (Charrier et al., 2011). IC₅₀ of this compounds for ATR was 0.62 μM, whereas that for ATM and DNA-PK was > 8 μM (Charrier et al., 2011). Then the derivatives of this compound were synthesized and subjected to test for ATR inhibition. VE-821, 3-amino-6-(4-(methylsulfonyl)phenyl)-N-phenylpyrazine-2-carboxamide, was found as most potent and selective inhibitor of ATR (Charrier et al., 2011). IC₅₀ of VE-821 for ATR was 0.026 μM, whereas that for ATM and DNA-PK was > 8 μM and 4.4 μM, respectively (Charrier et al., 2011).

NU6027, 2,6-diamino-4-cyclohexyl-methoxy-5-nitroso-pyrimidine, was initially developed as an inhibitor of cyclin-dependent kinases (CDKs) (Arris et al, 2000). NU6027 was recently found, however, to inhibit ATR more potently than CDK2 (Peasland et al., 2011). NU6027 inhibited *in cellulo* phosphorylation of Chk1 at Ser345 with IC₅₀ of 6.7 μM, whereas autophosphorylation of DNA-PKcs at Ser2056 and ATM at Ser1981 were not affected at 10 μM (Peasland et al., 2011). NU6027 sensitized cultured cells to hydroxyurea and cisplatin, but this effect was not observed in ATR-knocked down cells, showing that sensitization was mediated through ATR (Peasland et al., 2011).

It might be added that p53-deficient cells, than p53-proficient cells, exhibited greater extent of sensitization toward ionizing radiation and other DNA damaging agents by ATR inhibitors NVP-BEZ235, ETP-46464 (Toledo et al., 2011), VE-821 (Reaper et al., 2011) and NU6027 (Peasland et al., 2011). This could be due to simultaneous inactivation of two checkpoint pathways mediated through ATM and ATR, respectively, the former of which involves p53. As most of cancer cells lose p53 function, inhibition of ATR might be a promising approach to achieve selective killing of cancer cells, minimizing the effects to surrounding normal cells.

3. Other DSB repair enzymes as targets for radiosensitizer

3.1. MRN complex

Mirin, Z-5-(4-hydroxybenzylidene)-2-imino-1,3-thiazolidin-4-one (Fig.5), was identified in a screen for small molecules inhibiting MRN-ATM pathway (Dupre et al., 2009). Restriction enzyme-digested plasmid was added to cell-free extract prepared from *Xenopus laevis* egg in 96-well format and the phosphorylation of H2AX-mimicking peptide was quantified. Approximately 10,000 compounds, which had exhibited inhibition of p53 activity or interference with mitosis and spindle dynamics, were subjected to screen. Mirin inhibited H2AX phosphorylation in *Xenopus laevis* egg cell free extract with an IC_{50} of 66 μ M and also autophosphorylation of ATM at Ser1981 in human cells within 25 - 100 μ M range (Dupre et al., 2009). Mirin inhibited nuclease activity of Mre11, but did not affect DNA binding or DNA tethering activity of MRN complex (Dupre et al., 2009). Mirin also abrogated G2/M checkpoint, reduced homologous recombination and showed radiosensitizing effects in cultured human cells within 25 - 100 μ M range (Dupre et al., 2009).

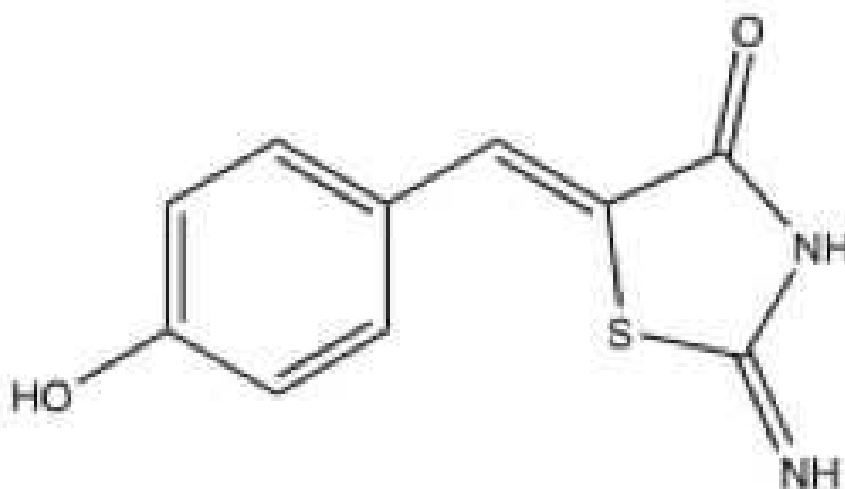


Figure 5. Structure of Mirin.

3.2. DNA ligase IV

Inhibitors of DNA ligases were searched in a database of 1.5 million commercially available low molecular weight chemicals by computer-aided drug design approach based on crystal structure of DNA ligase I (Chen et al., 2008). In this approach, L82 ((E)-2-((2-(2-((3,5-dibromo-4-methylphenyl)amino)ethyl)hydrazono)methyl)-4-nitrophenol, Fig.6 A), inhibiting DNA ligase I, L67 ((E)-4-chloro-5-(2-(4-hydroxy-3-nitrobenzylidene)hydrazinyl)pyridazin-3(2H)-one, Fig.6 B), inhibiting DNA ligases I and III, and L189 ((E)-6-amino-5-(benzylideneamino)-2-mercaptopyrimidin-4-ol, Fig.6 C), inhibiting DNA ligases I, III and IV, were identified. None of them inhibited the activity of T4 ligase (Chen et al., 2008). Kinetic analysis indicated that,

whereas L82 is non-competitive inhibitor, L67 and L189 competes with DNA substrate (Chen et al., 2008). L67 sensitized cultured human cancer cells to methylmethansulfonate at 3 μM (Chen et al., 2008). Similarly, L189 sensitized cultured human cancer cells to ionizing radiation at 20 μM (Chen et al., 2008). It might be noted that the sensitizing effects of L67 and L189 were not observed in non-cancer cells, suggesting is selective effects on cancer cells (Chen et al., 2008). These compounds can be a leading compounds for the development of more potent and/or more selective inhibitors of DNA ligases.

