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Targeting ATM pathway for therapeutic intervention in cancer

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Abstract

The Ataxia Telangiectasia Mutated gene encodes the ATM protein, a key element in the DNA damage response (DDR) signalling pathway responsible for maintaining genomic integrity within the cell. The ATM protein belongs to a family of large protein kinases containing the phosphatidylinositol-3 catalytic domain, including ATM, ATR and PI3K. ATM provides the crucial link between DNA damage, cell cycle progression and cell death by first sensing double stranded DNA breaks and subsequently phosphorylating and activating other downstream proteins functioning in DNA damage repair, cell cycle arrest and apoptotic pathways. Mammalian cells are constantly challenged by genotoxic agents from a variety of sources and therefore require a robust sensing and repair mechanism to maintain DNA integrity or activate alternative cell fate pathways. This review covers the role of ATM in DDR signalling and describes the interaction of the ATM kinase with other proteins in order to fulfil its various functions. Special emphasis is given to how the growing knowledge of the DDR can help identify drug targets for cancer therapy, thus providing a rationale for exploiting the ATM pathway in anticancer drug development. Moreover, we discuss how a network modelling approach can be used to identify and characterise ATM inhibitors and predict their therapeutic potential.

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Introduction

DNA repair is an essential physiological mechanism for maintaining genomic integrity in nucleated cells. The DNA damage response (DDR) pathway has evolved to be a complex, sensitive, highly integrated and interconnected pathway which can trigger a variety of cellular responses including DNA repair, cell cycle arrest and apoptosis [1]. Central to the DDR is the *Ataxia telangiectasia mutated* (ATM) gene product, a large protein kinase, which is responsible for the initiation of a signalling cascade that is triggered following double stranded DNA damage. The importance of the role of ATM is highlighted by its functional loss in the disease Ataxia Telangiectasia (A-T), also known as Louis-Barr syndrome, caused by hereditary mutations in the *ATM* gene. A-T is a rare autosomal recessive disorder characterized by immune deficiency, hypersensitivity to ionizing radiation (IR), and a predisposition to certain cancers [2]. A-T patients usually exhibit only partial deficiency of ATM protein, the residual levels varying between 1 and 17% of the normal level of ATM [3]. Usually, affected individuals present in early childhood with progressive cerebellar ataxia, which may initially be misdiagnosed as cerebral palsy of the ataxic type. Later, conjunctival telangiectasias and immune disturbances develop. Progressive neurological degeneration (without mental retardation) affects a significant proportion of older patients [4]. Thymus hypoplasia and complete loss or severe reduction in IgG2 or IgA levels are seen in the

majority of patients. Recurrent sinopulmonary infections, related to the accompanying immune deficiency and gonadal dysgenesis are also common among A-T patients [3, 5]. Predisposition to malignancy, especially haematological cancers, is one of the hallmarks of the A-T syndrome with cancer being the most frequent cause of death of A-T patients. This link between A-T and some forms of cancer was first observed by Reed WB et al in 1966 [7] who looked at incidences of familial and sporadic cancers in homozygous A-T patients as well as in heterozygous carriers. Normally, lymphomas in A-T patients tend to be of B-cell origin, whereas the leukemias are of T-cell origin [8]. Association between A-T status and susceptibility to cancer has been found in a variety of tumours to differing degrees and appears to be dependent on the type of *ATM* mutation, e.g. missense variants or truncating mutations [9] as well as the nature of the mutations e.g. germ line or somatic A-T [10, 11] At the cellular level, cultured A-T cells are abnormally resistant to inhibition of DNA synthesis by IR [6].

It is notable that heterozygous carriers of *ATM* mutation are relatively common amongst the general population, with estimates varying between 1.4 % and 2.2 %, and as high as 12.5 % in populations with a marked founder effect [5, 12, 13]. A-T patients from non-consanguineous families are usually compound heterozygotes. Carriers of defective *ATM* alleles, although generally considered asymptomatic, have been found to have an increased risk of death at any age due to all causes including cancer and ischemic heart disease [5, 13]. The role of defective *ATM* alleles has been clearly demonstrated in a proportion of familial breast cancer and colorectal cancer cases [14, 15]. Despite the heterogeneity in the clinical presentation of the A-T syndrome resulting in the initial finding of at least 4 complementation groups [16], subsequent linkage analysis showed that they all map to the same genomic location on chromosome 11 (11q22.3) containing the *ATM* gene [17, 18].

The *ATM* gene is extremely large - the full genomic region spans 150 Kb and consists of 66 exons [19]. ATM shares a bidirectional promoter with a gene called *NPAT*, which is located around 0.55 kB upstream of the ATM start codon. *NPAT* is required for progression through G1/S and entry into S-phase of cell cycle and has also been shown to positively regulate ATM [20, 21]. The ATM promoter region has been found to be TATA-less, but includes CCAAT boxes and several other important promoter sites including CREB, SP1, AP-2, GCF [22, 23, 24]. However, there is some evidence of the existence of another putative promoter region immediately upstream of the first coding exon which includes a TATA box [25]. This may contribute to the basal level expression of the *ATM*. In the past, several groups, including our own, have analysed the *ATM* promoter region to study its transcriptional activity and to elucidate *ATM* expression patterns and have found that the promoter activity is tissue specific, and inducible under certain conditions. For example, Gueven N et al in 2001 [26] reported downregulation of *ATM* expression upon treatment with Epidermal Growth Factor, while Hirai Y et al [27] in the same year demonstrated upregulation of *ATM* gene expression upon irradiation of lymphoblastoid cell lines. Recently, we reported induction of *ATM* expression upon inhibition of its kinase activity [28, 29]. Additional studies in vivo and in vitro as well as computer-aided mathematical modeling studies on *ATM* also revealed that *ATM* regulation could be tissue dependent, and may be subject to significant transcriptional regulation both at the promoter and mRNA level [30, 31, 32, 33].

The *ATM* gene encodes a Serine/Threonine protein kinase belonging to the **P**hosphatidy**I**nositol 3 **K**inase like **K**inase (PIKK) super family. The ATM protein is a ~350kD nuclear protein comprised of 3056 amino acids [34, 35] with its kinase activity located within the Phosphatidylinositol 3/4 catalytic domain and a FRAP-ATM-TRRAP (FAT) domain located towards the C-terminus (FATC) [35]. A leucine zipper motif has also been identified in the region spanning residues 1217 to 1238, which is thought to be involved in protein-protein interactions [36]. ATM is generally regarded as constitutively expressed protein and is held in an inactive homodimeric or higher order multimeric form. Upon DNA damage, for example by IR treatment, the pre-existing homodimeric ATM complexes undergo rapid autophosphorylation at residues Ser367, Ser1893 and Ser1981 within the FAT domain. This leads to dimer dissociation and the release of the active ATM monomers without an absolute increase in total ATM protein levels [37]. A complete list of known phosphorylated residues of ATM is given in Table 1.

Table 1. ATM phosphorylational mapping

Residue modified	Position	Reference
Serine	72	(40)
Serine	85	(40)
Serine	86	(40)
Serine	367	(38)
Serine	373	(40)
Serine	1883	(41)
Serine	1893	(38)
Serine	1981	(37)
Serine	1985	(40)
Serine	2996	(42)
Serine	72	(40)

However, of those listed, only the autophosphorylation sites at Serine 367, 1893 and 1981 have been fully characterised and play a role in ATM activation [37, 38, 39]. The remainder of the phosphorylation sites were identified through large scale proteomic analysis [40, 41, 42] and their role in regulating the function of the protein warrants further study. Once activated, ATM and other members of the PIKK family phosphorylate key substrates involved in DNA damage repair, DNA replication, cell cycle checkpoint arrest and apoptosis [43, 44], providing an important functional link between DNA damage recognition and appropriate cellular response to the damage.. In terms of its role in cell cycle regulation, ATM interacts with a number of downstream substrates including proteins that activate the G1, S or G2/M checkpoints, as set out in Table 2.

This functional link between the two pathways is very specific and tightly regulated at multiple levels. A simplified version of this pathway is shown in Figure 1. DNA damage activates sensor proteins and transducers that recognize, relay and amplify the damage signal following genotoxic insult, and triggers different cellular responses depending on the level of DNA damage. ATM also has a critical role in homologous repair during normal meiotic recombination events [45].

Table 2. ATM substrates in different phases of the cell cycle [14, 46]

G1	G1/S	S	G2/M
P53 Mdm2 Nbs1	P53 cAbl Rad51	RPA Chk2 FANCD2 H2AX BRCA1 CtIP MRN	Chk1 Chk2 Rad17(RFC)

Several early studies reported no quantitative changes in the expression levels and localisation of ATM throughout the cell cycle and upon DNA damage [47, 48]. However later studies reported that under certain conditions the expression of ATM may be altered, accompanied by a corresponding change in ATM activity [26, 32, 49].

