Novel targets for ATM-deficient malignancies

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Abbreviations: AP, apurinic/apyrimidinic; APE1, AP endonuclease 1; ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia and Rad3-related; BER, base excision repair; BRCA, breast cancer susceptibility gene; Chk, checkpoint kinase; Cdc25, cell division cycle 25 homolog; Cdk, cyclin-dependent kinase; CtIP, C-terminal-binding protein interacting protein; DDR, DNA damage response; DSB, double-strand break; DSBR, DSB repair; DNA-PK, DNA-dependent protein kinase; DNA-PKcs, catalytic subunit of DNA-PK; FA, Fanconi anemia; FANC, FA complementation group; FANCD2-Ub, monoubiquitinated FANC-D2; FISH, fluorescence in situ hybridization; HR, homologous recombination; ICL, interstrand crosslinking; IR, ionizing radiation; miRNA, micro-RNA; MRN, Mre11-Rad50-Nbs1; NER, nucleotide excision repair; NHEJ, non-homologous end joining; OSCC, oral squamous cell carcinoma; PAR, polymer of ADP-ribose; PARP1, Poly(adenosine diphosphate [ADP]–ribose) polymerase 1; PIKK, PI3K-like kinase; ROS, reactive oxygen species; RPA, replication Protein A; SL, synthetic lethality; SLI, synthetic lethal interactor; SSB, single-strand break; SSBR, SSB repair; XRCC, X-ray repair cross complementing

Conventional chemo- and radiotherapies for the treatment of cancer target rapidly dividing cells in both tumor and non-tumor tissues and can exhibit severe cytotoxicity in normal tissue and impair the patient’s immune system. Novel targeted strategies aim for higher efficacy and tumor specificity. The role of ATM protein in the DNA damage response is well known and ATM deficiency frequently plays a role in tumorigenesis and development of malignancy. In addition to contributing to disease development, ATM deficiency also renders malignant cells heavily dependent on other pathways that cooperate with the ATM-mediated DNA damage response to ensure tumor cell survival. Disturbing those cooperative pathways by inhibiting critical protein components allows specific targeting of tumors while sparing healthy cells with normal ATM status. We review druggable candidate targets for the treatment of ATM-deficient malignancies and the mechanisms underlying such targeted therapies.

Introduction

In response to the increasing number of patients with life-threatening malignant tumors, a major focus of the life sciences has become understanding how cancers develop in order to develop better treatments. Genome instability and mutations, together with tumor-promoting inflammation, are the enabling characteristics of cancers. The human body possesses well-organized systems to detect and respond to impaired genome integrity. These survey systems constantly signal and kill cells with an abnormal genome to avoid damage to the whole organism. Deficiencies in critical proteins that are involved in the response to DNA damage can disable these survey mechanisms and serve as potential biomarkers for cancers. Because of the crucial role of the ataxia telangiectasia mutated (ATM) protein in many pathways of the DNA damage response, defects in the ATM gene are considered driver deficiencies in malignancies. The functions of ATM in maintaining genome stability are well documented, and a vast number of studies using ATM-deficient cell lines and animal models have been published (for a review see ref. 2–4). Only recently, however, have attempts been made to translate results of these studies into personalized therapies for patients with defective ATM. The present review aims to give an update on how the Achilles’ heel of ATM-deficient malignancies can be targeted and explain the mechanisms underlying individual approaches.

Role of ATM in the DNA damage response

Genomic integrity is constantly being challenged by endogenous and exogenous factors. DNA lesions can be either caused by ubiquitous agents such as UV radiation, gamma radiation, or reactive oxygen species, or intentionally induced by treatment with chemotherapeutic drugs and radiation therapy. Cells recruit a host of proteins to the lesion site to sense and relay the damage signal. This cellular response, termed the DNA damage response (DDR), is crucial for the fate of the cells: the outcome of the DDR decides whether cells survive and re-enter the cell cycle or undergo programmed cell death (apoptosis). Cell cycle arrest, DNA repair, apoptosis, and chromatin remodeling are the four critical events of DDR that ensure and maintain genomic stability. These four events are not independent of each other, but share many common factors. Mutations in many of the genes associated with the DDR have been found in the germ line of patients suffering from cancer-prone syndromes.

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As an immediate response to DNA damage, checkpoint pathways are activated to prevent cell cycle progression. Cells can be arrested at the G1/S, intra-S, or G2/M phases, depending on the type of DNA lesion. Checkpoint pathways allow cells to slow their growth in order to repair the lesions and further ensure that the DNA has been fully repaired prior to replication and distribution to the daughter cells. Depending on the type of insult and the resulting lesion, different repair mechanisms are activated. At least four highly conserved, partially overlapping damage repair pathways operate in mammals – nucleotide excision repair (NER), base excision repair (BER), homologous recombination (HR), and non-homologous end joining (NHEJ). In addition, the chromatin is remodeled for better access of signaling and repair factors to sites of damage. If genotoxic stress is excessive and the damage is beyond repair, pathways leading to apoptosis are activated. In contrast to unicellular organisms, apoptosis in multicellular organisms is beneficial because the organism survives at the cost of a few somatic cells.

