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Impaired DNA Damage Response – an Achilles' Heel Sensitizing Cancer to Chemotherapy and Radiotherapy

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Abstract

Despite the progress in targeting particular molecular abnormalities specific to different cancers (targeted therapy), chemo- and radiotherapies are still the most effective of all anticancer modalities. Induction of DNA damage and inhibition of cell proliferation are the objects of most chemotherapeutic agents and radiation. Their effectiveness was initially thought to be due to the high rate of proliferation of cancer cells. However, normal cell proliferation rate in some tissues often exceeds that of curable tumors. Most tumors have impaired DNA damage response (DDR) and the evidence is forthcoming that this confers sensitivity to chemo- or radiotherapy. DDR is a complex set of events which elicits a plethora of molecular interactions engaging signaling pathways designed to: (a) halt cell cycle progression and division to prevent transfer of DNA damage to progeny cells; (b) increase the accessibility of the damaged sites to the DNA repair machinery; (c) engage DNA repair mechanisms and (d) activate the apoptotic pathway when DNA cannot be successfully repaired. A defective DDR makes cancer cells unable to effectively stop cell cycle progression, engage in DNA repair and/or trigger the apoptotic program when treated with DNA damaging drugs. With continued exposure to the drug, such cells accumulate DNA damage which leads to their reproductive death that may have features of cell senescence. Cancers with nonfunctional BRCA1 and BRCA2 are particularly sensitive to combined treatment with DNA damaging drugs and inhibitors of poly(ADP-ribose) polymerase. Antitumor strategies are being designed to treat cancers having particular defects in their DDR, concurrent with protecting normal cells.

Keywords

ATM; gammaH2AX; Chk1; Chk2; BRCA1; BRCA2

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1. Targeted therapy, cell proliferation rate and chemotherapy

The remarkable progress in molecular biology during the past two decades has led to the discovery and identification of a vast number of abnormalities associated with neoplastic growth. This progress stimulated attempts to develop targeted therapies aimed at selectively inhibiting growth of cancer cells by interfering with specific molecules or signal transduction pathways essential for their growth. Imatinib mesylate (Gleevec, also known as STI-571) developed to treat Philadelphia chromosome positive chronic myelogenous leukemia is an example of one of the more successful applications of the principle of targeted therapy (Muller, 2009). Its success stems from the fact that the target (Bcr-Abl tyrosine kinase) fulfils two criteria: (a) it is a protein essential for cell growth; and (b) it is unique to this type of leukemia and not present in normal cells. In a variety of other tumors, however, no such unique features have been detected thus far and attempts to treat patients based on this principle, especially targeting most common cancer types including solid tumors, have been less successful. For most tumors chemo- or radiotherapy is, and is likely to remain in the near future, the most effective of all anticancer modalities. Both chemo- and radiotherapy are designed to kill cancer cells by damaging nuclear DNA. While this review is focused primarily on chemotherapy, the issues discussed herein are relevant to radiotherapy as well.

The ultimate goal of chemotherapy is to selectively kill cancer cells while sparing normal cells. The tumors that have rapidly proliferating cells tend to be the most sensitive to chemotherapy. In addition, the proclivity of cells to undergo apoptosis, as exemplified by some leukemias, plays a key role in conferring sensitivity to chemotherapy. In instances such as testicular cancer, childhood acute lymphocytic leukemia, Ewing's sarcoma, Wilm's tumor or gestational choriocarcinoma, chemotherapy is highly effective. But chemotherapy's success rate varies depending on tumor type and grade, and in many instances the treatment is ineffective. In addition to targeting cancer cells, chemotherapy also kills non-tumor cells that rapidly proliferate such as in the bone marrow, digestive tract or hair follicles. This leads to the most common side-effects including myelosuppression, mucositis and alopecia. Thus, the success of chemotherapy is based on the delicate balance between the extent of the damage it induces in cancer cells versus the damage done to the normal, rapidly proliferating cells. As a result, treatment-induced toxicity can be lethal even in situations where cancer cells have already been eradicated.

The successes of chemotherapy were initially thought to occur as the result of the differences in the rate of cell proliferation of the cancer cells as compared with the normal cells of the patient. In fact, antitumor strategies were designed to schedule chemotherapy based on the cell cycle kinetics of tumors (Braunschweiger and Schiffer, 1980; Braunschweiger et al., 1979; Hill, 1975; Karp and Burke, 1976). Cytokinetic parameters were considered as predictive markers of tumor sensitivity to treatment (Amadori et al., 2004; Brons et al., 1993). Attempts at cell synchronization in treatment protocols aimed to effectively target cancer cells, however, did not yield the expected results (Kremer et al., 1976). One cause of the lack of success was the difficulty in accurately assessing cell proliferation rates in tumors. Heterogeneity, expressed as differences in proliferation rates of individual cells in the tumor, is another reason why the synchronization attempts were not as successful as initially anticipated.

In many organs, proliferation of normal cells is rapid, often more rapid than in tumors that can be successfully treated with chemotherapy. Turnover of hematopoietic cells in the bone marrow is particularly rapid and often exceeds that of the leukemic cells (Clarkson et al., 1967; Dormer et al., 1976). Epithelial cells in the mucosa of the gastrointestinal tract (Boyes et al., 1971; Lipkin, 1973) or epidermal skin and hair follicle cells are also characterized by rapid proliferation (Hoogduijn et al., 2006). There is a growing body of evidence that in the instances

of curable cancers other factors, in addition to the differences in cell proliferation rates, contribute to the selective elimination of tumor cells by chemotherapy.

The most effective chemotherapeutic agents target DNA, inducing different types of DNA damage. Among them are DNA topoisomerase I (TOP1) and topoisomerase II (TOP2) inhibitors, as well as DNA alkylating or crosslinking agents. DNA damage triggers the DNA damage response (DDR), an highly intricate set of events involving the induction of a plethora of post-translational modifications of proteins that trigger interactions between intracellular molecules and activate variety of interactive signaling pathways. The signaling pathways activated during DDR have three critical goals: (i) halting cell cycle progression and division to prevent transfer of DNA damage to progeny cells; (ii) increasing accessibility of the damage sites to- and engagement of- the DNA damage repair machinery, and (iii) triggering apoptosis to exterminate cells whose damaged DNA cannot successfully be repaired (reviews, Bakkenist and Kastan, 2003, 2004; Burma et al., 2001; Helt et al., 2005; Kastan, 2008; Kastan and Lim, 2000; Lee and Paul, 2005; Paull and Lee, 2005). In this review we discuss the evidence consistent with the notion that a defective DNA damage response, a feature that is common to many cancers, is a significant contributor to the success of chemotherapy.

2. DNA damage response

2.1. Activation of ATM

One of the early events of the DDR is remodeling of chromatin structure leading to its decondensation (relaxation) (Halicka et al. 2009b; Murga et al., 2007; Pandita and Richardson, 2009; Rouleau et al., 2004; Ziv et al., 2006). Relaxation of the torsional (topological) stress of the DNA double helix (DNA conformational change) resulting from DNA damage, particularly in the case of formation of DNA double-strand breaks (DSBs), triggers chromatin decondensation. The decondensation enhances the accessibility of the sites of DNA damage to the repair machinery and the presence of wt p53 appears to be required for the decondensation to occur (Rubbi and Milner, 2003). Translocation of the MRN complex consisting of Meiotic Recombination 11 Homolog A (Mre11), Rad50 homolog and Nijmegen Breakage Syndrome 1 (NMR1) into the damage site and activation of *Ataxia telangiectasia*, mutated (ATM) take place concurrently with chromatin decondensation (Abraham and Tibbetts, 2005; Downs and Cote, 2005; Paull and Lee, 2005) (Figure 1). The activation of ATM does not takes place at the exact site of the DSB but at some distance from it, and seems to be triggered by chromatin decondensation and relaxation of the torsional stress of the DNA double helix upon induction of the DSB (Kitagawa and Kastan, 2005).