The ATM signalling pathway during DDR

The ATM signalling pathway is triggered after double stranded DNA damage and provides a robust and very sensitive detection mechanism which generates responses that can cause cell cycle arrest, initiate DNA repair or induce apoptosis. The numerous substrates involved in the ATM signalling network are illustrated in Figure 2. There is considerable cross-talk between the different ATM substrates and several of them provide functions in more than one cellular response, which ensures a tightly co-ordinated and regulated cellular response. Following DNA damage, a variant form of histone, called H2AX, previously buried within the chromatin microenvironment, becomes exposed due to changes in the higher order of chromatin structure. This is caused by relaxation and unwinding of the local DNA supercoil due to the DNA breaks and recruitment of RAD51, exerting local topological changes. The phosphorylation of H2AX on Serine 139 (referred to as γH2AX) by ATM represents one of the earliest events in the DDR and acts as a docking site for a variety of other proteins involved in the DDR [56]. MRE11, RAD50 and NBS1 (collectively called the MRN complex) play a central and multifunctional role in the repair of double stranded DNA breaks and DNA recombination [56, 57, 58].

The MRE11 subunit of the complex has double strand exonuclease activity which excises the damaged DNA to allow for synthesis of a corrected strand. The RAD50 protein has ATPase activity which along with RAD51, 52 and 54, is thought to be involved in holding the two broken DNA ends together and also facilitate searches for homology in order to initiate recombination. NBS1 plays an important role of recruiting the MRN complex to the damaged site, binding to the γH2AX and also facilitating the binding of ATM to the complex and its subsequent autophosphorylation and activation [56]. Another ATM substrate involved in the DNA repair component of DDR is BRCA1, which acts as a scaffolding protein at the damaged site for recruitment of multiple protein complexes involved in DNA repair [59]. These initial events in turn activate other pre-existing inactive ATM molecules by exposing their binding sites and triggering dimer to monomer dissociation. The activation of ATM also involves its acetylation by a histone acetylase called Tip60 which was found to acetylate ATM on residue 3016, an event that is thought to precede autophosphorylation [60]. Activated ATM links the DNA damage response to the cell cycle pathway partly via the tumour suppressor protein P53. ATM causes the stabilization and activation of P53 primarily by phosphorylating P53 directly at Serine 15 [50]. ATM further contributes to P53 stabilisation by phosphorylating MDM2 at Serine 395, thereby triggering the degradation of this negative regulator of P53. Recently, in an *in-vivo* mouse study, the phosphorylation of the equivalent serine (Serine 394 in murine MDM2) by ATM was shown to be a critical event in the DNA damage induced stabilisation and activation of P53 [53]. In addition, ATM phosphorylates the Serine/Threonine checkpoint kinases Chk1 and Chk2 following DNA damage [61, 62], and these protein kinases in turn phosphorylate P53 on Serine 20 and contribute further to

its stabilization [63]. Once P53 is activated, it induces the expression of many proteins, including the endogenous inhibitors of the cyclin-cdk complexes e.g. p21Cip1/Waf1 and p27Kip [61, 63]. These early events in the DDR lead to checkpoint arrest in the G1 phase of the cell cycle. In addition to phosphorylating P53, checkpoint kinases Chk1 and Chk2 phosphorylate and inactivate Cdc25 phosphatases, which prevents the activation of cyclin-Cdk complexes and mitosis-promoting factor, leading to cell cycle arrest at the G2/M transition [62]. Figure 3 demonstrates the interplay between the DNA damage response and the cell cycle. Depending upon the level of DNA damage, activated ATM may also cause induction of apoptosis in a P53 dependent or independent manner, and involving the ATM substrates E2F1[52], checkpoint kinases Chk1 and Chk2 [63, 64], as well as p73 and c-Abl mediated pathways [65, 66] as shown in Figure 2.

ATM deficient cells are unable to detect the levels of DNA damage which would otherwise result in activation of the DNA repair machinery, leading to sustained genomic damage. Owing to the role of ATM in cell cycle arrest, ATM-deficient cells cannot induce checkpoint arrest following DNA damage, resulting in replication of damaged DNA and propagation of errors until the burden of damage becomes too severe for the genome and the cell is directed to a suicide route by an alternative, P53-independent mechanism [67]. This propensity for ATM-deficient cells to undergo apoptosis could explain the phenotype most commonly seen in A-T patients, due to death of cells in the neurons and the cells of the immune system, the main targets in the pathogenesis of the disease. The former are subjected to a massive (mainly oxidative) DNA damage which may not be promptly or completely repaired as neurons tend to inhibit selectively genome-wide DNA repair, focusing on the repair of actively transcribed genes [68]. During the formation of the T-cell receptor repertoire and the B-cell antibody repertoire in immune cells a significant amount of double-strand DNA breaks occurs naturally, which again, in ATM-deficient cells, triggers the apoptotic pathway [69].

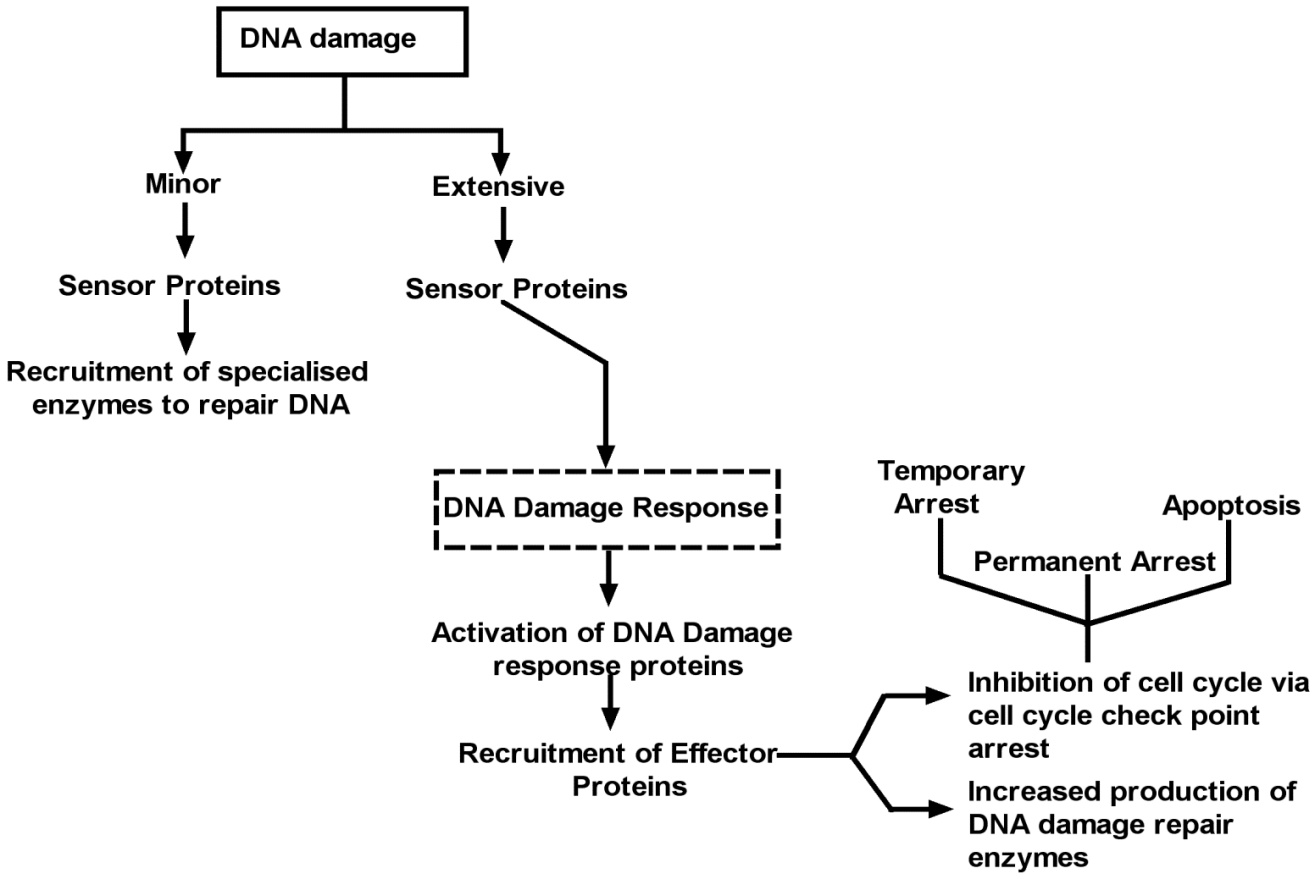

Figure 1: Cellular responses to DNA damage. Depending upon the scale of DNA damage, one of the two pathways is activated. If the DNA damage is minor, sensor proteins are activated which recruit specialized DNA repair enzymes to the damaged site and DNA is repaired. However, if the DNA damage is extensive, the DNA Damage Response (DDR) pathway is activated which activates specialized transducer proteins that amplify the damaged signal and activate effector proteins. The effector proteins cause cell cycle arrest and increase in the production of DNA damage repair enzymes. The arrest at the cell cycle checkpoints may result in temporary halting of the cell cycle, a permanent arrest or induction of apoptotic pathways.