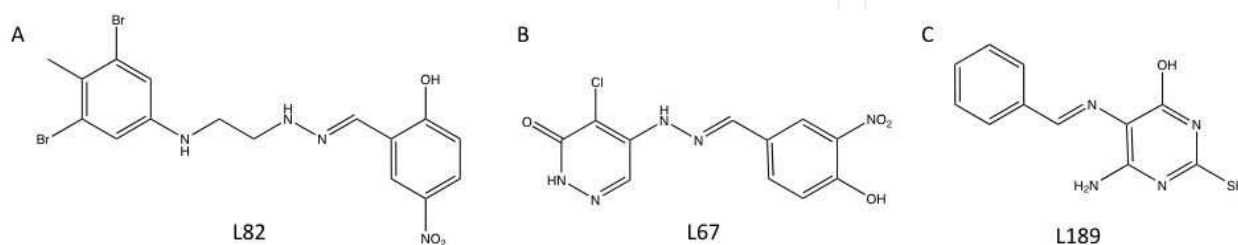


Figure 6. Structure of DNA ligase inhibitors.

3.3. DPYD as a new target

Gimeracil, 5-chloro-2,4-dihydropyridine (Fig.7), is an inhibitor of dihydropyrimidine dehydrogenase (DPYD) and used as a component of oral anti-cancer medicine S-1, in order to suppress degradation of 5-fluorouracil. The results of clinical trial of concurrent chemoradiotherapy using S-1 suggested possible radiosensitizing effect of S-1. Gimeracil increased radiosensitivity of cultured human cancer cells of various origin within 200 – 5,000 μM , being maximal within 1,000 – 5,000 μM range (Takagi et al., 2010). Cell lines deficient for DNA-PKcs or Ku86 were sensitized by gimeracil to radiation even to a greater extent than respective control cells (Takagi et al., 2010). On the other hand, radiosensitization was not observed in cell lines deficient for XRCC3, NBS1 or FANCD2 (Takagi et al., 2010). These observations collectively suggested that gimeracil exert radiosensitizing effects through inhibition of HR-mediated DSB repair. Gimeracil reduced the frequency of homologous recombination of chromosomal substrate including the restriction site of I-SceI by approximately 15% (Takagi et al., 2010). Gimeracil reduced the formation of ionizing radiation-induced foci of Rad51 and RPA but increased that of Nbs1, Mre11, Rad50 and FancD2 (Sakata et al., 2011). This observation suggested that gimeracil might have inhibited the step after strand resection by Mre11-Rad50-Nbs1 complex but before the loading of RPA and Rad51 onto single-stranded DNA. Although the role of DPYD in HR has not been described, treatment with siRNA for DPYD sensitized cells to ionizing radiation to a similar extent to gimeracil and also diminished the radiosensitization by gimeracil (Sakata et al., 2011). These results collectively indicate that gimeracil exerts radiosensitizing effects through inhibition of DPYD, which might have a novel role in HR.

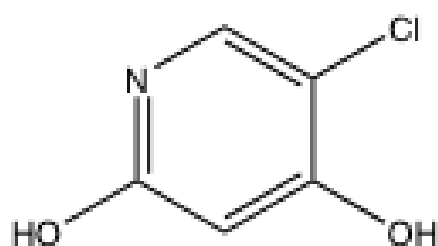


Figure 7. Structure of gimeracil.

4. Radiosensitization by hyperthermia

Hyperthermia, heating parts of body at 40 - 45 °C, has been used to treat cancer mostly combined with ionizing radiation. Hyperthermia is known to sensitize cells to ionizing radiation, inhibiting the repair of DNA damages including DSBs, but the molecular mechanism of radiosensitization by hyperthermia has remained to be clarified.

Effects of hyperthermia on DNA polymerases α and β have been studied for a long time. These studies suggested that DNA polymerase β was sensitive to hyperthermia and its inactivation was correlated to radiosensitization as well as to cell killing (Spiro et al., 1982). Later it was reported that DNA polymerase β knocked out cells or overexpressed cells exhibited radiosensitization by hyperthermia indifferent from control cells (Raaphorst et al., 2004). Elucidation of DSB repair mechanisms through NHEJ and HR provided clues to the mechanisms of radiosensitization by hyperthermia.

Among essential factors in NHEJ, Ku is shown to be affected by hyperthermia. Purified DNA-PK lost its activity upon incubation at 44°C for 5 - 30 min (Matsumoto et al., 1997). When DNA-PKcs and Ku were heated separately, heating of Ku, but not DNA-PKcs, lead to decrease in DNA-PK activity, suggesting that Ku, rather than DNA-PKcs, is heat sensitive component (Matsumoto et al., 1997). Inactivation of DNA-PK activity by hyperthermia was observed also *in cellulo*, *i.e.*, when culture cells were heated at 44 - 47°C (Burgman et al., 1997; Ihara et al., 1999; Umeda et al., 2003). It might be noted, however, that the extent of the loss of DNA-PK was greatly different between mouse, hamster and human cell, being greatest in mouse and least in human (Umeda et al. 2003). In murin cells, significant loss of DNA-PK activity was observed at lower temperatures, *i.e.*, 41°C or 42°C (our unpublished observations). DNA-PK activity could be restored by mixing the lysate of heated cells with the lysate of DNA-PKcs-deficient cells, but not with Ku86-deficient cells, indicating that *in cellulo* inactivation of DNA-PK by hyperthermia might be also due to the property of Ku rather than DNA-PKcs (Ihara et al., 1999). Moreover, Ku was identified as constitutive heat shock element-binding factor, CHBF, whose activity was lost by hyperthermia, allowing the binding of HSF1 (Kim et al., 1995). DNA binding activity of Ku correlated with extent of radiosensitization by hyperthermia (Burgman et al., 1997). Reduced solubility of Ku in aqueous buffer after hyperthermia was also reported, which might reflect aggregation (Beck and Dynlacht, 2001). However, the hypothesis

that radiosensitization by hyperthermia is due to inactivation of Ku or DNA-PK has been challenged by genetic studies, showing that cells deficient for Ku or DNA-PKcs could be radiosensitized by hyperthermia to a similar extent or even to a greater extent than control cells (Kampinga et al., 1993; Raaphorst et al., 1993; Woudstra, et al, 1999, Raaphorst et al., 2004), although there are studies, in contrast, showing no or reduced radiosensitization in Ku- or DNA-PKcs-deficient cells (Iliakis and Seaner, 1990; O'Hara et al., 1995). Moreover, chicken lymphocyte DT40 derivative lacking Ku70 and Rad54, therefore, deficient in both of NHEJ and HR, still showed radiosensitization by hyperthermia (Raaphorst et al., 2004; Yin et al., 2004).