ATM is the key component of the signal transduction pathways mobilized by DNA damage and is essential for safeguarding genomic integrity (Biton et al., 2008; Li and Zou, 2005; Shiloh, 2003). The MRN complex plays a key role in the process of ATM activation, since it recognizes DNA damage, recruits ATM to the damage site and also targets ATM to its respective substrates to initiate their phosphorylation (Lee and Paull, 2005). Activation of ATM occurs through autophosphorylation on *Ser1981* and it requires its prior acetylation which is mediated by the Tip60 histone acetyltransferase (Sun et al., 2005). *Ser1981* ATM phosphorylation causes dissociation of the inactive ATM dimers into monomers that have kinase catalytic activity (Bakkenist and Kastan, 2003, 2004). While ATM phosphorylation on *Ser1981* is required for dissociation of the dimer, the catalytic domain of ATM is located outside of the *Ser1981* site but becomes accessible to the substrates only if ATM is in its monomeric form (Bakkenist and Kastan, 2004).

Exposure of cells to ionizing radiation triggers activation of ATM which was shown to correlate strongly with the number of DSBs but not with the number of single-strand (ss) DNA breaks (SSBs) or other ss DNA lesions (Ismail et al., 2005). It should be noted that DSBs constitute

only a small fraction of radiation-induced DNA damage; SSBs and base damage account for over 98% of the overall damage. Interestingly, the cells in which DSBs are induced by certain radiomimetic agents such as eneidine C-1027 can activate DDR in the absence of ATM (Dziegielewski and Beerman, 2002).

2.2. Substrates phosphorylated (activated) by ATM

At the site of the DSB, ATM phosphorylates several substrates including NBS1, Structural Maintenance of Chromosomes 1 (SMC1) and Breast Cancer Susceptibility Gene 1 (BRCA1) protein. Phosphorylated NBS1 targets ATM towards checkpoint kinase 1 (Chk1), phosphorylated SMC1 engages the S-phase checkpoint momentarily halting DNA replication (Kitagawa et al., 2004; Wakeman et al., 2004; Yazdi et al., 2002), whereas BRCA1 phosphorylation is essential to activate this protein along the DNA repair pathway. The BRCA1 protein (E3-ubiquitin ligase) is implicated in several biochemical processes primarily related to DNA repair (Kastan, 2008; Kitagawa and Kastan, 2005). BRCA2 is essential for the localization of RAD51 [RAD51 homolog, RecA homolog, (*E. coli*) (*S. cerevisiae*)] to the sites of DNA damage and both proteins are involved in DNA repair by homologous recombination (HR) (Yuan et al., 1999). The mediator of DNA damage checkpoint 1 (MDC1) is also recruited to the site of DSBs. MDC1 activates intra-S phase and G₂/M phase cell cycle checkpoints (Stucki and Jackson, 2004). This nuclear protein interacts with phosphorylated histone H2AX near sites of DSBs and facilitates recruitment of ATM and other repair factors to the foci of DNA damage.

2.3. Activation of checkpoint kinases

Downstream target substrates phosphorylated by ATM also include p53 (TP53), checkpoint kinase 2 (Chk2) and histone H2A.X (Bakkenist and Kastan, 2004; Wakeman et al., 2004). The purpose of activation of the checkpoint pathways is to arrest progression through the cell cycle until the integrity to DNA is restored by the repair mechanisms (Ahn et al., Matsuoka et al., 1998; Zhou and Elledge, 2000). Chk 2 plays a central role in the response of the cell cycle progression machinery to DNA damage (Matsuoka et al., 1998). ATM activates Chk2 by phosphorylating *Thr68* of this protein which leads to its dimerization and acquirement of the kinase catalytic activity (Ahn et al., 2000; Ahn et al., 2002). In certain instances, such as in response to replication stress, phosphorylation of Chk2 on *Thr68* may be mediated by ATR (Matsuoka et al., 2002). Dimerization of Chk2 facilitates its subsequent intermolecular phosphorylation on *Thr383*, *Thr387* and *Ser516* which in turn leads to dissociation of the dimers and formation of monomers which unlike the *Thr68* phosphorylated dimers are also enzymatically active (Figure 2). Chk2 activated by damage to DNA undergoes dissociation from chromatin facilitating further signal amplification and translocation to soluble substrates (Li and Stern, 2005). Both, the enzymatically active dimers and monomers of Chk2 phosphorylate numerous substrates including Cdc25C and Cdc25A phosphatases which upon activation are responsible for cell arrest at the G₂ to M transition or in G₁ phase, respectively (Figure 2).

Chk2 also plays a role in regulating the response to DNA-damage by promoting apoptosis. In response to DNA damage by DNA TOP2 inhibitor such as etoposide, Chk2 phosphorylates and activates the E2F-1 transcription factor that increases a cell's proclivity to undergo apoptosis (Stevens et al., 2003). Phosphorylation of the tumor suppressor protein p53 by Chk2 may lead to upregulation of Bax, an apoptosis promoting event. On the other hand p53 phosphorylation can lead to upregulation of p21^{Waf1} thereby providing an additional means to arrest cells in G₁ (Lin et al., 1996). Furthermore, in response to DNA damage the checkpoint pathway scaffold proteins BCRA1 and Promyelocytic Leukemia (PML) may also be phosphorylated by Chk2 (Lee et al., 2000; Yang et al., 2002). As mentioned, phosphorylation of BRCA1 engages this protein in the DNA repair pathway while phosphorylation of PML

sensitizes cells to undergo apoptosis (Ahn et al., 2004). Chk2 also mediates stabilization of the FoxM1 transcription factor thereby stimulating expression of DNA repair genes (Tan et al., 2007). There is significant redundancy between Chk1 and Chk2 protein kinases as well as among all three isoforms of Cdc25 (Cdc25A, Cdc25B and Cdc25C) (Boutros et al, 2006; Peng et al, 1997; Rudolph, 2007).

3. Phosphorylation of histone H2A.X on serine 139 and tyrosine 142

As mentioned, one of substrates phosphorylated by ATM is histone H2A.X, the variant of histone H2A (Burma et al., 2001). H2A.X plays a critical role in maintaining genome integrity and its loss leads to genomic instability (Bonner et al., 2008; Celeste et al., 2001). H2A.X $-/-$ mice are radiation sensitive, growth retarded, and immuno-deficient. H2A.X haploinsufficiency compromises genetic integrity and enhances susceptibility to cancer (Celeste et al., 2003). In response to DNA damage involving formation of DSBs, H2A.X is phosphorylated by ATM on Ser139; phosphorylated H2A.X has been named γ H2A.X (Sedelnikova et al., 2002). H2A.X phosphorylation takes place in multiple nucleosomes spanning a megabase of DNA length on each side of the DSB. H2A.X can also be phosphorylated by other phosphoinositide 3-kinase related protein kinases (PIKKs) such as ATM and Rad3-related (ATR), and/or DNA dependent protein kinase (DNA-PKcs) (Helt et al., 2005; Cuadrado et al., 2006). For example, in cells subjected to replication stress by hydroxyurea or aphidicolin, H2A.X phosphorylation is mediated not by ATM but by ATR (Kurose et al., 2006; Ward et al., 2004). Phosphorylation of H2A.X by DNA-PKcs was reported in hypertonically-stressed cells (Raitsema et al., 2005) and during apoptosis (Mukherjee et al., 2006). However, during apoptosis, concurrent with the appearance of γ H2A.X, ATM is also strongly activated, indicating that ATM mediates H2A.X phosphorylation in response to the formation of DSBs during DNA fragmentation in apoptotic cells (Halicka et al., 2005; Huang et al, 2004a, 2004b; Rogakou et al., 2000; Tanaka et al., 2007). Similarly, photolysis of DNA that also generates DSBs triggers H2A.X phosphorylation mediated by ATM (Huang et al., 2004b). Strong evidence is emerging from a number of publications that H2A.X phosphorylation when it is mediated by ATM reports DNA damage that involves formation of DSBs (Abraham and Tibbets, 2005; Bakkenist and Kastan, 2003, 2004; Bartkova et al., 2005; Burma et al., Kastan and Lim, 2000; Lee and Paull, 2005). It should be noted however, that in response to DNA damage caused by ionizing radiation all three PIKKs, ATM, ATR and DNA-PKcs may function redundantly to phosphorylate H2A.X (Stiff et al., 2005; Wang et al., 2005).