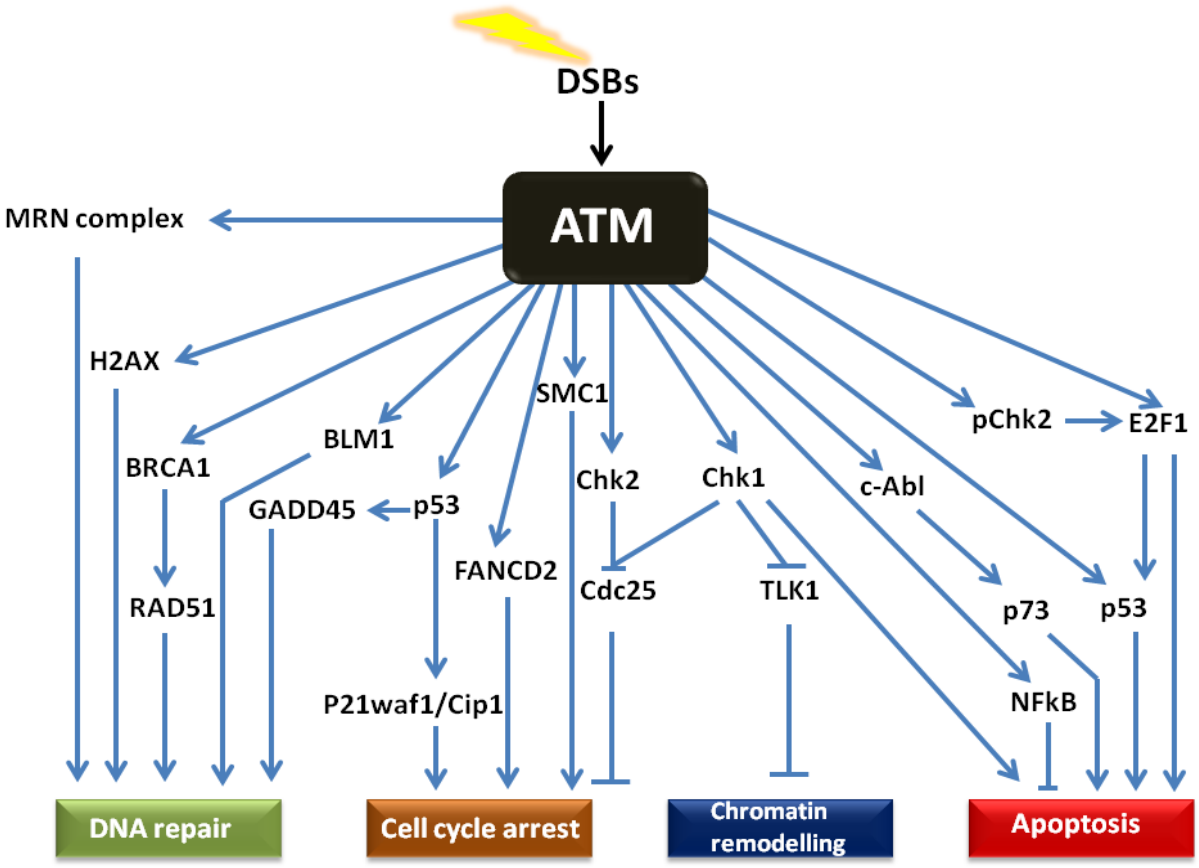

Figure 2: The ATM signalling pathway. ATM is present at the core of DNA damage pathway, activated upon DSBs and functions via multiple routes. While great deal of cross-talk exists between individual pathways, its major downstream substrates for DNA repair are MRN complex, BRCA1, RAD51 and, P53, for cell cycle arrest are SMC1, CIP/KIP family of proteins via P53 and checkpoint kinases, for chromatic remodelling are Chk1 and for apoptosis are c-Abl, P53, Chk2, E2F1, P73 and NFκB. The figure is designed on the basis of data from 50, 51, 52, 53, 54, 55.

Targeting ATM signalling pathway as an anticancer strategy

Decades of research efforts attempting to elucidate the DNA damage response pathways have added significant knowledge to our understanding of the mechanisms comprising DNA damage and repair signalling. These mechanisms involve sensing the DNA damage, generating a repair signal and amplification of the generated signal to elicit either a repair response, permanent arrest of the cell cycle, or apoptosis. Researchers have identified that cellular sensitivity to genotoxic agents in terms of cancer therapy can be achieved by modulating the function of key proteins involved in the above mentioned processes.

A key consideration in developing strategies for cancer therapy is to ensure the specificity of the cytotoxic action towards cancer cells as compared with their normal counterparts. This degree of specificity in terms of cellular response is theoretically achievable through identifying vital differences between normal and cancer cells and then basing the therapeutic approach on exploiting those differences.

Most, if not all cancer cells have mutations in genes affecting the cell cycle and DNA repair; these aberrations in vital cellular functions usually enhance their survival potential under stress, handicap their ability to sense the DNA damage and result in a failure to arrest the cell cycle to facilitate repair, leading to uncontrolled growth. However these abnormalities within cancer cells allow them to be distinguished from the surrounding normal cells and this can be exploited to achieve targeted cellular sensitivity. More than 50% of all cancers have P53 mutations [70]; these render the cancer cell incapable of executing P53-mediated G1/S cell cycle arrest and repair of DNA upon genotoxic insults. However, they do retain a functional G2/M checkpoint such that damaged cells can still be arrested allowing for DNA repair, thereby enabling them to survive. Therefore, abrogation of the G2/M checkpoint in cancer cells via ATM inhibition (ATM→Chk1/Chk2→G2/M arrest link) would disrupt the only available checkpoint and hence sensitise the cells against genotoxic agents [71]. In contrast, normal cells would be affected to a lesser extent by this treatment as they retain a wild type P53 and can therefore still exert G1/S arrest and repair of DNA. In such a scenario, ATM

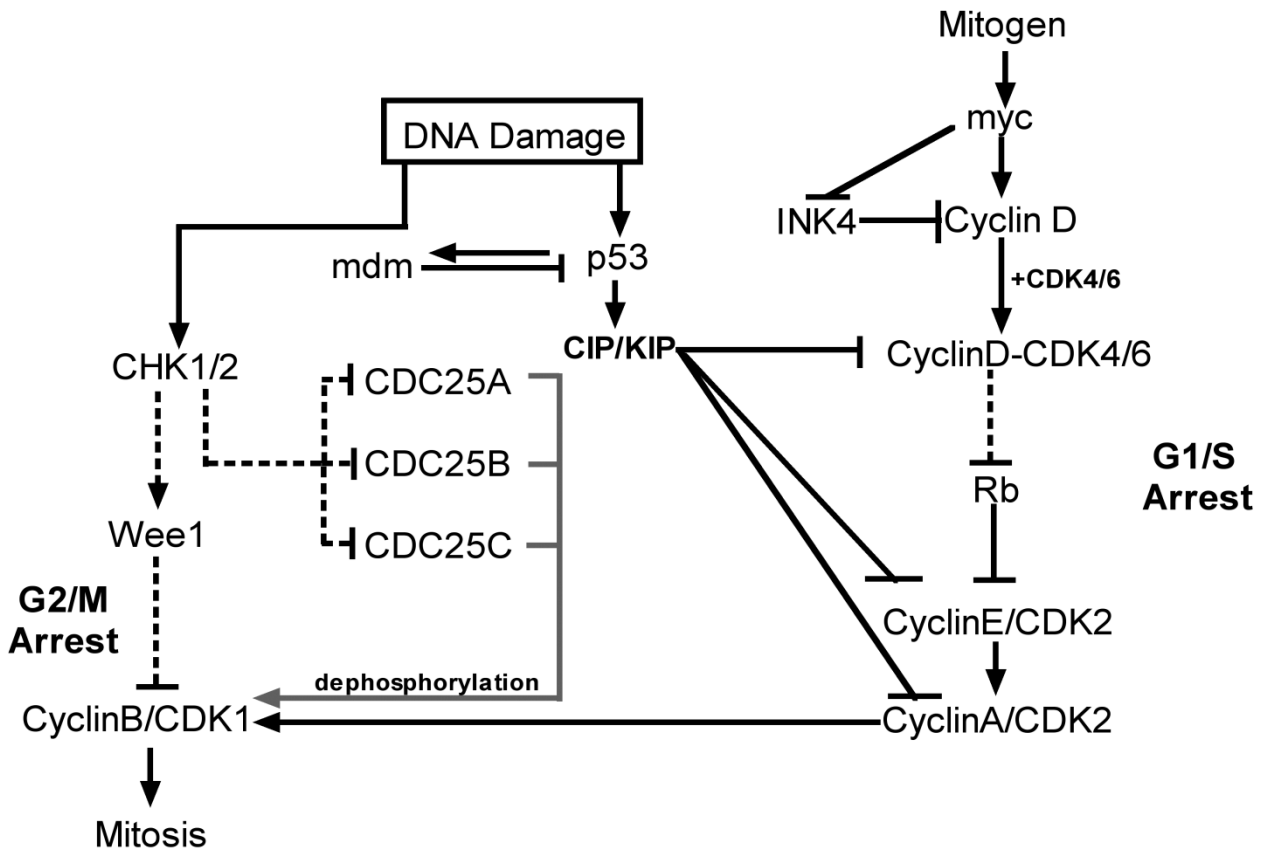

Figure 3: The DNA damage pathway and its crosstalk with the cell cycle. Lines with arrow heads represent activation while lines with bar heads represent deactivation, dotted lines with arrow heads indicate activation via phosphorylation and dotted lines with bar heads denote phosphorylational inactivation. Grey lines with arrow heads represent dephosphorylation. After DNA damage, P53 and checkpoint kinases Chk1/Chk2 are activated. P53 activation results in concomitant rise in the CIP/KIP proteins, P21 and P27 which inhibit cyclin/CDK complexes, blocking the cell cycle progression which results in G1/S arrest. The activated Chk1/Chk2 kinases activate Wee1 protein kinase as well as inhibit the function of Cdc25 group of phosphatases, which normally dephosphorylate and activate cyclin/cdk complexes. The inactivation of cyclinB/CDK1 activity via both phosphorylational inactivation by Wee1 as well as inhibition of its activating phosphatases by Chk1/Chk2 result in G2/M arrest. Thus in a normal cell, both G1/S and G2/M checkpoints are functional and may arrest the cell cycle upon DNA damage, to allow time for DNA repair.