There is also accumulating studies on the effects of hyperthermia on MRN complex. It was initially found that Mre11, Rad50 and Nbs1 exported from nucleus to cytoplasm upon hyperthermia at 42.5°C or 45.5°C (Zhu et al., 2001; Seno and Dynlacht, 2004). This nuclear export of MRN complex increased when cells were irradiated prior to hyperthermia (Zhu et al., 2001; Seno and Dynlacht, 2004). Similar phenomenon was observed in mild hyperthermia at 41.1°C (Xu et al., 2002). However, in a recent study, inhibition of nuclear export of MRN complex by leptomycin B did not diminish radiosensitization by hyperthermia at 45.5°C for 10 min (Dynlacht et al., 2011). It was also shown, nevertheless, that ATLD cells, which have mutated in Mre11, did not show radiosensitization by hyperthermia at 41.5°C for 2 hrs or at 45.5°C for 10 min (Dynlacht et al., 2011). On the other hand, radiosensitization by hyperthermia was observed in NBS cells and Rad50-knocked down cells (Dynlacht et al., 2011). Exonuclease activity of Mre11 was decreased to ~10% by 42.5°C treatment for 15 min (Dynlacht et al., 2011). These results collectively indicate Mre11 as target for radiosensitization by hyperthermia.

Hyperthermia is shown to affect BRCA1 and BRCA2. Heating cultured human cancer cells at 42°C for 1 - 2 hrs or more decreased the amount of BRCA1 (Ma et al., 2003). It might be caused by protein degradation, but various inhibitors of proteases, so far as tested, failed to suppress the decrease of BRCA1 (Ma et al., 2003). Alternatively, it might be caused by protein aggregation and reduced solubility in aqueous buffers. It was also shown that BRCA1 deficient cells were sensitive to hyperthermia (Ma et al., 2003). Recent study reported the degradation of BRCA2 induced by mild hyperthermia at 41°C to 42.5°C (Krawczyk et al., 2011). Rad54-deficient ES cells and cells treated with XRCC3 siRNA were not radiosensitized by mild hyperthermia (Krawczyk et al., 2011). Furthermore, mild hyperthermia showed synthetic lethality with PARP-1 inhibitor oraparib, like BRCA2 deficient cancer cells (Krawczyk et al., 2011). These data collectively indicated BRCA2 as a major target of mild hyperthermia.

Obviously, hyperthermia inactivates many enzymes and induces aggregation of many proteins. In this regard, hyperthermia is not specific on certain enzyme, unlike inhibitors described above. However, susceptibility to inactivation by hyperthermia might be greatly different among proteins. The extent of radiosensitization by hyperthermia can be greatly influenced by many factors, *e.g.*, cell type, genetic background, physiological conditions, heating temperature, duration of heating, sequence of heating and radiation and the interval between them. Further studies would be required to examine the effects of hyperthermia on various repair enzymes and its relationship to radiosensitizing effects under various conditions.

5. Concluding remarks and future perspectives

Because of great advances in our understanding of the molecular mechanisms of DSB repair in past two decades, extensive studies have been done to achieve radiosensitization by modification of DSB repair molecules. Especially, a number of inhibitors have been developed for DNA-PK, ATM and ATR protein kinases. We saw here that preceding studies on DNA-PK and on PI3K greatly facilitated the studies on ATM and ATR. It might be underscored that LY294002, preexisting inhibitor of PI3K, served as a leading compound and enabled the finding of potent and specific inhibitors like NU7441 and KU-55933.

Studies toward the clinical application of these compounds are underway. Preclinical studies of pharmacokinetics and metabolism in mice were conducted for NU7026 and NU7441. In the case of NU7026, the radiosensitizing effect on cultured cancer cell was marginal upon the treatment at 10 μ M for 2 hrs and could be increased by extending the treatment time up to 24 hrs (Nutley et al., 2005). On the other hand, however, NU7026 underwent rapid plasma clearance in mice, presumably because of oxidation and ring opening of morpholino group (Nutley et al., 2005). It was estimated that NU7026 should be administered four times per day at 100 mg/kg intraperitoneally in order to obtain radiosensitization (Nutley et al., 2005). In the case of NU7441, the radiosensitizing effect on cultured cancer cell could be obtained by treatment at 1 μ M for 1 hr (Zhao et al., 2006). The concentration of NU7441 required for radiosensitization could be maintained within tumor tissues for more than 4 hrs at nontoxic dose (Zhao et al., 2006). The administration of etoposide and NU7441 to mice bearing human tumor xenografts synergistically delayed tumor growth, indicating the chemosensitizing effect of NU7441 *in vivo* (Zhao et al., 2006). Studies are still going on to obtain compounds with better characteristics, *e.g.*, higher aqueous solubility (Cano et al., 2010).

Search for inhibitors of enzymes other than protein kinases has been difficult due to the absence of assay system suitable for highthroughput screening. However, inhibitors of other enzymes, *i.e.*, Mre11 nuclease and DNA ligase IV have been developed, although few at present. Now these compounds are obtained, more potent and specific inhibitors can be obtained by molecular evolution as in the case of DNA-PK, ATM and ATR protein kinases. Additionally, search for other inhibitors will be greatly facilitated by an aid of computer-based structural prediction and drug designing.

In addition to use of each chemicals alone, use of two or more chemicals together to inhibit two pathways of DSB repair or one of them with other repair mechanisms, which is called synthetic lethality approach, will be promising. Successful example is shown in the treatment of cancers arisen in the carriers of BRCA2 mutation with PARP-1 inhibitors. When PARP-1, which is essential for single-strand break (SSB) repair, is inactivated, SSB is converted to DSB, which requires BRCA2 to be repaired. As BRCA2 mutation is heterozygotic, normal cells retain BRCA2 function. On the other hand cancer cells have lost BRCA2 function and, therefore, shows extreme sensitivity to increased sensitivity to converted DSBs. This is instructive also to find a means to discriminate cancer cells and normal cells. As described in the previous section, although hyperthermia is not an approach to target a certain molecule specifically, it did show synthetic lethal effects with PARP-1 inhibitor. These examples underscores the

importance of the choice of agents based on the thorough consideration of biological characteristics and genetic background of each cancer and patient. In addition to continuing pursuit for the new radiosensitizing agents, extensive studies would be necessary regarding combinatorial approach and personalized medicine.