The development of the phospho-specific γ H2A.X Ab (Sedelnikova et al., 2002) made it possible to assess H2A.X phosphorylation in individual cells. In the case of H2A.X phosphorylation triggered by formation of DSBs, γ H2A.X is expressed in nuclei in the form of distinct immunofluorescent foci (IF), each focus representing a single DSB. Thus, it is possible to quantify DSBs by counting the foci; while in most studies γ H2A.X IF foci were counted manually, cell imaging and computer algorithms have also been used to provide their quantitative analysis (Nakamura et al., 2006). Immunocytochemical detection of γ H2A.X offered a means to quantify DNA damage in individual cells in relation to the cell cycle phase and induction of apoptosis by multiparameter flow cytometry (Halicka et al., 2005, 2009a; Huang et al., 2004a, 2004b; MacPhail et al., 2003; Olive, 2004; Tanaka et al., 2007a; 2007b; Zhao et al., 2007, 2008). It should be noted that in addition to activation by exogenous genotoxins whether they be antitumor drugs (Smart et al., 2008; Tanaka et al, 2007a, 2007b), environmental mutagens (Albino et al., 2004; 2006; Pietrzak et al., 2008), or supravital bioprobes (Zhao et al. 2009) H2A.X is also phosphorylated in response to DNA damage induced by endogenous oxidants, likely generated during oxidative metabolic activity in mitochondria (Tanaka et al., 2006; Zhao et al., 2007).

It was recently reported that phosphorylation of H2A.X also serves as a signal to induce apoptosis (Cook et al., 2009; Lukas and Bartek, 2009; Xiao et al., 2009). Specifically, in normal untreated cells the tyrosine 142 (*Tyr142*) on the COOH-terminus of H2A.X remains phosphorylated by the WSTF protein kinase. Upon induction of DSB the Eya1/3 phosphatase replaces WSTF kinase de-phosphorylating *Tyr142*. Dephosphorylation of *Tyr142* facilitates phosphorylation of *Ser139* by ATM which, as described above, leads to the recruitment of MDC1 and other repair factors required for initiation of DNA repair. This is the scenario that unfolds when DNA damage is repairable. However, if DNA damage is extensive and beyond repair, *Tyr142* undergoes re-phosphorylation. Phosphorylated *Tyr142* prevents retention of the repair factors and instead attracts the c-Jun NH2-terminal kinase type 1 (JNK1) complex that promotes apoptosis (Cook et al., 2009; Lukas and Bartek 2009).

4. Impaired DDR in cancer

Susceptibility to cancer is often associated with syndromes that have altered DDR (review: Kastan, 2008). Some of the syndromes that increase the likelihood of cancer development have defects in specific DNA repair mechanisms. Fanconi's anemia, for example, is characterized by the mutation of genes involved in repair of DNA cross-links such that patients are prone to myeloid leukemias (de Winter and Joenje, 2008). Individuals with *Xeroderma pigmentosum* have defective genes involved in DNA excision repair and this makes them sensitive to UV-induced DNA damage (Dergoglio and Magnaldo, 2008). Hereditary nonpolyposis colon cancer syndrome that sensitizes individuals to colorectal, uterine and other sporadic cancers is characterized by a defect in the DNA mismatch repair (MMR) mechanism (Hsieh and Yamane, 2008). Mutations of genes in other syndromes predisposing patients to cancer affect not only specific DNA repair mechanisms but also impair activation of cell cycle checkpoints. A classic example is *Ataxia-telangiectasia* (A-T), a syndrome caused by mutations of ATM, which predisposes individuals to a variety of cancers and neurodegenerative diseases (Lavin, 2008). ATM is upstream of so many regulatory pathways that a defect in this gene not only impairs DNA repair processes but can also perturb cell cycle progression at any phase of the cell cycle as well as affect the cell's propensity to undergo apoptosis (Biton et al., 2008). Nijmegen breakage syndrome to a certain extent resembles ataxia-telangiectasia, in that it also is characterized by defects in DNA repair and cell cycle checkpoints (Difilippantonio and Nussenzweig 2007). Mutation of either the BRCA1 or BRCA2 gene strongly predisposes to cancer, particularly of the breast and ovary (Ashworth, 2008; Fackenthal and Olopade, 2007).

Listed above are the classic examples of cancers that originate in patients with distinct germline abnormalities of the DNA damage response machinery. However, many primary cancers that develop in patients who do not have germline mutations in these genes can also have impaired DDR (Stawinska et al., 2008). In fact, tumor progression is often associated with progressive impairment of DDR (Seoane et al., 2008; Wang and Figg, 2008). Furthermore, in early pre-malignant lesions the oncogene-driven DNA replication causes replication stress in which the stalling of replication forks cause DNA breakage, formation of DSBs and "constitutive" activation of DDR (Bartkova et al., 2005; 2007). This leads to the damage and inactivation of many genes, among them the ones coding for proteins involved in DDR, and allows the genetically unstable cancer cells to survive and acquire further genomic instability. In exceptional cases however, such as testicular germ cell tumors, similar as in their precursors gonocytes, this process of constitutive DDR activation is suppressed. Accumulation of DNA defects and progression of genomic instability is therefore minimal in these tumors, and this may contribute to their exceptional curability (Bartkova et al., 2007). It should be noted that genomic instability is further amplified, and the cell capability to effectively carry out the DDR progressively deteriorates, during chemo- or radiotherapy (Borst et al., 2008; Wynne et al., 2007). It is safe to assume therefore that regardless of the mechanism responsible for the

impairment of DDR a large majority of cancers to be clinically treated have defects in one or more elements of the DDR machinery.

5. Targeting DDR in cancer treatment

Cancer appears to be a multi-heeled monster and many of its heels can provide therapeutic targets. A search of the literature in PubMed under the query “*Achilles heel - cancer*” reveals 118 publications. Of that number, about 100 pertain to cancer treatment. Altered mitochondria, telomeres, tumor vasculature, hypoxia, mutated p53 and a variety of oncogenes or tumor suppressor genes each have been identified in these publications as cancer’s “*Achilles’ heel*” (e.g. Blagosklonny, 2001), a potential target for anticancer therapy. Only four of those articles list the impaired DDR collectively as an “*Achilles heel*” that can be exploited in chemo- or radiotherapy (Caldecott and Chalmers, 2005; Eastman, 2004; Kyle et al., 2008; Lord et al., 2006).

Several variants of the anti-cancer strategy that targets DDR have been proposed and some were tested in preclinical and clinical trials. One variant is aimed towards disruption of the DDR to further impair the effectiveness of DNA repair (Lieberman, 2008; Martin et al., 2008). This approach utilizes inhibitors of particular elements of the DNA repair machinery to be used concurrently with chemotherapy to augment the extent of DNA damage. One of the ATM inhibitors (KU55933) was shown to effectively sensitize cancer cells to DNA damaging drugs (Hickson et al., 2004). Likewise, several inhibitors of DNA-PKcs have been tested and found to enhance the cytotoxicity of chemotherapeutics both in vitro and in vivo in mice xenograft models (Ding et al., 2006; Madhusudan and Middleton, 2005). Downstream of ATM, the inhibitors of Chk1 and Chk2 protein kinases have also been proposed to enhance the cytotoxicity of antitumor drugs (Collins and Garrett, 2005; Eastman, 2004; Garber, 2005; O’Connor et al., 2007; Syljuasen et al., 2004; Tao and Lin, 2006). Further downstream of ATM, inhibitors of Cdc25 phosphatases have been considered either as antitumor agents acting alone (Boutros et al., 2007; Brezak et al., 2008; Wang et al., 2008), or as enhancers of cell sensitivity to DNA damaging drugs (Aressy et al., 2008; Deep and Agarwal, 2008).