inhibition in P53 mutated cell lines (representing the majority of cancer cells) would theoretically cause more cytotoxicity after the same level of genotoxic insult as compared to cells carrying wild type P53 (normal cells). The working principle of this strategy is illustrated in Figure 4. Another potential substrate of ATM which could constitute a potential anticancer target is NF-kB, a transcription factor that is activated as part of the stress response and induces the transcription of genes that promote survival. In cancer cells, elevated or deregulated NF-kB activation is commonly found. Inhibition of ATM activity could prevent NF-kB-mediated cell survival and lead to NF-kB dependent sensitisation of such cells to IR triggering apoptosis [72].

The concept of ATM inhibition-based anticancer therapeutic approach appears quite straightforward; nevertheless there are a number of challenges to overcome. Theoretically, inhibition of ATM function would disable the DDR mediated cell cycle arrest, DNA repair and apoptotic pathways, but there are other protein kinases sharing overlapping functions with ATM, such as DNA-PK and ATR. Thus, it may be necessary to inhibit all essential DDR kinases. However, a less selective inhibitor may be more cytotoxic in normal cells. Secondly, ATM itself can induce apoptosis via P53 dependent and independent pathways [51, 73]. In these instances, ATM inhibition may rather desensitise the cells against genotoxic agents by enhancing, rather than reducing, survival. Hence, the design of an ATM inhibition-based strategy for maximising cellular sensitivity to genotoxic agents, requires dual consideration; firstly it is essential to demonstrate, for any particular genotoxic agent, that the subsequent DNA repair and cell cycle arrest pathways, require ATM function. The second requirement is to carefully characterise the cellular response to the specific agent in terms of kinetics and dose response as well as the precise role of ATM in that context, in order to determine the

most appropriate time to administer an ATM inhibitor, to maximise cancer cell cytotoxicity.

In our own studies, we have determined the consequence of ATM inhibition on cell survival after subjecting a normal breast epithelial cell line, MCF10A and a breast cancer cell line, MCF7 to different levels of double stranded DNA damage. Time course, and dose response experiments were conducted with the DNA damaging agent, Doxorubicin (Dox) in the presence or absence of the small molecule ATM inhibitor KU55933 (KU), and the extent of cell death was analysed using the Neutral red (NR) uptake assay (Supplementary fig. 1 A-D).

Time series treatment of 0.5μM Dox with and without ATM inhibition revealed different outcomes on cellular sensitivity towards genotoxicity and indicated context dependent dual roles of ATM in the following way: Dox treatment alone resulted in decreasing NR uptake indicating cell death to varying degrees depending upon time of treatment and starting at the first time point in both cell lines. While MCF7 cell line was more sensitive to Dox at early time points of treatment with 50% cell death reaching at 12hr of Dox treatment (Supplementary fig. 1B), MCF10A was less sensitive with 50% cell death at 20hr of treatment (Supplementary fig.1 A). However, at a final time point of 24 hr Dox treatment, we observed comparable cell deaths between the two cell lines tested. Interestingly, addition of 10μM ATM inhibitor (KU) along with Dox resulted in higher apoptosis (hence sensitisation to the genotoxic agent) only at earlier time points of 2, 4, 8 and 12 hr of treatments as compared to Dox alone in both cell lines. Furthering the treatment regime contrastingly resulted in a lower cell death with the addition of ATM inhibitor than without, at 16, 20 and 24 hr time points (Supplementary figures 1A & 1B). Hence, it is indicated that at earlier time points, ATM kinase may mainly perform a cytoprotective function by triggering cell cycle arrest and DNA repair pathways as it ensured greater cell survival until 12 hr time point, as compared to that in ATM inhibited state. On the other hand, at a greater extent of DNA damage caused by longer genotoxic treatment beyond 12 hr, its function may have switched towards apoptosis, as there was higher cell death in functional ATM state than in inhibited state.

Dose dependent treatment of Dox ranging from 0.1 to 7μM with and without ATM inhibition similarly suggested a cytoprotective function of ATM at lower (0.1-0.25μM), while a role in apoptosis at higher (≥0.5μM) Dox levels in both MCF10A and MCF7 cell lines in the following way: Addition of KU with 0.1 and 0.25μM Dox resulted in greater sensitivity towards the genotoxic agent, and hence greater cell death in both cell lines as compared to Dox treatment alone. Increasing the dosage

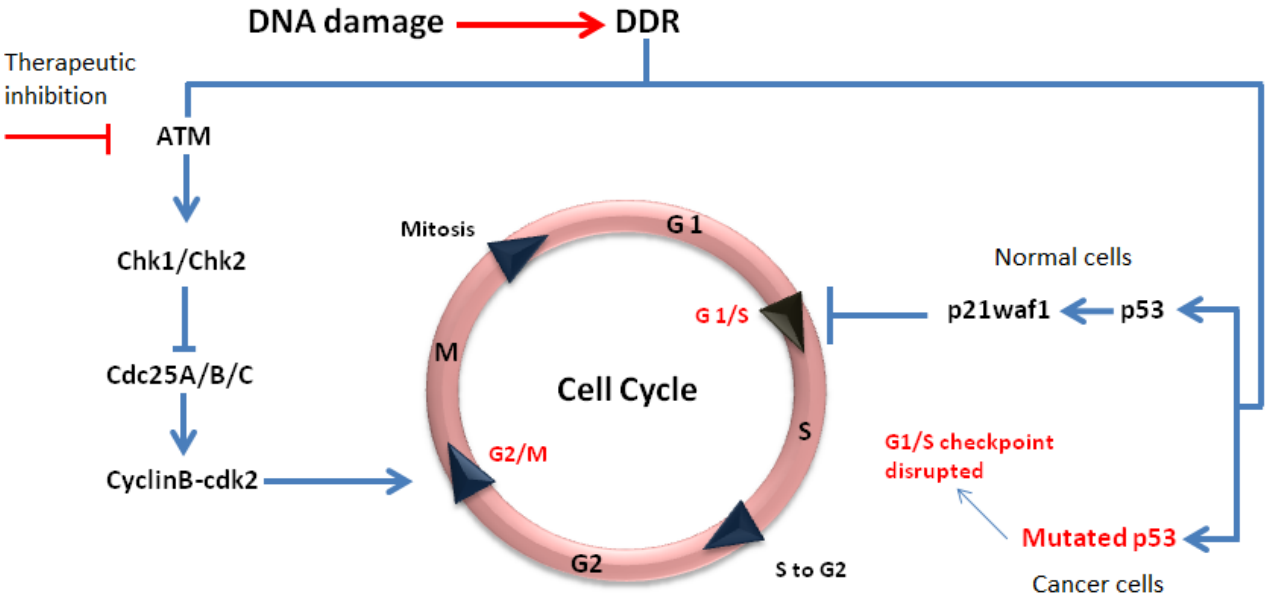

Figure 4: The principle of targeting ATM pathway for achieving targeted cellular sensitivity in cancer therapy. Lines with arrow heads indicate activation, while lines with bar heads indicate inhibition. Red arrow indicates DNA damage caused by radio or chemotherapy while red line with bar head indicates therapeutic inhibition of ATM. Cancer cells with P53 loss of function mutation have a dysfunctional G1/S checkpoint whereas the G2/M checkpoint may still be functional. When cells are exposed to genotoxic agents, DDR pathway is activated which will cause G1/S arrest via P53 pathway and G2/M arrest via checkpoint kinases Chk1 and Chk2 pathway. Normal cells can be arrest in either of these pathways allowing themselves time for DNA repair. However, cancer cells can only be arrested in G2/M pathway via ATM→Chk1/Chk2 pathway. Disruption of G2/M checkpoint by way of ATM inhibition would result in failure of cancer cell to arrest in any checkpoint causing more sensitivity to genotoxic agents while normal cells with functional P53 would still be arrested and repaired.

to and above 0.5μM Dox showed reduced cell death in ATM inhibited state than ATM active state. For example, when ATM was inhibited, 50% cell death occurred at a relatively higher concentration of 5μM Dox, as compared to 1μM in ATM active state (Supplementary fig. 2A).