Note

We apologize for not citing many important literatures because of space limitation.

Acknowledgements

Our study was supported in part by Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sport, Science and Technology of Japan to YM. MKS is supported by Takeda Science Foundation, Japan Society for Promotion of Sciences and Tokyo Biochemistry Research Foundation.

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References

- [1] Arris, C.E., Boyle, F.T., Calvert, A.H., Curtin, N.J., Endicott, J.A., Garman, E.F., Gibson, A.E., Golding, B.T., Grant, S., Griffin, R.J., Jewsbury, P., Johnson, L.N., Lawrie, A.M., Newell, D.R., Noble, M.E., Sausville, E.A., Schultz, R. & Yu, W. (2000) Identification of novel purine and pyrimidine cyclin-dependent kinase inhibitors with distinct molecular interactions and tumor cell growth inhibition profiles. *J. Med. Chem.*, 43: 2797-2804.
- [2] Banin, S., Moyal, L., Shieh, S.-Y. Taya, Y., Anderson, C. W., Chessa, L., Smorodinsky, N. I., Prives, C., Reiss, Y., Shiloh, Y., & Ziv, Y. (1998) Enhanced phosphorylation of p53 by ATM in response to DNA damage. *Science*, 281:1674-1677.
- [3] Beck, B.D. & Dynlacht, J.R. (2001) Heat-induced aggregation of XRCC5 (Ku80) in nontolerant and thermotolerant cells. *Radiat. Res.*, 156: 767-774.
- [4] Blunt, T.; Finnie, N.; Taccioli, G.; Smith, G.; Demengeot, J.; Gottlieb, T.; Mizuta, R.; Varghese, A.; Alt, F.; Jeggo, P. & Jackson, S.P. (1995) Defective DNA-dependent protein

- kinase activity is linked to V(D)J recombination and DNA repair defects associated with the murine scid mutation. *Cell*, 80: 813-823.
- [5] Burgman, P., Ouyang, H., Peterson, S., Chen, D.J. & Li, G.C. (1997) Heat inactivation of Ku autoantigen: possible role in hyperthermic radiosensitization. *Cancer Res.*, 57: 2847-2850.
- [6] Canman, C. E., Lim, D.-S., Cimprich, K. A., Taya, Y., Tamai, K., Sakaguchi, K., Appella, E., Kastan, M. & Siliciano, J. D. (1998) Activation of the ATM kinase by ionizing radiation and phosphorylation of p53. *Science*, 281: 1677-1679.
- [7] Cano, C., Barbeau, O.R., Bailey, C., Cockcroft, X.-L., Curtin, N.J., Guggan, H., Frigerio, M., Golding, B.T., Hardcastle, I.R., Hummersone, M.G., Knights, C., Menear, K.A., Newell, D.R., Richardson, C.J., Smith, G.C.M., Spittle, B. & Griffin, R.J. (2010) DNA-dependent protein kinase (DNA-PK) inhibitors. Synthesis and biological activity of quinolin-4-one and pyridopyrimidin-4-one surrogates for the chromen-4-one chemotype. *J. Med. Chem.*, 53: 8498-8507.
- [8] Carter, T., Vancurova, I., Sun, I., Lou, W. & DeLeon, S. (1990) A DNA-activated protein kinase from HeLa cell nuclei. *Mol. Cell. Biol.*, 10: 6460-6471.
- [9] Charrier, J.D., Durrant, S.J., Golec, J.M.C., Kay, D.P., Knegt, R.M.A., MacCormick, S., Mortimore, M., O'Donnell, M.E., Pinder, J.L., Reaper, P.M., Rutherford, A.P., Wang, P.S.H., Young, S.C. & Pollard, J.R. (2011) Discovery of potent and selective inhibitors of ataxia telangiectasia mutated and Rad3 related (ATR) protein kinase as potential anticancer agents. *J. Med. Chem.*, 54: 2320-2330.
- [10] Chen, X., Zhong, S., Zhu, X., Dziegielewska, B., Ellenberger, T., Wilson, G.M., MacKerell, A.D. & Tomkinson, A.E. (2008) Rational design of human DNA ligase inhibitors that target cellular DNA replication and repair. *Cancer Res.*, 68: 3169-3177.
- [11] Chernikova, S.B., Wells, R.L. & Elkind, M.M. (1999) Wortmannin sensitizes mammalian cells to radiation by inhibiting the DNA-dependent protein kinase-mediated rejoining of double-strand breaks. *Radiat. Res.*, 151: 159-166.
- [12] Choi, S., Toledo, L.I., Fernandez-Capetillo, O. & Bakkenist, C.J. (2011) CGK733 does not inhibit ATM or ATR kinase activity in H460 human lung cancer cells. *DNA Repair*, 10: 1000-1001.
- [13] Cimprich, K.A., Shin, T.B., Keith, C.T. & Shreiber, S.L. (1996) cDNA cloning and gene mapping of a candidate human cell cycle checkpoint protein. *Proc. Natl. Acad. Sci. USA*, 93: 2850-2855.
- [14] Dvir, A., Peterson, S.R., Knuth, M.W., Lu, H. & Dynan, W.S. (1992) Ku autoantigen is the regulatory component of a template-associated protein kinase that phosphorylates RNA polymerase II. *Proc. Natl. Acad. Sci. USA*, 89: 11920-11924
- [15] Dvir, A., Stein, L.Y., Calore, B.L. & Dynan, W.S. (1993) Purification and characterization of a template-associated protein kinase that phosphorylates RNA polymerase II. *J. Biol. Chem.*, 268: 10440-10447.