While this approach of using inhibitors of DDR in conjunction with chemotherapy does enhance cell sensitivity to the treatment, it does not provide for selective eradication of cancer cells. Since normal cells are exposed to these inhibitors as well, their sensitivity to the chemotherapeutic agents is being increased thereby contributing to the chemotherapy-induced side-effects. Because of the lack of cancer-selectivity it is difficult to define such strategies as targeting the cancer’s “*Achilles’ heel*”. While this approach may lower the drug or radiation doses necessary to induce a tumor response, the sensitivity of normal cells is also enhanced and the *differential* tumor versus normal cell response (“therapeutic window”) may not be significantly improved (Helleday, 2008).

5.1. Therapeutic advantage of BRCA mutations; the “synthetic lethality” opportunity

In contrast to the above, cancers bearing BRCA1 and BRCA2 mutations offer an attractive *Achilles heel* to be exploited. The cells deficient in BRCA1 or BRCA2s, due to their intrinsically impaired ability to repair DNA damage, are highly sensitive to radiation (Shen et al., 1998) and to certain DNA damaging drugs such as cisplatin (Kennedy et al., 2004; Powell and Kachnie, 2008; Tassone et al., 2009). Clinical observations indicate that survival of patients with BRCA-associated ovarian cancers treated with cisplatin is improved relative to the patients with cancers without BRCA mutations (Cass et al., 2003; Pal et al., 2007; Quinn et al., 2009). In vitro studies in which transformed normal and breast cancer cell lines expressing no, low or normal levels of BRCA1 were exposed to DNA crosslinking agents such as mitomycin C or cisplatin demonstrated that loss of BRCA1 expression correlated with

increased drug sensitivity leading to premature senescence and reproductive death rather than to apoptosis (Santarosa et al., 2009).

An additional therapeutic advantage can be achieved by treatment of BRCA1 or BRCA2 cancers with poly(ADP-ribose) polymerase (PARP) inhibitors (Ashworth, 2008; Caldecott and Chalmers, 2005; Lord and Ashworth, 2008; Lord et al., 2006). Namely, the combination of a non-functional BRCA1 or BRCA2 with inhibition of PARP activity creates a “synthetic lethality” condition that can be exploited to selectively eliminate cancer cells (Kaelin 2005). The “synthetic lethality” principle, described over a half a century ago (Dobzhansky, 1946), defines a state in which mutation of either of two genes has no effect whereas the combined mutation of both genes is lethal. The absence of functional BRCA1 or BRCA2 impairs the cells ability to repair DNA damage through the mechanism of homologous recombination (HR). Any DNA damage would then have to be repaired by the alternative, although error-prone, mechanism of nonhomologous end-joining (NHEJ), or by single-strand annealing (SSA), utilizing PARP. The loss of PARP activity increases the formation of DNA lesions that otherwise would be repaired by the BRCA2 pathway. A combination of a nonfunctional BRCA2 repair pathway with inhibition of PARP creates a “synthetic lethality” status for the cancer cells dramatically expanding the “therapeutic window” (Ashworth, 2008). Inactivation of BRCA1 or BRCA2 is relatively common in breast and ovarian cancer and this strategy therefore may have significant clinical implementation.

6. Final reflections

It is quite evident from the data discussed above that DNA damage in normal non-tumor cells triggers very efficient machinery that momentarily halts cell cycle progression, engages the DNA repair mechanisms and when the damage is severe prepares cells to undergo apoptosis. Cell cycle progression can be stopped at each phase of the cell cycle and the mechanisms arresting the cell progression are highly redundant. As mentioned, there is strong redundancy between ATM, ATR and DNA-PKcs, between Chk1 and Chk2 protein kinases as well as between all three isoforms of Cdc25 phosphatases. Their activation stops cell progression in G₁ as well as in G₂. Another back-up/redundant mechanism of arrest in G₁ involves upregulation of the cyclin-dependent kinases inhibitor p21^{WAF1}. The cell arrest in S phase can be mediated by phosphorylation of SMC1.

Concurrent with the arrest of cell cycle progression, the mechanisms preparing the cell to undergo apoptosis are also mobilized. These mechanisms also have several redundant and back-up pathways, such as upregulation of Bax through phosphorylation of p53, maintenance of the phosphorylation of Tyr142 on histone H2AX and/or activation of the E2F-1 transcription factor. The critical decision to undergo apoptosis depending on whether DNA damage is repairable or not appears to be under the control of histone H2A.X phosphorylation on Tyr142 versus Ser139 (Cook et al., 2009; Lukas and Bartek, 2009). To recapitulate, the induction of DNA damage to normal cells momentarily freezes their progression through the cycle preventing further accumulation of DNA damage. In contrast, in cancer cells with an impaired DDR, progression through the cycle in the presence of the genotoxic agent augments the extent of the damage which ultimately may lead to reproductive cell death.

It should be noted that the therapeutic window for chemo- or radiotherapy can be additionally improved by halting cell cycle progression of normal cells just prior to treatment. Since many tumors have impaired regulation of cell cycle control, it is possible to arrest cell cycle progression of normal cells without affecting progression of cancer cells. DNA damage is then additionally amplified preferentially in cancer cells. This approach, initially proposed by Pardee (Pardee, 1974, Pardee and James, 1975), has more recently been promoted in several

publications (Blagosklonny and Darzynkiewicz, 2002; Blagosklonny and Pardee, 2002; Bruno et al., 1992).

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References

- Abraham RT, Tibbetts RS. Guiding ATM to broken DNA. *Science* 2005;308:510–511. [PubMed: 15845843]
- Ahn JY, Schwartz JK, Piwnica-Worms H, Canman CE. Threonine 68 phosphorylation by ataxia telangiectasia mutated is required for efficient activation of Chk2 in response to ionizing radiation. *Cancer Res* 2000;60:5934–5936. [PubMed: 11085506]
- Ahn JY, Li X, Davis HL, Canman CE. Phosphorylation of threonine 68 promotes oligomerization and autophosphorylation of Chk2 protein kinase via the forkhead-associated domain. *J Biol Chem* 2002;277:19389–19395. [PubMed: 11901158]
- Ahn J, Urist M, Prives C. The Chk2 protein kinase. *DNA Repair* 2004;3:1039–1047. [PubMed: 15279791]
- Albino AP, Huang X, Yang J, Gietl D, Jorgensen E, Traganos F, Darzynkiewicz Z. Induction of histone H2AX phosphorylation in A549 human pulmonary epithelial cells by tobacco smoke and in human bronchial epithelial cells by smoke condensate: A new assay to detect the presence of potential carcinogens in tobacco. *Cell Cycle* 2004;3:1062–1068. [PubMed: 15254392]
- Albino AP, Huang X, Jorgensen E, Gietl D, Traganos F, Darzynkiewicz Z. Induction of DNA double-strand breaks in A549 and normal human pulmonary epithelial cells by cigarette smoke is mediated by free radicals. *Int J Oncol* 2006;28:1491–1505. [PubMed: 16685450]
- Amadori D, Volpi A, Maltoni R, Nanni O, Amaducci L, Amadori A, Giunchi DC, Vio A, Saragoni A, Silvestrini R. Cell proliferation as a predictor of a response to chemotherapy in metastatic breast cancer: A prospective study. *Breast Canc Res Treat* 2004;43:7–14.
- Aressy B, Bugler B, Valette A, Biard D, Ducommun B. Moderate variation in CDC25B protein levels modulate the response to DNA damaging agents. *Cell Cycle* 2008;7:2234–2240. [PubMed: 18635965]
- Ashworth A. A synthetic lethal therapeutic approach: poly(ADP)ribose polymerase inhibitors for the treatment of cancers deficient in DNA double-strand break repair. *J Clin Oncol* 2008;26:3785–3790. [PubMed: 18591545]
- Bakkenist CJ, Kastan MB. DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. *Nature* 2003;421:499–506. [PubMed: 12556884]
- Bakkenist CJ, Kastan MB. Initiating cellular stress responses. *Cell* 2004;118:9–17. [PubMed: 15242640]
- Bartkova J, Bakkenist CJ, Rajpert-De Meyts E, Skakkebaek NE, Sehested M, Lukas J, Kastan MB, Bartek J. ATM activation in normal human tissues and testicular cancer. *Cell Cycle* 2005;4:838–845. [PubMed: 15846060]
- Bartkova J, Rajpert-De Meyts E, Skakkebaek NE, Lukas J, Bartek J. DNA damage response in human testes and testicular germ cell tumours: biology and implications for therapy. *Int J Androl* 2007;30:282–291. [PubMed: 17573848]
- Biton S, Barzilay A, Shiloh Y. The neurological phenotype of ataxia-telangiectasia: solving a persistent puzzle. *DNA Repair* 2008;7:1028–1038. [PubMed: 18456574]
- Blagosklonny MV. Hypoxia-inducible factor: Achilles' heel of antiangiogenic cancer therapy (review) *Int. J Oncol* 2001;19:257–262.
- Blagosklonny MV, Darzynkiewicz Z. Cyclotherapy: Protecting of normal cells and unshielding of cancer cells. *Cell Cycle* 2002;1:375–382. [PubMed: 12548008]
- Blagosklonny MV, Pardee AB. The restriction point of the cell cycle. *Cell Cycle* 2002;1:103–110. [PubMed: 12429916]
- Bonner WM, Redon CE, Dickey JS, Nakamura AJ, Sedelnikova OA, Solier S, Pommier Y. γ H2AX and cancer. *Nat Reviews* 2008;8:957–967.