Taken together, this data clearly demonstrates the different consequences of inhibiting ATM depending on the duration of treatment and dose of Dox used in the experiments. While these results are preliminary, they could have an impact on how DDR modulating drugs could be employed in the clinic. For example, in normal clinical practice, the physiologically achievable Dox concentration is maintained at approximately 100nM for up to 96hr by continuous infusion of the drug [74]. In our experiments, administration of an ATM inhibitor with 100nM Dox for 12 hours resulted in greater cell death than Dox alone, implying that ATM was indeed performing a cytoprotective function under these conditions but not at 0.5μM concentration of Dox. This has significant implications for clinical use of ATM inhibitors. However it should be borne in mind that, these treatments were only performed for up to 12hr, thus additional experiments extending the time-course to 96hr, as is used in the clinic, would be worthwhile. Although these results are preliminary, these experiments serve to illustrate the necessity to carefully characterise the role of ATM or any other enzyme functioning in DDR signalling, in the context of the experimental setting before reaching the clinical setting.

Inhibition of ATM activity: Research strategies employed

Since ATM operates at the core of a signalling network that governs cell fate decision, manipulation of ATM function has become of increasing interest as a potential anticancer drug target. Theoretically, the attenuation of the DDR pathway should handicap the cellular machinery responsible for repairing damaged DNA, sensitise cancer cells to genotoxic insults and promote cell death after genotoxic treatments as illustrated in previous sections. This sensitisation of cancer cells could facilitate the use of reduced treatment regimens, which are less cytotoxic to normal cells but would still be sufficient to kill the cancer cells and achieve the desired efficacy. Such an approach would diminish the detrimental side effects of the drug on normal tissues and importantly has been demonstrated at the cellular level as well as in *in vivo* studies. Hence, one important therapeutic strategy is the identification of inhibitors of PIKK super family of kinases central to the decision making process of cell fate after genotoxic intervention. The discovery and characterization of these inhibitors has not only helped to elucidate the function of the relevant proteins but also to identify the most therapeutically relevant targets.

Table 3 is a list of recent inhibitors whose targets lie within the DNA damage response pathway. It is noteworthy that many of those listed in the table are either currently in clinical trials or have been in clinical trials. For example the Chk1 inhibitors AZD7762 and PF-00477736, and the broader spectrum Chk1 and Chk2 inhibitors UCN-01 and XL-844 as well as the PARP-1 inhibitor AZD2281 have completed clinical trials whilst the DNA-PK inhibitor, CC-115, the alkyl guanine transferase inhibitor, O^6 BG, and a specific Chk1 inhibitor, LY2606368, are currently recruiting patients for clinical trials. In terms of exploiting the DNA damage response pathway for therapeutic intervention, the inhibitors of Poly ADP ribose polymerases have made the greatest progress and many novel drugs targeting these enzymes are already in clinical trials [93]. As yet, small molecule inhibitors of ATM have not reached any clinical trials.

There are several reports that demonstrate increased cellular sensitivity towards genotoxic agents by way of modulating the ATM signalling pathway through inhibition of ATM kinase specifically. Upon DNA damage for example after treatments with chemotherapeutic agents or radiotherapy, some tumours undergo a premature senescence also called stress-induced senescence. Although this permanent arrest of tumour cells is beneficial and prevents tumour metastasis, such senescent tumour cells resist apoptosis and may re-enter the cell cycle [94]. In order to identify the underlying signalling pathway, Crescenzi et al [95] studied drug induced tumour senescence in breast, lung and colon carcinoma cell lines and identified the ATM/ATR pathway to be constitutively active in these cells. Addition of the ATM kinase specific inhibitor KU55933 [75] or another ATM / ATR dual inhibitor CGK733 [79], caused increased apoptosis in these cell lines. Of considerable note is that while this treatment was cytotoxic to cancer cells, it did not cause apoptosis in the normal senescent human fibroblasts.

Morgan SE et al [35] over-expressed a mutated ATM protein encoding a truncated version of its functionally important Leucine zipper domain; this molecule had a dominant negative effect on ATM function. Overexpression of this functionally defective version of ATM in colon cancer cells increased the number of chromosomal breakages in such cells and resulted in the abrogation of the S-phase checkpoint, which led to an enhanced level of radiosensitivity.

Inhibition of ATM expression, via ATM antisense RNA or siRNA mediated gene silencing, is another strategy adopted by several researchers. Guha C et al [96] explored the use of antisense RNA directed against the kinase domain of ATM for the attenuation of ATM levels in human glioblastoma cells. They found that attenuation of ATM levels resulted in increased radiosensitivity in

Table 3. Different inhibitors that target the major proteins involved in DNA Damage response

Target	Compound	Details	Reference
ATM	KU55933	ATM is a critical kinase of the DNA damage response and acts as a sensor and signal transducer for the damage pathway. KU55933 is the first potent and specific inhibitor of ATM.	75
ATM	KU60019	An improved specific ATM kinase inhibitor	76
ATM	CP466722	CP466722 is a reversible and specific inhibitor of ATM	77
ATR	NU6027	A CDK2 and ATR inhibitor	78
ATM and ATR	CGK73	ATM and ATR are important DNA damage response kinases that link the response to cell cycle. CGK733 is a potent but reversible inhibitor or both ATR and ATM.	79
ATM and ATR	Caffeine	Inhibits both ATM and ATR kinase activity leading to radiosensitisation.	80
DNA-PK	NU7441	DNA-PK is a DNA dependent kinase identifying double strand breaks activated in non-homologous end joining repair. NU7441 is a specific DNA-PK inhibitor.	81
DNA-PK	LY294002	A general inhibitor of PI3-Kinases used to inhibit DNA-PK activity	82
ATM and DNA-PK	Wortmannin	A non-specific inhibitor of PI3-Like kinases	83
DNA-PK	CC-115	A dual DNA-PK and TOR kinase inhibitor	84
Chk1	AZD7762	An ATM/ATR dependent kinase with role in S and G2 arrest in response to DNA damage. AZD7762 is ATP-competitive inhibitor of Chk1	85
Chk1	PF-00477736	ATP competitive small molecular inhibitor of Chk1	86
Chk2	C3742	Small molecular inhibitor of Chk2	87
Chk1 and Chk2	UCN-01	A general protein kinase inhibitor	88
Chk1 and Chk2	XL-844	An inhibitor of the checkpoint kinases	89
Chk1	LY2606368	A selective inhibitor of Chk1 kinase activity	90
PARP-1	AZD2281	Poly-ADP-Ribose polymerase 1is involved in S-phase related DNA repair.	91
O6-alkylguanine-DNA alkyl transferase (AGT)	O6-BG	AGT is a repair factor in the direct repair. O6-BG is the first specific and potent inhibitor of AGT and sensitises several cancer types to different DNA damaging drugs	92

such cells independent of the status of P53. The resulting cells showed higher expression of P53 and P21 proteins, aberrant G2 checkpoint control and enhanced radiosensitivity after irradiation with a clinically relevant 2 Gy dose.

Collis GC et al [97] in a similar approach assessed the therapeutic potential of siRNA against ATM, ATR and DNA dependent protein kinase to test for their potential as radiotherapy and chemotherapy sensitizing agents against human prostate cancer cells. This approach reduced the respective protein levels by 90% after 48 hours post treatment, accompanied by a significant increase in sensitivity of such cells towards radiotherapy. Interestingly, they also confirmed that such siRNA mediated silencing was a more potent sensitizing agent then some of the inhibitors of these kinases, e.g. wortmannin or LY294002.

Recently, Neijenhuis S et al [98] reported that lung carcinoma cells expressing an aberrant β-Polymerase, a key enzyme involved in base excision repair, became

dependent on homologous recombination repair after IR treatment. Blockade of ATM function in these cells, via KU55933 treatment, led to an increased radiosensitivity due to failure of cells to repair the damage by homologous recombination repair.

Truman JP et al [99] exploited the link between the protein kinase C activator, 12-O-tetradecanoylphorbol 12-acetate (TPA), and ATM in the radiosensitization of an otherwise radioresistant human prostate cancer cell line and to induce apoptosis. They reported that treatment of cells with TPA increased the levels of an apoptotic inducing regulatory enzyme ceramide synthase (CS) which was accompanied by a decrease in ATM activity, resulting in apoptosis induction after treatment with IR. This group also used antisense ATM oligonucleotides to suppress ATM expression in cancer cells. This resulted in higher levels of CS activation and apoptosis even with low radiation doses of 1Gy in the radio resistant prostrate cancer cells.

In another study, Gueven N et al. [26] established a negative link between EGF and ATM expression. The authors observed that the treatment of human lymphoblasts and fibroblasts with EGF down regulated the expression of both ATM and DNA-dependent protein kinases which, in turn, resulted in increased radiosensitization in cells with wild type ATM. As expected, no further radiosensitization was seen in irradiated A-T cells, which are already inherently radiosensitive. The group further demonstrated that the reduction of ATM protein levels following EGF treatment resulted from transcriptional suppression of ATM at the promoter level.