- [16] Du, L., Zhou, L.-J., Pan, X.-J., Wang, Y.-X., Xu, Q.-Z., Yang, Z.-H., Wang, Y., Liu, X.-D., Zhu, M.-X. & Zhou, P.-K. (2010) Radiosensitization and growth inhibition of cancer cells mediated by an scFv antibody gene against DNA-Pkcs in vitro and in vivo. *Radiat. Oncol.*, 5: 70.
- [17] Dupre, A., Boyer-Chatenet, Sattler, R.M., Modi, A.P., Lee, J.-H., Nicolette, M.L., Kopelovich, L., Jasin, M., Baer, R., Paull, T.T. & Gautier, J. (2009) A forward chemical genetic screen reveals an inhibitor of the Mre11-Rad50-Nbs1 complex. *Nat. Chem. Biol.*, 4: 119-125. (Corrington Vol. 5, pp.1)
- [18] Durant, S. & Karran, P. (2003) Vanillins – a novel family of DNA-PK inhibitors. *Nucleic Acids Res.*, 31: 5501-5512.
- [19] Dynlacht, J.R., Batuello, C.N., Lopez, J.T., Kim, K.K. & Turchi, J.J. (2011) Identification of Mre11 as a target for heat radiosensitization. *Radiat. Res.*, 176: 323-332.
- [20] Golding, S.E., Rosenberg, E., Valerie, N., Hussaini, I., Frigerio, M., Cockcroft, X.F., Chong, W.Y., Hummersone, M., Rigoreau, L., Menear, K.A., O'Connor, M., Povirk, L., van Meter, T. & Valerie, K. (2009) Improved ATM kinase inhibitor KU-60019 radiosensitizes glioma cells, compromises insulin, AKT and ERK prosurvival signaling, and inhibits migration and invasion. *Mol. Cancer Ther.*, 8: 2894-2902.
- [21] Gottlieb, T.M. & Jackson, S.P. (1993) The DNA-dependent protein kinase: requirement for DNA ends and association with Ku antigen. *Cell*, 72: 131-142.
- [22] Griffin, R.J., Fontana, G., Golding, B.T., Guiard, S., Hardcastle, I.R., Leahy, J.J.J., Martin, N., Richardson, C., Rigoreau, L., Stockley, M. & Smith, G.C.M. (2005) Selective benzopyranone and pyrimido[2,1-a]isoquinolin-4-one inhibitors of DNA-dependent protein kinase: synthesis, structure-activity studies, and radiosensitization of a human tumor cell line in vitro. *J. Med. Chem.*, 48: 569-585.
- [23] Hall-Jackson, C.A., Cross, D.A.E., Morrice, N. & Smythe, C. (1999) ATR is a caffeine-sensitive, DNA-activated protein kinase with a substrate specificity distinct from DNA-PK. *Oncogene*, 18: 6707-6713.
- [24] Hartley, K.; Gell, D.; Smith, C.; Zhang, H.; Divecha, N.; Connelly, M.; Admon, A.; Lees-Miller, S.; Anderson, C. & Jackson, S. (1995) DNA-dependent protein kinase catalytic subunit: a relative of phosphatidylinositol 3-kinase and the ataxia telangiectasia gene product. *Cell*, 82: 849-856.
- [25] Hardcastle, I.R., Cockcroft, X., Curtin, N.J., El-Murr, M. D., Leahy, J.J.J., Stockley, M., Golding, B.T., Rigoreau, L., Richardson, C., Smith, G.C.M. & Griffin, R.J. (2005) Discovery of potent chromen-4-one inhibitors of the DNA-dependent protein kinase (DNA-PK) using a small-molecule library approach. *J. Med. Chem.*, 48: 7829-7846.
- [26] Hashimoto, M., Rao, S., Tokuno, O., Yamamoto, K., Takata, M., Takeda, S. & Utsumi, H. (2003) DNA-PK: the major target for wortmannin-mediated radiosensitization by the inhibition of DSB repair via NHEJ pathway. *J. Radiat. Res.*, 44: 151-159.

- [27] Hickson, I., Zhao, Y., Richardoson, C.J., Green, S.J., Martin, N.M.B., Orr, A.I., Reaper, P.M., Jackson, S.P., Curtin, N.J. & Smith, G.C.M. (2004) Identification and characterization of a novel and specific inhibitor of the ataxia-telangiectasia mutated kinase ATM. *Cancer Res.*, 64: 9152-9159.
- [28] Hollick, J.J., Rigoreau, L.J.M., Cano-Soumillac, C., Cockcroft, X., Curtin, N.J., Frigerio, M., Golding, B.T., Guiard, S., Hardcastle, I.R., Hickson, I., Hummersone, M.G., Menear, K.A., Martin, N.M.B., Matthews, I., Newell, D.R., Ord, R., Richardson, C.J., Smith, G.C.M. & Griffin, R.J. (2007) Pyranone, thiopyranone, and pyridone inhibitors of phosphatidylinositol 3-kinase related kinases. Structure-activity relationships for DNA-dependent protein kinase inhibition, and identification of the first potent and selective inhibitor of the ataxia telangiectasia mutated kinase. *J. Med. Chem.*, 50: 1958-1972.
- [29] Hosoi, Y., Matsumoto, Y., Enomoto, A., Morita, A., Green, J., Nakagawa, K., Naruse, K. & Suzuki, N. Suramin sensitizes cells to ionizing radiation by inactivating DNA-dependent protein kinase. *Radiat. Res.* 162: 308-314 (2004).
- [30] Hosoi, Y., Matsumoto, Y., Tomita, M., Enomoto, A., Morita, A., Sakai, K. Umeda, N., Zhao, H.-J., Nakagawa, K., Ono, T. & Suzuki, N. (2002) Phosphorothioate oligonucleotides, suramin and heparin inhibit DNA-dependent protein kinase activity. *Brit. J. Cancer*, 86: 1143-1149.
- [31] Hosoi, Y., Miyachi, H., Matsumoto, Y., Ikehata, H., Komura, J., Ishii, K., Zhao, H.J., Yoshida, M., Takai, Y., Yamada, S., Suzuki, N. & Ono, T. (1998) A phosphatidylinositol 3-kinase inhibitor wortmannin induces radioresistant DNA synthesis and sensitizes cells to bleomycin and ionizing radiation. *Int. J. Cancer*, 78: 642-647.
- [32] Ihara, M., Suwa, A., Komatsu, K., Shimasaki, T., Okaichi, K., Hendrickson, E.A. & Okumura, Y. (1999) Heat sensitivity of double-stranded DNA-dependent protein kinase (DNA-PK) activity. *Int. J. Radiat. Biol.*, 75: 253-258.
- [33] Iliakis, G. & Seamer, R. (1990) A DNA double-strand break repair-deficient mutant of CHO cells shows reduced radiosensitization after exposure to hyperthermic temperatures in the plateau phase of growth. *Int. J. Hyperthermia*, 6: 801-812.
- [34] Ismail, I.H., Marternsson, S., Moshinsky, D., Rice, A., Tang, C., Howlett, A., McMahon, G. & Hammarste, O. (2004) SU11752 inhibits the DNA-dependent protein kinase and DNA double-strand break repair resulting in ionizing radiation sensitization. *Oncogene*, 23: 873-882.
- [35] Izzard, R.A., Jackson, S.P. & Smith, G.C.M. (1999) Competitive and noncompetitive inhibition of the DNA-dependent protein kinase. *Cancer Res.*, 59: 2581-2586.
- [36] Kamping, H.H., Kanon, B., Konings, A.W.T., Stackhouse, M.A. & Bedford, J.S. (1993) Thermal radiosensitization in heat- and radiation-sensitive mutants of CHO cells. *Int. J. Radiat. Biol.*, 64: 225-230.