- Borst P, Rottenberg S, Jonkers J. How do real tumors become resistant to cisplatin? *Cell Cycle* 2008;7:1353–1359. [PubMed: 18418074]
- Boutros R, Dozier C, Ducommun B. The when and wheres of CDC25 phosphatases. *Curr Opin Cell Biol* 2006;18:185–191. [PubMed: 16488126]
- Boutros R, Lobjois V, Ducommun B. CDC25 phosphatases in cancer cells: key players? Good targets? *Nat Rev Cancer* 2007;7:495–507. [PubMed: 17568790]
- Boyes BE, Crean GP, Watkinson G. Studies in the rates of epithelial cell exfoliation from the gastric mucosa in normal and in ulcer subjects. *Gut* 1971;12:867–869. [PubMed: 5123296]
- Braunschweiger PG, Schenken LL, Schiffer LM. The cytokinetic basis for the design of efficacious radiotherapy protocols. *Int J Radiat Oncol Biol Phys* 1979;5:37–47. [PubMed: 422414]
- Braunschweiger PG, Schiffer LM. Cell kinetic-directed sequential chemotherapy with cyclophosphamide and adriamycin in T1699 mammary tumors. *Cancer Res* 1980;40:737–743. [PubMed: 7471093]
- Brezak MC, Kasprzyk PG, Galcera MO, Lavergne O, Prevost GP. CDC25 inhibitors as anticancer agents are moving forward. *Anticancer Agents Med Chem* 2008;8:857–862. [PubMed: 19075568]
- Brons PP, Haanen C, Boezeman JB, Muus P, Holdrinet RR, Pennings AH, Wessels HM, de Witte T. Proliferation patterns in acute myeloid leukemia: leukemic clonogenic growth and in vivo cell kinetics. *Ann Hematol* 1993;66:225–233. [PubMed: 8507717]
- Bruno S, Ardelt B, Skierski JS, Traganos F, Darzynkiewicz Z. Different effects of staurosporine, an inhibitor of protein kinases, on the cell cycle and chromatin structure of normal and leukemic lymphocytes. *Cancer Res* 1992;52:470–473. [PubMed: 1728418]
- Burma S, Chen BP, Murphy M, Kurimasa A, Chen DJ. ATM phosphorylates histone H2AX in response to DNA double-strand breaks. *J Biol Chem* 2001;276:42462–42467. [PubMed: 11571274]
- Caldecott KW, Chalmers A. An Achilles' heel for breast cancer? *Nat Struct Molec Biol* 2005;12:387–398. [PubMed: 15870726]
- Cass I, Baldwin RL, Varkey T, Moslehi R, Narod SA, Karlan BY. Improved survival in women with BRCA-associated ovarian carcinoma. *Cancer* 2003;97:2187–2195. [PubMed: 12712470]
- Celeste A, Paterson S, Romanienko PJ, Fernandez-Capetillo O, Chen HT, Sedelnikova A, Reina-San MB, Coppola V, Meffre E, Difilippantonio MJ, Redon C, Pilch DR, Oлару A, Eckhaus M, Camerini-Otero RD, Tessarollo L, Livak F, Manova K, Bonner WM, Nussenzweig MC, Nussenzweig A. Genomic instability in mice lacking histone H2AX. *Science* 2001;296:922–927. [PubMed: 11934988]
- Celeste A, Difilippantonio S, Fernandez-Capetillo O, Pilch DR, Sedelnikova O, Eckhaus M, Ried T, Bonner WM, Nussenzweig A. H2AX haploinsufficiency modifies genomic stability and tumor susceptibility. *Cell* 2003;114:371–383. [PubMed: 12914701]
- Clarkson B, Ohkita T, Ota K, Fried J. Studies of cellular proliferation in human leukemia. I Estimation of growth rates of leukemic and normal hematopoietic cells in two adults with acute leukemia given a single injection of tritiated thymidine. *J Clin Invest* 1967;46:506–525. [PubMed: 5227717]
- Collins I, Garrett MD. Targeting the cell division cycle in cancer: CDK and the cell cycle checkpoint kinase inhibitors. *Curr Opin Pharmacol* 2005;5:366–373. [PubMed: 15964238]
- Cook PJ, Ju BG, Telese F, Wang X, Glass C, Rosenfeld MG. Tyrosine dephosphorylation of H2AX modulates apoptosis and survival decisions. *Nature* 2009;458:591–596. [PubMed: 19234442]
- Cuadrado, M.; Martinez-Pastor, B.; Fernandez-Capetillo, O. ATR activation in response to ionizing radiation: still ATM territory; *Cell Division*. 2006. p. 7<http://www.celldiv.com/content/1/1/7>
- Deep G, Agarwal R. New combination therapies with cell cycle agents. *Curr Opin Invest Drugs* 2008;9:591–604.
- Dergogio V, Magnaldo T. Nucleotide excision repair and related human diseases. *Genome Dyn* 2006;1:35–52. [PubMed: 18724052]
- de Winter JP, Joenje H. The genetic and molecular basis of Fanconi anemia. *Mutat Res*. 2008 Nov 14; (Epub)
- Difilippantonio S, Nussenzweig A. The NBS1-ATM connection revisited. *Cell Cycle* 2007;6:2366–2370. [PubMed: 17881893]
- Ding J, Miao ZH, Meng LH, Geng MY. Emerging cancer therapeutic opportunities target DNA-repair systems. *Trends Pharmacol Sci* 2006;27:338–344. [PubMed: 16697054]