A-T cells, which are highly chemo- and radio-sensitive have disrupted NFκB expression. Exploitation of the link between ATM and NFκB pathway regulation (Fig. 2) is an interesting anticancer target and experiments have been successful using either a dominant negative form of the NFκB inhibitor, I Kappa B alpha or inhibitors of ATM to disrupt the NFκB pathway in achieving chemosensitization and apoptosis of cancer cells [72].

The body of data described here has compelled many pharmaceutical and biotechnology companies to fund projects in this area, and the large number of DDR inhibitors currently being studied in clinical trials serves to illustrate the significance of this strategy in ongoing efforts to combat cancer.

New perspectives: Role of systems biology in the development and use of ATM inhibitors for anticancer treatment

A prerequisite of a molecularly targeted anticancer approach is a detailed understanding of the underlying molecular mechanisms contributing to tumour development. Elucidation of these mechanisms would facilitate a better understanding of cancer biology, but would also provide for the identification of potential drug targets that would also afford cancer selectivity. The latter, as mentioned before, is based on characterising differences between normal and cancer cells. These differences are not only limited to DDR signalling pathways, but may also involve adaptive trafficking mechanisms, alteration in cell surface receptors, differential microRNA expression levels and changes in both the types and extents of molecular interactions governing key processes in a cell, all of which may influence drug efficacy. Additionally, a thorough understanding of the extracellular microenvironment, proliferation, growth and stress signals, serum levels and of the phase of the cell cycle are vital before a successful anticancer approach could be actualised. Owing to a central role of ATM in performing multiple functions after genotoxic insults, it is becoming clear that targeting the ATM signalling pathway may sensitise cancer cells to the genotoxic agents used in cancer therapy. However, recent reports have identified greater complexity in terms of ATM function and regulation [28, 29, 33] than was previously realised. In this more complex setting, the effect of manipulating ATM activity is not fully predictable. Thus, the efficacy of potential ATM inhibitors would have to be assessed in terms of the effects it exerts, not only on ATM activity, but on a number of other key proteins in the DNA repair, cell cycle and apoptotic pathways.

We believe that this complexity in determining the absolute effects of manipulation of ATM activity could be best addressed by employing a systems biology approach to the problem, involving mathematical modelling [100]. Such a model could be capable of incorporating information from multiple cellular processes in which ATM is involved and which influence survival after genotoxic intervention. The model should be able to predict the global phosphorylation status of all the important substrates of ATM that function in different signalling pathways as well as the activity levels of a number of checkpoint and pro and anti-apoptotic proteins and then ultimately predict the cellular response generated by the cell after the intervention. The model would also need to provide for varying levels of drug input over variable time courses as well as being capable of incorporating combinations of intervention strategies e.g. involving both IR and DNA damaging agents. The model could be calibrated initially using information gleaned from experimental assays. This would involve the quantitative measurement, at the molecular level, of a large number of inputs in terms of drug concentration, radiation dose, culture media, as well as measurement of the corresponding outputs with respect to a range of key molecules on the relevant pathways. A secondary calibration stage could be carried out along with results from initial clinical trials. This

would permit the model to be developed as a clinical application to be used in conjunction with the drug treatment regimes. Such an application would enable clinicians to determine the optimum timing and level of drug dose and radiation for use in the individual patient where the DDR pathway status has already been established by the measurement of key protein levels in that patient.

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References

1. Kastan MB, Bartek, J. Cell cycle checkpoints and cancer. *Nature* 2004; **432**: 316-323
2. Perlman S, Becker-Catania S, Gatti RA. Ataxia-telangiectasia: diagnosis and treatment. *Semin Pediatr Neurol* 2003; **10**(3): 173-82.
3. Gilad S, Chessa L, Khosravi R, Russell P, Galanty Y, Piane M, et al. Genotype-phenotype relationships in ataxia-telangiectasia and variants. *Am J Hum Genet* 1998; **62**(3): 551-61.
4. Gatti RA, Swift M. Ataxia-telangiectasia: Genetics, Neuropathology, and Immunology of a Degenerative Disease of Childhood. New York: Alan R. Liss 1985.
5. Gilad S, Bar-Shira A, Harnik R, Shkedy D, Ziv Y, Khosravi R et al. Ataxia-telangiectasia: founder effect among North African Jews. *Hum Mol Genet* 1996; **5**(12): 2033-7.
6. McKinnon PJ. Ataxia-telangiectasia: an inherited disorder of ionizing-radiation sensitivity in man. *Hum Genet* 1987; **75**(3): 197-208.
7. Reed WB, Epstein WL, Boder E, Sedgwick R. Cutaneous manifestations of ataxia-telangiectasia. *JAMA* 1966; **195**(9): 746-53.
8. Boultwood J. Ataxia telangiectasia gene mutations in leukaemia and lymphoma. *J Clin Pathol* 2001; **54**(7): 512-6.
9. Telatar M, Teraoka S, Wang Z, Chun HH, Liang T, Castellvi-Bel S et al. Ataxia-telangiectasia: identification and detection of founder-effect mutations in the ATM gene in ethnic populations. *Am J Hum Genet* 1998; **62**(1): 86-97.
10. Athma P, Rappaport R, Swift M. Molecular genotyping shows that ataxia-telangiectasia heterozygotes are predisposed to breast cancer. *Cancer Genet Cytogenet* 1996; **92**(2): 130-4.
11. Thompson D, Duedal S, Kirner J, McGuffog L, Last J, Reiman A, Byrd P et al. Cancer risks and mortality in heterozygous ATM mutation carriers. *J Natl Cancer Inst* 2005; **97**(11): 813-22.
12. Swift M. Genetic aspects of ataxia-telangiectasia. *Immunodefic Rev* 1990; **2**(1): 67-81.
13. Su Y, Swift M. Mortality rates among carriers of ataxia-telangiectasia mutant alleles. *Ann Intern Med* 2000; **133**(10): 770-8.
14. Prokopcova J, Kleibl Z, Banwell CM, Pohlreich P. The role of ATM in breast cancer development. *Breast Cancer Res Treat* 2007; **104**(2): 121-8.
15. Gao Y, Hayes RB, Huang WY, Caporaso NE, Burdette L, Yeager M et al. DNA repair gene polymorphisms and tobacco smoking in the risk for colorectal adenomas. *Carcinogenesis* 2011; **32**(6): 882-7.
16. Sanal O, Wei S, Foroud T, Malhotra U, Concannon P, Charmley P et al. Further mapping of an ataxia-telangiectasia locus to the chromosome 11q23 region. *Am J Hum Genet* 1990; **47**(5): 860-866.
17. Chen X, Yang L, Udar N, Liang T, Uhrhammer N, Xu S et al. CAND3: a ubiquitously expressed gene immediately adjacent and in opposite transcriptional orientation to the ATM gene at 11q23.1. *Mamm Genome* 1997; **8**(2): 129-33.
18. Gatti RA, Berkel I, Boder E, Braedt G, Charmley P, Concannon P et al.. Localization of an ataxia-telangiectasia gene to chromosome 11q22-23. *Nature* 1988; **336**(6199): 577-80.
19. Uziel T, Savitsky K, Platzer M, Ziv Y, Helbitz T, Nehls M et al. Genomic Organization of the ATM gene. *Genomics* 1996; **33**(2): 317-20.
20. Imai T, Yamauchi M, Seki N, Sugawara T, Saito T, Matsuda Y et al. Identification and characterization of a new gene physically linked to the ATM gene. Genome Res. 1996; **6**(5): 439-47.
21. Imai T, Sugawara T, Nishiyama A, Shimada R, Ohki R, Seki N et al. The structure and organization of the human NPAT gene. *Genomics* 1997; **42**(3): 388-92.
22. Byrd PJ, Cooper PR, Stankovic T, Kullar HS, Watts GD, Robinson PJ et al. A gene transcribed from the bidirectional ATM promoter coding for a Serine rich protein: amino acid sequence, structure and expression studies. *Hum Mol Genet* 1996; **5**(11): 1785-91.
23. Gueven N, Keating K, Fukao T, Loeffler H, Kondo N, Rodemann HP et al. Site-directed mutagenesis of the ATM promoter: consequences for response to proliferation and ionizing radiation. *Genes Chromosomes Cancer* 2003; **38**(2): 157-67.
24. Gentilini F, Turba ME, Forni M, Cinotti S. Complete sequencing of full-length canine ataxia telangiectasia mutated mRNA and characterization of its putative promoter. *Vet Immunol Immunopathol* 2009; **128**(4): 437-40.
25. Platzer M, Rotman G, Bauer D, Uziel T, Savitsky K, Bar-Shira A, Gilad S, Shiloh Y, Rosenthal A. 1997. Ataxia-telangiectasia locus: sequence analysis of 184 kb of human genomic DNA containing the entire ATM gene. Genome Res. **7**(6): 592-605.
26. Gueven N, Keating KE, Chen P, Fukao T, Khanna KK, Watters D et al. Epidermal growth factor sensitises cells to ionizing radiation by down-regulating protein mutated in ataxia-telangiectasia. *J Biol Chem* 2001; **276**(12): 8884-91.
27. Hirai Y, Hayashi T, Kubo Y, Hoki Y, Arita I, Tatsumi K et al.. X-irradiation induces up-regulation of ATM gene expression in wild-type lymphoblastoid cell lines, but not in their heterozygous or homozygous ataxia-telangiectasia counterparts. *Jpn J Cancer Res* 2001; **92**(6): 710-7.
28. Khalil HS, Tummala H, Oluwaseun OA, Zhelev N. Novel insights of Ataxia Telangiectasia Mutated (ATM) regulation and its potential as a target for therapeutic intervention in cancer. *Curr Opin Biotechnol* 2011; **22**(1): S115-S116.
29. Khalil HS, Tummala H, Hupp TR, Zhelev N. Pharmacological inhibition of ATM by KU55933 stimulates ATM transcription. *Exp Biol Med (Maywood)* 2012; **237**: 622-34.
30. Savitsky K, Platzer M, Uziel T, Gilad S, Sartiel A, Rosenthal A et al. Ataxia-telangiectasia: structural diversity of untranslated sequences suggests complex post-transcriptional regulation of ATM gene expression. *Nucleic Acids Res* 1997; **25**(9): 1678-84.
31. Gueven N, Fukao T, Luff J, Paterson C, Kay G, Kondo N et al. Regulation of the Atm promoter in vivo. *Genes Chromosomes Cancer* 2006; **45**(1): 61-71.
32. Fukao T, Kaneko H, Birrell G, Gatei M, Tashita H, Yoshida T et al. ATM is upregulated during the mitogenic response in peripheral blood mononuclear cells. *Blood* 1999; **94**(6): 1998-2006
33. Clyde RG, Craig AL, de Breed L, Bown JL, Forrester L, Vojtesek B et al.. A novel ataxia-telangiectasia mutated autoregulatory feedback mechanism in murine embryonic stem cells. *J R Soc Interface* 2009; **6**(41): 1167-77.