- [37] Kashishian, A., Douangpanya, H., Clark, D., Schlachter, S.T., Eary, C.T., Schiro, J.G., Huang, H., Burgess, L.E., Kesicki, E.A. & Halbrook, J. (2003) DNA-dependent protein kinase inhibitors as drug candidates for the treatment of cancer. *Mol.Cancer Ther.*, 2: 1257-1264.
- [38] Keegan, K.S., Holzman, D.A., Plug, A.W., Christenson, E.R., Brainerd, E.E., Flaggs, G., Bentley, N.J., Taylor, E.M., Meyn, M.S., Moss, S.B., Carr, A.M., Ashley, T. & Hoekstra, M.F. (1996) The Atr and Atm protein kinases associate with different sites along meiotically pairing chromosomes. *Genes Dev.*, 10: 2423-2437.
- [39] Kim, D., Ouyang, H., Yang, S.H., Nussenzweig, A., Burgman, P. & Li, G.C. (1995) A constitutive heat shock element-binding factor is immunologically identical to the Ku autoantigen. *J. Biol. Chem.*, 270: 15277-15284.
- [40] Kirchgessner, C.; Patil, C.; Evans, J.; Cuomo, C.; Fried, L.; Carter, T.; Oettinger, M. & Brown, M. (1995) DNA-dependent kinase (p350) as a candidate gene for the murine SCID defect. *Science*, 267: 1178-1183.
- [41] Knight, Z.A., Chiang, G.G., Alaimo, P.J., Kenski, D.M., Ho, C.B., Coan, K., Abraham, R.T. & Shokat, K.M. (2004) Isoform-specific phosphoinositide 3-kinase inhibitors from an arylmorpholine scaffold. *Bioorg. Med. Chem.*, 12: 4749-4759.
- [42] Krawczyk, P.M., Eppink, B., Essers, J., Stap, J., Rodermond, H., Odijk, H., Zelensky, A., van Bree, C., Stalpers, L.J., Buist, M.R., Soullie, T., Rens, J., Verhagen, H.J., O'Connor, M.J., Franken N.A., ten Hagen, T.L., Kanaar, R. & Aten, J.A. (2011) Mild hyperthermia inhibits homologous recombination, induces BRCA2 degradation, and sensitizes cancer cells to poly (ADP-ribose) polymerase-1 inhibition. *Proc. Natl. Acad. Sci. USA*, 108: 9851-9856.
- [43] Kruszewski, M., Wojewódzka, M., Iwaneńko, T, Szumiel, I. & Okuyama, A. (1998) Differential inhibitory effect of OK-1035 on DNA repair in L5178Y murine lymphoma sublines with functional or defective repair of double strand breaks. *Mutat. Res.*, 409: 31-36.
- [44] Lau, A., SwinBank, K.M., Ahmed, P.S., Taylor, D.L., Jackson, S.P., Smith, G.C.M. & O'Connor, M.J. (2005) Suppression of HIV-1 infection by a small molecule inhibitor of the ATM kinase. *Nat. Cell Biol.*, 7: 493-500.
- [45] Leahy, J.J.J., Golding, B.T., Griffin, R.J., Hardcastle, I.R., Richardson, C., Rigoreau, L. & Smith, G.C.M. (2004) Identification of a highly potent and selective DNA-dependent protein kinase (DNA-PK) inhibitor (NU7441) by screening of chromenone libraries. *Bioorg. Med. Chem. Lett.*, 14: 6083-6087.
- [46] Lees-Miller, S.P., Chen, Y.-R. & Anderson, C.W. (1990) Human cells contain a DNA-activated protein kinase that phosphorylates simian virus 40 T antigen, mouse p53, and the human Ku autoantigen. *Mol. Cell. Biol.*, 10: 6472-6481.