- Dobzhansky T. Genetics of natural populations: XIII. Recombination and variability of in populations of *Drosophila Pseudoobscura*. *Genetics* 1946;31:269–290. [PubMed: 17247197]
- Dormer P, Hegemann F, Brinkmann W. Proliferation and production of hemopoietic cells in two stages of disease: preleukemia and overt leukemia. *Klin Wochenschr* 1976;54:461–466. [PubMed: 1064731]
- Downs JA, Cote J. Dynamics of chromatin during the repair of DNA double-strand breaks. *Cell Cycle* 2005;4:1373–1376. [PubMed: 16205111]
- Dziegielewska J, Beerman TA. Cellular responses to the DNA strand-scission enedine C-1027 can be independent of ATM, ATR and DNA-PK kinases. *J Biol Chem* 2002;277:20549–50554. [PubMed: 11927575]
- Eastman A. Cell cycle checkpoints and their impact on anticancer therapeutic strategies. *J Cell Biochem* 2004;91:223–231. [PubMed: 14743382]
- Fackenthal JD, Olopade OJ. Breast cancer risk associated with BRCA1 and BRCA2 in diverse populations. *Nat Rev Cancer* 2007;7:937–948. [PubMed: 18034184]
- Halicka HD, Huang X, Traganos F, King MA, Dai W, Darzynkiewicz Z. Histone H2AX phosphorylation after cell irradiation with UV-B: Relationship to cell cycle phase and induction of apoptosis. *Cell Cycle* 2005;4:339–345. [PubMed: 15655354]
- Halicka HD, Ozkaynak MF, Levendoglu-Tugal O, Sandoval C, Seiter K, Kajstura M, Traganos F, Jayabose S, Darzynkiewicz Z. DNA damage response as a biomarker in treatment of leukemias. *Cell Cycle* 2009 Jun 30;8:2009a[Epub]
- Halicka HD, Zhao H, Podhorecka M, Traganos F, Darzynkiewicz Z. Cytometric detection of chromatin relaxation, an early reporter of DNA damage response. *Cell Cycle* 2009 Jul 15;8:2009b[Epub]
- Helleday T. Amplifying tumour-specific replication lesions by DNA repair inhibitors – A new era in targeted cancer therapy. *Eur J Cancer* 2008;44:921–927. [PubMed: 18374562]
- Helt CE, Cliby WA, Keng PC, Bambara RA, O'Reilly MA. Ataxia telangiectasia mutated (ATM) and ATM and Rad3-related protein exhibit selective target specificities in response to different forms of DNA damage. *J Biol Chem* 2005;280:1186–1192. [PubMed: 15533933]
- Hickson I, Zhao Y, Richardson CJ, Green SJ, Martin NM, Orr AI, Reaper PM, Jackson SP, Curtin NJ, Smith GC. Identification and characterization of a novel and specific inhibitor of the ataxia-telangiectasia mutated kinase ATM. *Cancer Res* 2004;64:9152–9159. [PubMed: 15604286]
- Hill BT. Cancer chemotherapy. The relevance of certain concepts of cell cycle kinetics. *Biochem Biophys Acta* 1975;516:389–417. [PubMed: 361085]
- Hoogduijn MJ, Gorjup J, Genever PG. Comparative characterization of hair follicle dermal stem cells and bone marrow mesenchymal stem cells. *Stem Cells Develop* 2006;15:49–60.
- Hsieh P, Yamane K. DNA mismatch repair: molecular mechanism, cancer, and ageing. *Mech Ageing Dev* 2008;129:391–407. [PubMed: 18406444]
- Huang X, Okafuji M, Traganos F, Luther E, Holden E, Darzynkiewicz Z. Assessment of histone H2AX phosphorylation induced by DNA topoisomerase I and II inhibitors topotecan and mitoxantrone and by DNA crosslinking agent cisplatin. *Cytometry A* 2004a;58A:99–110.
- Huang X, King M, Halicka DH, Traganos F, Okafuji M, Darzynkiewicz Z. Histone H2AX phosphorylation induced by selective photolysis of BrdU-labeled DNA with UV light: relationship to cell cycle phase. *Cytometry A* 2004b;62A:1–7.
- Ismail IH, Nystrom S, Nygren J, Hammersten O. Activation of ataxia telangiectasia mutated by DNA strand break-inducing agents correlates closely with the number of DNA double strand breaks. *J Biol Chem* 2005;280:4649–4655. [PubMed: 15546858]
- Kaelin WG. The concept of synthetic lethality in the context of anticancer therapy. *Nat Rev Cancer* 2005;5:689–698. [PubMed: 16110319]
- Karp JE, Burke PJ. Enhancement of drug cytotoxicity by recruitment of leukemic myeloblasts with humoral stimulation. *Cancer Res* 1976;36:3600–3603. [PubMed: 1066183]
- Kastan MB. DNA damage responses: Mechanisms and roles in human disease. 2007 G.H.A. Cloves Memorial Award Lecture. *Mol Cancer Res* 2008;6:517–524. [PubMed: 18403632]
- Kastan MB, Lim DS. The many substrates and functions of ATM. *Nat Rev Mol Cell Biol* 2000;1:179–186. [PubMed: 11252893]

- Kennedy RD, Quinn JE, Mullan PB, Johnston PG. The role of BRCA1 in the cellular response to chemotherapy. *JNCI* 2004;96:1659–1668. [PubMed: 15547178]
- Kitagawa R, Bakkenist CJ, McKinnon PJ, Kastan MB. Phosphorylation of SMC1 is a critical downstream event in the ATM-ANBS1-BRCA1 pathway. *Genes Dev* 2004;18:1423–1438. [PubMed: 15175241]
- Kitagawa R, Kastan MB. The ATM-dependent DNA damage signaling pathway. *Cold Spring Harb Symp Quant Biol* 2005;70:99–109. [PubMed: 16869743]
- Kremer WB, Vogler WR, Chan YK. An attempt at synchronization of marrow cells in acute leukemia: relationship to therapeutic response. *Cancer* 1976;37:390–403. [PubMed: 1082365]
- Kurose A, Tanaka T, Huang X, Traganos F, Dai W, Darzynkiewicz Z. Effects of hydroxyurea and aphidicolin on phosphorylation of ATM on Ser 1981 and histone H2AX on Ser 139 in relation to cell cycle phase and induction of apoptosis. *Cytometry A* 2006;69A:212–221. [PubMed: 16528719]
- Kyle S, Thomas HD, Mitchell J, Curtin NJ. Exploiting the Achilles heel of cancer: the therapeutic potential of poly(ADP-ribose) polymerase inhibitors in BRCA2-defective cancer. *Br J Radiol* 81 Spec No 2008;1:S6–11.
- Lavin MF. Ataxia-telangiectasia: from a rare disorder to a paradigm for cell signaling and cancer. *Nat Rev Mol Cell Biol* 2008;9:759–769. [PubMed: 18813293]
- Lee JH, Paull TT. ATM activation by DNA double-strand breaks through the Mre11-Rad50-Nbs1 complex. *Science* 2005;308:551–554. [PubMed: 15790808]
- Lee JS, Collins KM, Brown AL, Lee CH, Chung JH. hCds1-mediated phosphorylation of BRCA1 regulates the DNA damage response. *Nature* 2000;404:201–204. [PubMed: 10724175]
- Li J, Stern DF. DNA damage regulates Chk2 association with chromatin. *J Biol Chem* 2005;280:37948–37956. [PubMed: 16150728]
- Li L, Zou L. Sensing signaling and responding to DNA damage: organization of checkpoint pathways in mammalian cells. *J Cell Biochem* 2005;94:298–306. [PubMed: 15578575]
- Lieberman HB. DNA damage repair and response proteins as targets for cancer therapy. *Curr Med Chem* 2008;15:360–367. [PubMed: 18288990]
- Lin J, Reichner C, Wu X, Levine AJ. Analysis of wild-type and mutant p21WAF-1 gene activities. *Mol Cell Biol* 1996;16:1786–1793. [PubMed: 8657154]
- Lipkin M. Proliferation and differentiation of gastrointestinal cells. *Physiol Rev* 1973;53:891–915. [PubMed: 4583283]
- Lipkin M, Sherlock P, Bell B. Cell proliferation kinetics in the gastrointestinal tract of man. II Cell renewal in stomach, ileum, colon, and rectum. *Gastroenterology* 1966;45:721–729. [PubMed: 14134007]
- Lord CJ, Ashworth A. Targeted therapy for cancer using PARP inhibitors. *Curr Opin Pharmacol* 2008;8:363–369. [PubMed: 18644251]
- Lord CJ, Garrett MD, Ashworth A. Targeting the double-strand DNA break repair pathway as a therapeutic strategy. *Clin Cancer Res* 2006;12:4463–4468. [PubMed: 16899589]
- Lukas J, Bartek J. New tales of an old tail. *Nature* 2009;458:581–583. [PubMed: 19340068]
- MacPhail SH, Banath JP, Yu TY, Chu EH, Lambur H, Olive PL. Expression of phosphorylated histone H2AX in cultured cell lines following exposure to X-rays. *Int J Radiat Biol* 2003;79:351–358. [PubMed: 12943243]
- Madhusudan S, Middleton MR. The emerging role of DNA repair proteins as predictive, prognostic and therapeutic targets in cancer. *Cancer Treat Rev* 2005;31:603–617. [PubMed: 16298073]
- Martin SA, Lord CJ, Ashworth A. DNA repair deficiency as a therapeutic target in cancer. *Curr Opin Genet Dev* 2008;18:80–86. [PubMed: 18343102]
- Matsuoka S, Huang M, Elledge SJ. Linkage of ATM to cell cycle regulation by the Chk2 protein kinase. *Science* 1998;282:1893–1897. [PubMed: 9836640]
- Matsuoka S, Rotman G, Ogawa A, Shiloh K, Tamai SJ, Elledge SJ. Ataxia telangiectasia-mutated phosphorylates Chk2 in vivo and in vitro. *Proc Natl Acad Sci USA* 2000;97:10289–10394.
- Mukherjee B, Kessinger C, Kabayashi J, Chen BP, Chen DJ, Chatterjee A, Burma S. DNA-PK phosphorylates histone H2AX during apoptotic DNA fragmentation in mammalian cells. *DNA Repair (Amst)* 2006;10:575–590. [PubMed: 16567133]
- Muller BA. Imatinib and its successors – how modern chemistry has changed drug development. *Curr Pharm Des* 2009;15:120–133. [PubMed: 19149608]