34. Chen G, Lee EYHP. The product of the ATM gene is a 370-kDa nuclear phosphoprotein. *J Biol Chem* 1996; **271**(52): 33693-7.
35. Morgan SE, Lovly C, Pandita TK, Shiloh Y, Kastan MB. Fragments of ATM which have dominant-negative or complementing activity. *Mol Cell Biol* 1997; **17**(4): 2020-9.
36. Shafman T, Khanna KK, Kedar P, Spring K, Kozlov S, Yen T et al. Interaction between ATM protein and c-Abl in response to DNA damage. *Nature* 1997; **387**(6632): 520-3.
37. Bakkenist CJ, Kastan MB. DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. *Nature* 2003; **421**(6922): 499-506.
38. Kozlov SV, Graham ME, Peng C, Chen P, Robinson PJ, Lavin MF. Involvement of novel autophosphorylation sites in ATM activation. *EMBO J* 2006; **25**(15): 3504-14.
39. Kozlov SV, Graham ME, Jakob B, Tobias F, Kijas AW, Tanuji M et al.. Autophosphorylation and ATM activation: additional sites add to the complexity. *J Biol Chem* 2011; **286**(11): 9107-19.
40. Matsuoka S, Ballif BA, Smogorzewska A, McDonald ER 3rd, Hurov KE, Luo J et al. ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. *Science* 2007; **316**(5828): 1160-1166.
41. Oppermann FS, Gnad F, Olsen JV, Hornberger R, Greff Z, Kéri G et al. Large-scale proteomics analysis of the human kinome. *Mol Cell Proteomics* 2009; **8**(7): 1751-64.
42. Daub H, Olsen JV, Bairlein M, Gnad F, Oppermann FS, Körner R et al. Kinase-selective enrichment enables quantitative phosphoproteomics of the kinome across the cell cycle. *Mol Cell* 2008; **31**(3): 438-48.
43. Kurz EU, Lees-Miller SP.. DNA damage-induced activation of ATM and ATM-dependent signaling pathways. *DNA Repair (Amst)* 2004; **3**(8-9): 889-900.
44. Chaturvedi P, Eng WK, Zhu Y, Mattern MR, Mishra R, Hurle MR et al.. Mammalian Chk2 is a downstream effector of the ATM-dependent DNA damage checkpoint pathway. *Oncogene* 1999; **18**(28): 4047-54.
45. Barlow C, Liyanage M, Moens PB, Tarsounas M, Nagashima K, Brown K et al.. ATM deficiency results in severe meiotic disruption as early as leptonema of prophase I. *Development* 1998; **125**(20): 4007-17.
46. Xu B, O'Donnell AH, Kim ST, Kastan MB. Phosphorylation of serine 1387 in BRCA1 is specifically required for the Atm-mediated S-phase checkpoint after ionizing irradiation. *Cancer Res* 2002; **62**(16): 4588-91.
47. Brown KD, Ziv Y, Sadanandan SN, Chessa L, Collins FS, Shiloh Y et al. The ataxia-telangiectasia gene product, a constitutively expressed nuclear protein that is not up-regulated following genome damage. *Proc Natl Acad Sci U S A* 1997; **94**(5): 1840-5.
48. Watters D, Khanna KK, Beamish H, Birrell G, Spring K, Kedar P et al. Cellular localisation of the ataxia-telangiectasia (ATM) gene product and discrimination between mutated and normal forms. *Oncogene* 1997; **14**(16): 1911-21
49. Fang ZM, Lee CS, Sarris M, Kearsley JH, Murrell D, Lavin MF et al. Rapid radiation-induction of ATM protein levels in situ. *Pathology* 2001; **33**(1): 30-6.
50. Banin S, Moyal L, Shieh S, Taya Y, Anderson CW, Chessa L et al. Enhanced phosphorylation of p53 by ATM in response to DNA damage. *Science* 1998; **281**(5383): 1674-7.
51. Westphal CH, Rowan S, Schmaltz C, Elson A, Fisher DE, Leder P. ATM and p53 cooperate in apoptosis and suppression of tumorigenesis, but not in resistance to acute radiation toxicity. *Nat Genet* 1997; **16**(4): 397-401.
52. Powers, J.T., Hong, S., Mayhew, C.N., Rogers, P.M., Knudsen, E.S., Johnson, D.G., E2F1 uses the ATM signaling pathway to induce p53 and Chk2 phosphorylation and apoptosis. *Mol Cancer Res* 2004; **2**(4): 203-14.
53. Gannon HS, Woda BA, Jones SN.. ATM Phosphorylation of Mdm2 Ser394 Regulates the Amplitude and Duration of the DNA Damage Response in Mice. *Cancer Cell* 2012; **21**(5): 668-79.
54. Xu Y, Baltimore D. Dual roles of ATM in the cellular response to radiation and in cell growth control. *Genes Dev* 1996; **10**(19): 2401-10.
55. Chong MJ, Murray MR, Gosink EC, Russell HR, Srinivasan A, Kapsetaki M, et al. Atm and Bax cooperate in ionizing radiation-induced apoptosis in the central nervous system. *Proc Natl Acad Sci U S A* 2000; **97**(2): 889-94.
56. Carney JP, Maser RS, Olivares H, Davis EM, Le Beau M, Yates JR 3rd et al. The hMre11/hRad50 protein complex and Nijmegen breakage syndrome: linkage of double-strand break repair to the cellular DNA damage response. *Cell* 1998; **93**(3): 477-86.
57. 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**(45): 42462-7.
58. Hopfner KP, Karcher A, Craig L, Woo TT, Carney JP, Tainer JA. Structural biochemistry and interaction architecture of the DNA double-strand break repair Mre11 nuclease and Rad50-ATPase. *Cell* 2001; **105**(4): 473-85.
59. Wang Q, Zhang H, Fishel R, Greene MI. BRCA1 and cell signaling. *Oncogene* 2000; **19**(53): 6152-8.
60. Sun Y, Xu Y, Roy K, Price BD. DNA damage-induced acetylation of lysine 3016 of ATM activates ATM kinase activity. *Mol Cell Biol* 2007; **27**(24): 8502-9.
61. Matsuoka S, Rotman G, Ogawa A, Shiloh Y, Tamai K, Elledge SJ. Ataxia telangiectasia-mutated phosphorylates Chk2 in vivo and in vitro. *Proc Natl Acad Sci U S A* 2000; **97**(19): 10389-94.
62. Gatei M, Sloper K, Sorensen C, Syljuäsen R, Falck J, Hobson K et al. Ataxia-telangiectasia-mutated (ATM) and NBS1-dependent phosphorylation of Chk1 on Ser-317 in response to ionizing radiation. *J Biol Chem* 2003; **278**(17): 14806-11.
63. Shieh SY, Ahn J, Tamai K, Taya Y, Prives C. The human homologs of checkpoint kinases Chk1 and Cds1 (Chk2) phosphorylate p53 at multiple DNA damage-inducible sites. *Genes Dev* 2000; **14**(3): 289-300.
64. Sahu RP, Batra S, Srivastava SK. Activation of ATM/Chk1 by curcumin causes cell cycle arrest and apoptosis in human pancreatic cancer cells. *Br J Cancer* 2009; **100**(9): 1425-33.
65. Stiewe T, Pützer BM. Role of the p53-homologue p73 in E2F1-induced apoptosis. *Nat Genet* 2000; **26**(4): 464-9.
66. Wang JY. Regulation of cell death by the Abl tyrosine kinase. *Oncogene* 2000 **19**(49): 5643-50.
67. Thompson LH, Schild D. Recombinational DNA repair and human disease. *Mutat Res* 2002; **509**(1-2): 49-78.
68. Nouspikel TP, Hyka-Nouspikel N, Hanawalt PC. Transcription domain-associated repair in human cells. *Mol Cell Biol* 2006; **6**(23): 8722-30.
69. Chakarov S, Russev G.. DNA repair and differentiation – does getting older means getting wiser as well? *Biotechnol Biotech Eq* 2010; **24**(2): 1804-6.
70. Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. *Nature* 2000; **408**(6810): 307-10.
71. Smith J, Tho LM, Xu N, Gillespie DA. The ATM-Chk2 and ATR-Chk1 pathways in DNA damage signaling and cancer. *Adv Cancer Res* 2010; **108**: 73-112.