- [47] Lees-Miller, S., Godbout, R., Chan, D., Weinfeld, M., Day III, R., Barron, G. & Allalunis-Turner, J. (1995). Absence of p350 subunit of DNA-activated protein kinase from a radiosensitive human cell line. *Science*, 267: 1183-1185.
- [48] Lees-Miller, S., Sakaguchi, K., Ullrich, S., Appella, E. & Anderson, C. (1992) Human DNA-activated protein kinase phosphorylates serines 15 and 37 in the amino-terminal transactivation domain of human p53. *Mol. Cell. Biol.*, 12: 5041-5049.
- [49] Li, S., Takeda, Y., Wragg, S., Barrett, J., Phillips, A. & Dynan, W.S. (2003) Modification of the ionizing radiation response in living cells by an scFv against the DNA-dependent protein kinase. *Nucleic Acids Res.*, 31: 5848-5857.
- [50] Ma, Y.X., Fan, S., Yuan, R., Meng, Q., Gao, M., Goldberg, I.D., Fuqua, S.A., Pestell, R.G. & Rosen, E.M. (2003) Role of BRCA1 in heat shock response. *Oncogene*, 22: 10-27.
- [51] Maira, S.M., Stauffer, F., Brueggen, J., Furet, P., Schnell, C., Fritsch, C., Brachmann, S., Chene, P., De Pover, A., Schoemaker, K., Fabbro, D., Gabriel, D., Simonen, M., Murphy, L., Finan, P., Sellers, W. & Garcia-Echeverria, C. (2008) Identification and characterization of NVP-BEZ235, a new orally available dual phosphatidylinositol 3-kinase/mammalian target of rapamycin inhibitor with potent in vivo antitumor activity. *Mol. Cancer Ther.*, 7: 1851-1863.
- [52] Matsumoto, Y., Suzuki, N., Sakai, K., Morimatsu, A., Hirano, K. & Murofushi, H. (1997) A possible mechanism for hyperthermic radiosensitization mediated through hyperthermic lability of Ku subunits in DNA-dependent protein kinase. *Biochem. Biophys. Res. Commun.*, 234: 568-572.
- [53] Mukherjee, B., Tomimatsu, N., Amancherla, K., Camacho, C.V., Pichamoorthy, N. & Burma, S. (2012) The dual PI3K/mTOR inhibitor NVP-BEZ235 is a potent inhibitor of ATM- and DNAPKcs-mediated DNA damage responses. *Neoplasia*, 14: 34-43.
- [54] Nishida, H., Tatewaki, N., Nakajima, Y., Magara, T., Ko, K.M., Hamamori, Y. & Konishi, T. (2009) Inhibition of ATR protein kinase activity by schisandrin B in DNA damage response. *Nucleic Acids Res.*, 37: 5678-5689.
- [55] Nutley, B.P., Smith, N.F., Hayes, A., Kelland, L.R., Brunton, L., Golding, B.T., Smith, G.C.M., Martin, N.M.B., Workman, P., & Raynaud, F.I. (2005) Preclinical pharmacokinetics and metabolism of a novel prototype DNA-PK inhibitor NU7026. *Brit. J. Cancer*, 93: 1011-1018.
- [56] O'Hara, M.D., Pollard, M.D., Wheatley, G., Regine, W.F., Mohiuddin, M. & Leeper, D. B. (1995) Thermal response and hyperthermic radiosensitization of scid mouse bone marrow CFU-C. *Int. J. Radiat. Oncol. Biol. Phys.*, 31: 905-910.
- [57] Peasland, A., Wang, L.-Z., Rowling, E., Kyle, S., Chen, T., Hopkins, A., Cliby, W.A., Sarkaria, J., Beale, G., Edmondson, R.J. & Curtin, N.J. (2011) Identification and evaluation of a potent novel ATR inhibitor NU6027, in breast and ovarian cancer cell lines. *Brit. J. Cancer*, 105: 372-381.

- [58] Peterson, S., Kurimasa, A., Oshimura, M., Dynan, W., Bradbury, E. & Chen, D. (1995) Loss of the catalytic subunit of the DNA-dependent protein kinase in DNA double-strand-break-repair mutant mammalian cells. *Proc. Natl. Acad. Sci. USA*, 92: 3171-3174.
- [59] Price, B.D. & Youmell, M.B. (1996) The phosphatidylinositol 3-kinase inhibitor wortmannin sensitizes murine fibroblast and human tumor cells to radiation and blocks induction of *p53* following DNA damage. *Cancer Res.*, 56: 246-250.
- [60] Raaphorst, G.P., Maude-Leblanc, J. & Li, L. (2004) Evaluation of recombination repair pathways in thermal radiosensitization. *Radiat. Res.*, 161: 215-218.
- [61] Raaphorst, G.P., Thakar, M. & Ng, C.E. (1993) Thermal radiosensitization in two pairs of CHO wild-type and radiation-sensitive mutant cell lines. *Int. J. Hyperthermia*, 9: 383-391.
- [62] Raaphorst, G.P., Yang, D.P. & Niedbala, G. (2004) Is DNA polymerase beta important in thermal radiosensitization? *Int. J. Hyperthermia*, 20: 140-143.
- [63] Rainey, M.D., Charlton, M.E., Stanton, R.V. & Kastan, M.B. (2008) Transient inhibition of ATM kinase is sufficient to enhance cellular sensitivity to ionizing radiation. *Cancer Res.*, 68: 7466-7474.
- [64] Reaper, P.M., Griffiths, M.R., Long, J.M., Charrier, J.-D., MacCormick, S., Charlton, P.A., Golec, J.M.C. & Pollard, J.R. (2011) Selective killing of ATM- or *p53*-deficient cancer cells through inhibition of ATR. *Nat. Chem. Biol.*, 7: 428-430.
- [65] Rosenzweig, K.E., Youmell, M.B., Palayoor, S.T. & Price, B.D. (1997). Radiosensitization of human tumor cells by the phosphatidylinositol3-kinase inhibitors wortmannin and LY294002 correlates with inhibition of DNA-dependent protein kinase and prolonged G2-M delay. *Clin. Cancer Res.*, 3: 1149-1156.
- [66] Sakata, K., Someya, M., Matsumoto, Y., Tauchi, H., Kai, M., Toyota, M., Takagi, M., Hareyama, M. & Fukushima, M. (2011) Gimeracil, an inhibitor of dihydropyrimidine dehydrogenase, inhibits the early step in homologous recombination. *Cancer Sci.*, 102: 1712-1716.
- [67] Sarkaria, J.N., Busby, E.C., Tibbetts, R.S., Roos, P., Taya, Y., Karnitz, L.M. & Abraham, R.T. (1999) Inhibition of ATM and ATR kinase activities by the radiosensitizing agent, caffeine. *Cancer Res.*, 59: 4375-4382.
- [68] Sarkaria, J.N., Tibbetts, R.S., Busby, E.C., Kennedy, A.P., Hill, D.E. & Abraham, R.T. (1998) Inhibition of phosphoinositide 3-kinase related kinases by the radiosensitizing agent wortmannin. *Cancer Res.*, 58: 4375-4382.
- [69] Savitsky, K.; Bar-Shira, A.; Gilad, S.; Rotman, G.; Ziv, Y.; Vanagaite, L.; Tagle, D.A.; Smith, S.; Uziel, T.; Sfez, S.; Ashkenazi, M.; Pecker, I.; Frydman, M.; Harnik, R.; Patanjali, S.R.; Simmons, A.; Clines, G.A.; Sartiel, A.; Jaspers, N.G.J.; Taylor, A.M.R.; Arlett, C.F.; Miki, T.; Weissman, S.M.; Lovett, M.; Collins, F.S. & Shiloh, Y. (1995) A single ataxia telangiectasia gene with a product similar to PI-3 kinase. *Science*, 268: 1749-1753.