- Murga M, Jaco I, Soria R, Martinez-Pastor B, Cuadrado M, Yang SM, Blasco MA, Skoultschi AI, Fernandez-Capetillo O. Global chromatin compaction limits the strength of the DNA damage response. *J Cell Biol* 2007;178:1101–1108. [PubMed: 17893239]
- Nakamura A, Sedelnikova OA, Redon C, Pilch DR, Sinogeeva NI, Shroff R, Lichten M, Bonner WM. Techniques for gamma-H2AX detection. *Methods Enzymol* 2006;409:236–250. [PubMed: 16793405]
- O'Connor MJ, Martin NM, Smith GC. Targeted cancer therapies based on the inhibition of DNA strand break repair. *Oncogene* 2007;26:7816–7824. [PubMed: 18066095]
- Olive PL. Detection of DNA damage in individual cells by analysis of histone H2AX phosphorylation. *Methods Cell Biol* 2004;75:355–373. [PubMed: 15603433]
- Pal T, Permuth-Way J, Kapoor R, Cantor R, Sutphen R. Improved survival in BRCA2 carriers with ovarian cancer. *Fam Cancer* 2007;6:113–119. [PubMed: 17160431]
- Pandita TK, Richardson C. Chromatin remodeling finds its place in the DNA double-strand breaks. *Nucleic Acids Res* 2009;1–15. [PubMed: 19033363](Advanced ePub)
- Pardee AB. A restriction point for control of normal animal cell proliferation. *Proc Natl Acad Sci USA* 1974;71:1286–1290. [PubMed: 4524638]
- Pardee AB, James LJ. Selective killing of transformed baby hamster kidney (BHK) cells. *Proc Natl Acad Sci USA* 1975;72:4994–4998. [PubMed: 174089]
- Paull TT, Lee JH. The Mre11/Rad50/Nbs1 complex and its role as a DNA-double strand break sensor for ATM. *Cell Cycle* 2005;4:737–740. [PubMed: 15908798]
- Peng CY, Graves PR, Thoma RS, Wu Z, Shaw AS, Piwnicka-Worms H. Mitotic and G2 checkpoint control: regulation of 14-3-3 protein binding by phosphorylation of Cdc25C on serine-216. *Science* 1997;277:1501–1505. [PubMed: 9278512]
- Pietrzak M, Halicka HD, Wieczorek Z, Wieczorek J, Darzynkiewicz Z. Attenuation of acridine mutagen ICR 191-DNA interactions and DNA damage by the mutagen interceptor chlorophyllin. *Biophys J* 2008;135:69–75.
- Powell SN, Kachnie LA. Therapeutic exploitation of tumor cell defects in homologous recombination. *Anticancer Agents Med Chem* 2008;8:448–460. [PubMed: 18473729]
- Quinn JE, Carser JE, James CR, Kennedy RD, Harkin DP. BRCA1 and implications for response to chemotherapy in ovarian cancer. *Gynecol Onc* 2009;113:134–142.
- Raitsema T, Klovov D, Banath JP, Olive PL. DNA-PK is responsible for enhanced phosphorylation of histone H2AX under hypertonic conditions. *DNA Repair (Amst)* 2005;28:1172–1181.
- Rogakou EP, Nieves-Neira W, Boon C, Pommier Y, Bonner WM. Initiation of DNA fragmentation during apoptosis induces phosphorylation of H2AX histone at serine 139. *J Biol Chem* 2000;275:9390–9395. [PubMed: 10734083]
- Rouleau M, Aubin RA, Poirier GG. Poly(ADP-ribosyl)ated chromatin domains: access granted. *J Cell Sci* 2004;117:815–825. [PubMed: 14963022]
- Rubbi CP, Milner J. p53 is a chromatin accessibility factor for nucleotide excision repair of DNA damage. *The EMBO J* 2003;22:975–986.
- Rudolph J. Cdc25 phosphatases: Structure, specificity and mechanism. *Biochemistry* 2007;46:3595–3604. [PubMed: 17328562]
- Santarosa M, Del Col L, Tonin E, Caragnano A, Viel A, Maestro R. Premature senescence is a major response to DNA cross-linking agents in BRCA-1 defective cells: implication for tailored treatments of BRCA1 mutation carriers. *Mol Cancer Ther* 2009;8:844–854. [PubMed: 19372557]
- Sedelnikova OA, Rogakou EP, Panuytin IG, Bonner W. Quantitative detection of ¹²⁵IUdr-induced DNA double-strand breaks with γ -H2AX antibody. *Radiation Res* 2002;158:486–492. [PubMed: 12236816]
- Seoane M, Iglesias P, Gonzalez T, Dominguez F, Fraga M, Aliste C, Forteza J, Costoya JA. Retinoblastoma loss modulates DNA damage response favoring tumor progression. *PLoS ONE* 2008;3:e3632. [PubMed: 18985151]Epub Nov5
- Shen SX, Weaver Z, Xu X, Li C, Weinstein M, Chen L, Guan XY, Ried T, Deng CX. A targeted disruption of the murine Brca1 gene causes gamma-irradiation hypersensitivity and genetic instability. *Oncogene* 1998;17:3115–3124. [PubMed: 9872327]