72. Jung M, Dritschilo A. NF-kappa B signalling pathway as a target for human tumor radiosensitisation. *Semin Radiat Oncol* 2001; **11**(4): 346-51.
73. Rogoff HA, Pickering MT, Frame FM, Debatis ME, Sanchez Y, Jones S, Kowalik TF. Apoptosis associated with deregulated E2F activity is dependent on E2F1 and Atm/Nbs1/Chk2. *Mol Cell Biol* 2004; **24**(7): 2968-77.
74. Gajewski E, Gaur S, Akman SA, Matsumoto L, van Balgooy JN, Doroshow JH. Oxidative DNA base damage in MCF-10A breast epithelial cells at clinically achievable concentrations of doxorubicin. *Biochem Pharmacol* 2007; **73**(12): 1947-56.
75. 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**(24): 9152-9.
76. Golding SE, Rosenberg E, Valerie N, Hussaini I, Frigerio M, Cockcroft XF, Chong WY, Hummersone M, Rigoreau L, Menear KA, O'Connor MJ, Povirk LF, van Meter T, Valerie K. Improved ATM kinase inhibitor KU-60019 radiosensitises glioma cells, compromises insulin, AKT and ERK prosurvival signaling, and inhibits migration and invasion. *Mol Cancer Ther* 2009; **8**(10): 2894-902.
77. Rainey MD, Charlton ME, Stanton RV, Kastan MB. Transient inhibition of ATM kinase is sufficient to enhance cellular sensitivity to ionizing radiation. *Cancer Res* 2008; **68**(18): 7466-74.
78. Peasland A, Wang LZ, Rowling E, Kyle S, Chen T, Hopkins A, Cliby WA, Sarkaria J, Beale G, Edmondson RJ, Curtin NJ. Identification and evaluation of a potent novel ATR inhibitor, NU6027, in breast and ovarian cancer cell lines. *Br J Cancer* 2011; **105**(3): 372-81.
79. Alao JP, Sunnerhagen P. The ATM and ATR inhibitors CGK733 and caffeine suppress cyclin D1 levels and inhibit cell proliferation. Radiat Oncol 2009; **10**; 4: 51.
80. Sarkaria JN, Busby EC, Tibbetts RS, Roos P, Taya Y, Karnitz LM, Abraham RT. Inhibition of ATM and ATR kinase activities by the radiosensitizing agent, caffeine. *Cancer Res* 1999; **59**(17): 4375-82.
81. Zhao Y, Thomas HD, Batey MA, Cowell IG, Richardson CJ, Griffin RJ, Calvert AH, Newell DR, Smith GC, Curtin NJ. Preclinical evaluation of a potent novel DNA-dependent protein kinase inhibitor NU7441. *Cancer Res* 2006; **66**(10): 5354-62.
82. Vlahos CJ, Matter WF, Hui KY, Brown RF. A specific inhibitor of phosphatidylinositol 3-kinase, 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one (LY294002). *J Biol Chem* 1994; **269**(7): 5241-8.
83. Sarkaria JN, Randal ST, Ericka CB, Amy PK, David EH, Robert TA.. Inhibition of Phosphoinositide 3-Kinase Related Kinases by the Radiosensitizing Agent Wortmannin. *Cancer Research* 1998; **58**(19): 4375-82.
84. Postel-Vinay S, Vanhecke E, Olaussen KA, Lord CJ, Ashworth A, Soria JC. The potential of exploiting DNA-repair defects for optimizing lung cancer treatment. *Nat Rev Clin Oncol* 2012; **9**(3): 144-55.
85. Zabludoff SD, Deng C, Grondine MR, Sheehy AM, Ashwell S, Caleb BL, Green S, Haye HR, Horn CL, Janetka JW, Liu D, Mouchet E, Ready S, Rosenthal JL, Queva C, Schwartz GK, Taylor KJ, Tse AN, Walker GE, White AM. AZD7762, a novel checkpoint kinase inhibitor, drives checkpoint abrogation and potentiates DNA-targeted therapies. *Mol Cancer Ther* 2008; **7**(9): 2955-66.
86. Blasina A, Hallin J, Chen E, Arango ME, Kraynov E, Register J, Grant S, Ninkovic S, Chen P, Nichols T, O'Connor P, Anderes K. Breaching the DNA damage checkpoint via PF-00477736, a novel small-molecule inhibitor of checkpoint kinase 1. *Mol Cancer Ther* 2008; **7**(8): 2394-404.
87. Arienti KL, Brunmark A, Axe FU, McClure K, Lee A, Blevitt J, Neff DK, Huang L, Crawford S, Pandit CR, Karlsson L, Breitenbucher JG. Checkpoint kinase inhibitors: SAR and radioprotective properties of a series of 2-arylbenzimidazoles. *J Med Chem* 2005; **48**(6): 1873-85.
88. Kawabe T. G2 checkpoint abrogators as anticancer drugs. *Mol Cancer Ther* 2004; **3**(4): 513-9.
89. Riesterer O, Matsumoto F, Wang L, Pickett J, Molkentine D, Giri U, Milas L, Raju U. A novel Chk inhibitor, XL-844, increases human cancer cell radiosensitivity through promotion of mitotic catastrophe. *Invest New Drugs* 2011; **29**(3): 514-22.
90. McNeely SC, Burke TF, DurlandBusbice SS, Barnard DS, Marshall MS, Bence AK et al. LY2606368, a second generation Chk1 inhibitor, inhibits growth of ovarian carcinoma xenografts either as monotherapy or in combination with standard-of-care agents. *Molecular Cancer Therapeutics* 2011; **10**(11) A108.
91. Fong PC, Boss DS, Yap TA, Tutt A, Wu P, Mergui-Roelvink M et al. Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. *N Engl J Med* 2009; **361**(2): 123-34.
92. Dolan ME, Stine L, Mitchell RB, Moschel RC, Pegg AE. Modulation of mammalian O6-alkylguanine-DNA alkyltransferase in vivo by O6-benzylguanine and its effect on the sensitivity of a human glioma tumor to 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea. *Cancer Commun* 1990; **2**(11): 371-7.
93. Plummer R, Jones C, Middleton M, Wilson R, Evans J, Olsen A et al. Phase I study of the poly(ADP-ribose) polymerase inhibitor, AG014699, in combination with temozolomide in patients with advanced solid tumors. *Clin Cancer Res* 2008; **14**(23): 7917-23.
94. te Poele RH, Okorokov AL, Jardine L, Cummings J, Joel SP. DNA damage is able to induce senescence in tumor cells in vitro and in vivo. *Cancer Res* 2002; **62**(6): 1876-83.
95. Crescenzi E, Palumbo G, de Boer J, Brady HJ. Ataxia telangiectasia mutated and p21CIP1 modulate cell survival of drug-induced senescent tumor cells: implications for chemotherapy. Clin Cancer Res 2008; **14**(6): 1877-87.
96. Guha C, Guha U, Tribius S, Alfieri A, Casper D, Chakravarty P et al. Antisense ATM gene therapy: a strategy to increase the radiosensitivity of human tumors. *Gene Ther* 2000; **7**(10): 852-8.
97. Collis SJ, Swartz MJ, Nelson WG, DeWeese TL.. Enhanced radiation and chemotherapy-mediated cell killing of human cancer cells by small inhibitory RNA silencing of DNA repair factors. *Cancer Res* 2003; **63**(7): 1550-4.
98. Neijenhuis S, Verwijs-Janssen M, van den Broek LJ, Begg AC, Vens C.. Targeted radiosensitization of cells expressing truncated DNA polymerase {beta}. *Cancer Res* 2010; **70**(21): 8706-14.
99. Truman JP, Gueven N, Lavin M, Leibel S, Kolesnick R, Fuks Z et al.. Down-regulation of ATM protein sensitises human prostate cancer cells to radiation-induced apoptosis. *J Biol Chem* 2005; **280**(24): 23262-72.
100. Idowu M, Goltsov A, Khalil HS, Tummala H, Zhelev N, Bown J.. Cancer research and personalised medicine: a new approach to modelling time-series data using analytical methods and Half systems. *Curr Opin Biotechnol* 2011; **22**(S1): S59.