- [70] Seno, J.D. & Dynlacht, J.R. (2004) Intracellular redistribution and modification of proteins of Mre11/Rad50/Nbs1 DNA repair complex following irradiation and heat-shock. *J. Cell. Physiol.*, 199: 157-170.
- [71] Smider, V., Rathmell, W.K., Lieber, M.R. & Chu, G. (1994) Restoration of X-ray resistance and V(D)J recombination in mutant cells by Ku cDNA. *Science*, 266: 288-291.
- [72] Spiro, I.J., Denman, D.L. & Dewey, W.C. (1982) Effect of hyperthermia on CHO DNA polymerase alpha and beta. *Radiat. Res.*, 89: 134-149.
- [73] Stockley, M., Clegg, W., Fontana G., Golding, B.T., Martin, N., Rigoreau, L.J., Smith, G.C., & Griffin, R.J. (2001) Synthesis, crystal structure determination, and biological properties of the DNA-dependent protein kinase (DNA-PK) inhibitor 3-cyano-6-hydrazonomethyl-5-(4-pyridyl)pyridin-2-one (OK-1035). *Bioorg. Med. Chem. Lett.*, 11: 2837-2841.
- [74] Taccioli, G.E., Gottlieb, T.M., Blunt, T., Priestley, A., Demengeot, J., Mizuta, R., Lehmann, A.R., Alt, F.W., Jackson, S.P. & Jeggo, P.A. (1994) Ku80: product of the XRCC5 gene and its role in DNA repair and V(D)J recombination. *Science*, 265: 1442-1445.
- [75] Takagi, M., Sakata, K., Someya, M., Tauchi, H., Iijima, K., Matsumoto, Y., Torigoe, T., Takahashi, A., Hareyama, M. & Fukushima, M. (2010) Gimeracil sensitizes cells to radiation via inhibition of homologous recombination. *Radiother. Oncol.*, 96: 259-266.
- [76] Take, Y., Kumano, M., Hamano, Y., Fukatsu, H., Teraoka, H., Nishimura, S. & Okumura, A. (1995) OK-1035, a selective inhibitor of DNA-dependent protein kinase. *Biochem. Biophys. Res. Commun.*, 215: 41-47.
- [77] Take, Y.; Kumano, M.; Teraoka, H.; Nishimura, S. & Okuyama, A. (1996) DNA-dependent protein kinase inhibitor (OK-1035) suppresses p21 expression in HCT116 cells containing wild-type p53 induced by adriamycin. *Biochem. Biophys. Res. Commun.*, 221: 207-212.
- [78] Toledo, L.I., Murga, M., Zur, R., Soria, R., Rodriguez, A., Martinez, S., Oyarzabal, J., Pastor, J., Bischoff, J.R. & Fernandez-Capetillo, O. (2011) A cell-based screen identifies ATR inhibitors with synthetic lethal properties for cancer-associated mutations. *Nat. Struct. Mol. Biol.*, 18: 721-727.
- [79] Veuger, S.J., Curtin, N.J., Richardson, C.J., Smith, G.C.M. & Durkacz, B.W. (2003) Radiosensitization and DNA Repair Inhibition by the Combined Use of Novel Inhibitors of DNA-dependent Protein Kinase and Poly(ADP-Ribose) Polymerase-1. *Cancer Res.*, 63: 6008-6015.
- [80] Walker, A.I., Hunt, T., Jackson, R.J. & Anderson, C.W. (1985) Double-stranded DNA induces the phosphorylation of several proteins including the 90 000 mol. wt. heat-shock protein in animal cell extracts. *EMBO J.*, 4: 139-145.
- [81] Woudstra, E.C., Konings, A.W.T., Jeggo, P.A. & Kampinga, H.H. (1999) Role of DNA-PK subunits in radiosensitization by hyperthermia. *Radiat. Res.*, 152: 214-218.

- [82] Xiong, H., Lee, R.J., Haura, E.B., Edwards, J.G., Dynan, W.S. & Li, S. (2012) Intranuclear delivery of a novel antibody-derived radiosensitizer targeting the DNA-dependent protein kinase catalytic subunit. *Int. J. Radiat. Oncol. Biol. Phys.*, 83: 1023-1030.
- [83] Xiong, H., Li, S., Yang, Z., Burgess, R.B., & Dynan, W.S. (2009) E. coli expression of a soluble, active single-chain antibody variable fragment containing a nuclear localization signal. *Protein Expr. Purif.*, 66: 172-180.
- [84] Xu, M., Myerson, R.J., Straube, W.L., Moros, E.G., Lagroye, I., Wang, L.L., Lee, J.T. & Roti Roti, J.L. (2002) Radiosensitization of heat resistant human tumour cells by 1 hour at 41.1°C and its effect on DNA repair. *Int. J. Hyperthermia*, 18: 385-403.
- [85] Yin, H.-L., Suzuki, Y., Matsumoto, Y, Tomita, M, Furusawa, Y, Enomoto, A, Morita, A, Aoki, M, Yatagai, F, Suzuki, T, Hosoi, Y, Ohtomo, K & Suzuki N. (2004) Radiosensitization by hyperthermia in chicken B lymphocyte cell line DT40 and its derivatives lacking non-homologous end-joining and/or homologous recombination pathways of DNA double-strand break repair. *Radiat Res* 162: 433-441.
- [86] Zhao, Y., Thomas, H.D., Matey, M.A., Cowell, I.G., Rihardson, C.J., Griffin, R.J., Calvert, A.H., Newell, D.R., Smith, G.C.M. & Curtin, N.J. (2006) Preclinical evaluation of a potent novel DNA-dependent protein kinase inhibitor NU7441. *Cancer Res.*, 66: 5354-5362.
- [87] Zhu, W.-G., Seno, J.D., Beck, B.D. & Dynlacht, J.R. (2001) Translocation of MRE11 from the nucleus to the cytoplasm as a mechanism of radiosensitization by heat. *Radiat. Res.*, 156: 95-102.
- [88] Zou, L & Ellege, S.J. (2003) Sensing DNA damage through ATRIP recognition of RPA-ssDNA complex. *Science*, 300: 1542-1548.