- Shiloh Y. ATM and related protein kinases: safeguarding genome integrity. *Nat Rev Cancer* 2003;22:5834–5868.
- Smart DJ, Halicka HD, Schmuck G, Traganos F, Darzynkiewicz Z, Williams GM. Assessment of DNA double-strand breaks and γ H2AX induced by the topoisomerase II poisons etoposide and mitoxantrone. *Mutation Res* 2008;641:43–47. [PubMed: 18423498]
- Stawinska M, Cygankiewicz A, Trzcinski R, Mik M, Dziki A, Krajewska WM. Alterations of Chk1 and Chk2 expression in colon cancer. *Int J Colorectal Dis* 2008;23:1243–1249. [PubMed: 18679694]
- Stevens C, Smith L, La Thangue NB. Chk2 activates E2F-1 in response to DNA damage. *Nat Cell Biol* 2003;5:4465–4479.
- Stiff TO, Driscoll M, Rief N, Iwabuchi K, Lobrich M, Jeggo PA. ATM and DNA-PK function redundantly to phosphorylated H2AX after exposure to ionizing radiation. *Cancer Res* 2004;64:2390–2396. [PubMed: 15059890]
- Stucki M, Jackson SP. MDC1/NFBD1: a key regulator of the DNA damage response in higher eukaryotes. *DNA Repair (Amst)* 2005;3:953–957. [PubMed: 15279781]
- Sun Y, Jiang X, Chen S, Fernandes N, Price BD. A role for the Tip60 histone acetyltransferase in the acetylation and activation of ATM. *Proc Natl Acad Sci USA* 2005;102:13182–13187. [PubMed: 16141325]
- Syliuasen RG, Sorensen CS, Nylandsted J, Lukas C, Lukas J, Bartek J. Inhibition of Chk1 by CEP-3891 accelerates mitotic nuclear fragmentation in response to ionizing radiation. *Cancer Res* 2004;64:9035–9040. [PubMed: 15604269]
- Tan Y, Raychaudhuri P, Costa RH. Chk2 mediates stabilization of the FoxM1 transcription factor to stimulate expression of DNA repair genes. *Mol Cell Biol* 2007;27:1007–1016. [PubMed: 17101782]
- Tanaka T, Halicka HD, Huang X, Traganos F, Darzynkiewicz Z. Constitutive histone H2AX phosphorylation and ATM activation, the reporters of DNA damage by endogenous oxidants. *Cell Cycle* 2006;5:1940–1945. [PubMed: 16940754]
- Tanaka T, Halicka HD, Traganos F, Seiter K, Darzynkiewicz Z. Induction of ATM activation, histone H2AX phosphorylation and apoptosis by etoposide: relation to the cell cycle phase. *Cell Cycle* 2007a;6:371–376. [PubMed: 17297310]
- Tanaka T, Huang X, Halicka HD, Zhao H, Traganos F, Albino AP, Dai W, Darzynkiewicz Z. Cytometry of ATM activation and histone H2AX phosphorylation to estimate extent of DNA damage induced by exogenous agents. *Cytometry A* 2007b;71A:648–661.
- Tao ZF, Lin NH. Chk1 inhibitors for novel cancer treatment. *Anticancer Agents Med Chem* 2006;6:377–388. [PubMed: 16842237]
- Tassone P, Martino MT, Ventura M, Pietragalla A, Cuccinotto I, Calimeri T, Bulotta A, Neri P, Caraglia M, Tagliaferri P. Loss of BRCA1 function increases the antitumor activity of cisplatin against human breast cancer xenografts in vivo. *Cancer Biol Ther* 2009 Apr 28;8(7)[Epub]
- Wakeman TP, Kim WJ, Callens S, Chu A, Brown KD, Xu B. The ATM-SMC1 pathway is essential for activation of chromium [VI]-induced S-phase checkpoint. *Mutat Res* 2004;554:241–251. [PubMed: 15450422]
- Wang H, Wang M, Wang H, Bocker W, Illiakis G. Complex H2AX phosphorylation patterns by multiple kinases including ATM and DNA-PK in human cells exposed to ionizing radiation and treated with kinase inhibitors. *J Cell Phys* 2005;202:492–502.
- Wang W, Figg WD. Secondary BRCA1 and BRCA2 alterations and acquired chemoresistance. *Cancer Biol Ther* 2008;7:1004–1007. [PubMed: 18720553]
- Wang Z, Kar S, Carr BI. Cdc25A protein phosphatase: a therapeutic target for liver cancer therapies. *Anticancer Agents Med Chem* 2008;8:863–871. [PubMed: 19075569]
- Ward IM, Minn K, Chen J. UV-induced ataxia-telangiectasia-mutated and Rad3-related (ATR) activation requires replication stress. *J Biol Chem* 2004;279:9677–9680. [PubMed: 14742437]
- Wynne P, Newton C, Ledermann JA, Olaitan A, Mould TA, Hartley JA. Enhanced repair of DNMA interstrand crosslinking in ovarian cancer cells from patients following treatment with platinum-based chemotherapy. *Br J Cancer* 2007;97:927–933. [PubMed: 17848946]

- Xiao A, Li H, Schechter D, Ahn SH, Fabrizio LA, Erdjument-Bromage H, Ishibe-Murakami S, Wang B, Tempst P, Hofmann K, Patel DJ, Elledge SJ, Allis CD. WSTF regulates the H2A.X DNA damage response via novel tyrosine kinase activity. *Nature* 2009;457:57–62. [PubMed: 19092802]
- Yang S, Kuo C, Bisi JE, Kim MK. PML-dependent apoptosis after DNA damage is regulated by the checkpoint kinase hCds/Chk2. *Nat Cell Biol* 2002;4:865–870. [PubMed: 12402044]
- Yuan SS, Lee SE, Chen G, Song M, Tomlinson GE, Lee FY. BRCA2 is required for ionizing radiation-induced assembly of Rad51 complex in vivo. *Cancer Res* 1999;59:3547–3551. [PubMed: 10446958]
- Yazdi PT, Wang Y, Zhao S, Patel N, Lee EY, Qin J. SMC1 is a downstream effector in the ATM/NBS1 branch of the human S-phase checkpoint. *Genes Dev* 2002;16:571–582. [PubMed: 11877377]
- Zhao H, Tanaka T, Halicka HD, Traganos F, Zarebski M, Dobrucki J, Darzynkiewicz Z. Cytometric assessment of DNA damage by exogenous and endogenous oxidants reports the aging-related processes. *Cytometry A* 2007;71A:905–914. [PubMed: 17879239]
- Zhao H, Traganos F, Darzynkiewicz Z. Kinetics of histone H2AX phosphorylation and Chk2 activation in A549 cells treated with topotecan and mitoxantrone in relation to the cell cycle phase. *Cytometry A* 2008;73A:480–489. [PubMed: 18459160]
- Zhao H, Traganos F, Dobrucki J, Wlodkowic D, Darzynkiewicz Z. Induction of DNA damage response by the supravital probes of nucleic acids. *Cytometry A* April 15;2009 75AEpub
- Zhou BB, Elledge SJ. The DNA response: putting checkpoints in perspective. *Nature* 2000;408:433–439. [PubMed: 11100718]
- Ziv Y, Bielopolski D, Galanty Y, Lukas C, Taya Y, Schultz DC, Lukas J, Bekker-Jensen S, Bartek J, Shiloh Y. Chromatin relaxation in response to DNA double-strand breaks is modulated by a novel ATM- and KAP-1 dependent pathway. *Nat Cell Biol* 2006;8:870–876. [PubMed: 16862143]

Fig. 1. The model of the ATM signaling pathway in response to induction of DSBs, as proposed by Kitagawa et al., (2004)

Induction of DSB causes unwinding and relaxation of torsional stress of the DNA double helix, which leads to local decondensation of chromatin and recruitment of the MRE11, RAD50 and NBS1 proteins (MRN complex), as well as BRCA1 to the DSB site. This event activates ATM by triggering its *Ser139* auto-phosphorylation which causes dissociation of the ATM dimer into two enzymatically active monomers. Activated ATM is then recruited to the site of DSB at which point it phosphorylates several substrates including NBS1, BRCA1 and SMC1. Phosphorylation of NBS1 targets ATM to Chk1, and is also essential for targeting ATM to phosphorylate Chk2. Phosphorylation of SMC1 activates the S-phase checkpoint while BRCA1 phosphorylation engages this protein in DSB repair. ATM also phosphorylates other substrates such as E2F1, Chk1, p53, Mdm2, Chk2, and H2AX. Activated p53 induces transcription of p21 and/or Bax p53 genes whose products arrest cells in G₁ or promote apoptosis, respectively.