The DDR consists of two main branches: the ATM pathway and the ataxia telangiectasia and Rad3-related (ATR) pathway. The ATR pathway is activated by single-strand breaks (SSBs) and bulky DNA lesions induced by UV-light and stalled replication forks during S-phase, whereas the ATM pathway is activated in response to DNA double-strand breaks (DSBs), either primary DSBs such as those induced by ionizing radiation (IR) or topoisomerase II inhibitors, or secondary DSBs resulting from replication of SSBs or collapsed replication forks. Rapid and effective repair of DSBs is of utmost importance to cell survival because a single unrepaired DSB can be lethal. The ATM and ATR pathways have distinct functions but partially overlap. For example, during processing of a DSB, ATM activity is required to generate a single-strand intermediate, which may result in activation of the ATR pathway. Furthermore, in response to UV treatment or replication fork stalling, ATM is activated in an ATR-dependent manner and cooperates with ATR to ensure an effective G2/M checkpoint. Interplay between the two pathways occurs particularly when one pathway is partially or completely deficient and the other pathway is able to execute the damage response through phosphorylation of their common DDR effectors (for a review see ref. 2).

ATM is a master regulator of the DDR. The ATM gene was first discovered as an allele that was mutated and inactivated in patients with ataxia telangiectasia (A-T). A-T patients are radiosensitive and have a predisposition to cancers, especially lymphoid malignancies (for a review see refs. 3,10). ATM is a member of the PI3K-like kinase (PIKK) family and has a highly conserved C-terminal kinase domain; other family members include ATR, DNA-PK, and mTOR. PIKK family members phosphorylate substrates at serine or threonine residues in the context of SQ/TQ consensus sites. The substrates of ATM, including ATM itself, histone H2AX, checkpoint kinase 1 and 2 (Chk1, Chk2), p53, and BRCA1, can also be phosphorylated by ATR. This sharing of common substrates forms the basis for the interplay between ATM and ATR.

DSBs are immediately sensed by the Mre11-Rad50-Nbs1 (MRN) complex and ATM is recruited by the C-terminus of Nbs1, thereby activating ATM kinase. Upon autophosphorylation and acetylation, the conformation of ATM changes from an inactive dimer to an activated monomer that can then function in the DDR.

ATM is engaged in all four pathways of the DDR through phosphorylation of different effectors. Beginning with autophosphorylation and H2AX phosphorylation, a cascade of phosphorylation events and protein recruitment is initiated to form ionizing radiation-induced foci (IRIF) in the vicinity of the break. IRIFs maintain and amplify DSB signals for the recruitment of checkpoint and repair factors in situ. Cell cycle arrest is the first cellular response to DSBs. ATM first effects a G1/S phase arrest that blocks replication of damaged DNA and thus prevents amplification of damaged DNA. Together with its substrate Chk2, ATM phosphorylates p53, which then acts as a transcription factor to induce p21 expression. p21 in turn inhibits the cyclin-dependent kinases (Cdk2, Cdk4, or Cdk6, which are required for entry into S phase. G1/S phase arrest forms a barrier prior to replication to ensure that damaged DNA is not amplified. ATM can also effect a later G2/M phase arrest as a final barrier to ensure that DNA lesions are repaired prior to mitosis, when unrepaired DNA damage would cause mitotic catastrophe and cell death. Entry into mitosis is controlled by Cdk1, which is active in its dephosphorylated cyclin B-bound state. The phosphatase cell division cycle 25 homolog (Cdc25) dephosphorylates and thus activates Cdk1, whereas the kinase Weel counteracts Cdc25 by phosphorylating and inactivating Cdk1. The ATM/Chk2 complex phosphorylates both Weel and Cdc25, leading to inactivation of Cdk1 and preventing mitotic entry. At later stages of the DSB response or in ATM-deficient cells, ATR/Chk1 kinases can be activated to phosphorylate Cdc25 and Weel, which then synergistically effect a G2/M arrest.

Once the cell cycle is stalled, cells activate two major pathways for repairing DSBs: non-homologous end joining and homologous recombination. NHEJ is a rapid repair pathway in which the Ku70 and Ku80 subunits of the DNA-dependent protein kinase (DNA-PK) complex first bind and protect the broken termini. DNA-bound Ku70/80 recruits additional NHEJ proteins to the site of the lesion, including the catalytic subunit of DNA-PK (DNA-PKcs), X-ray repair cross complementing protein 4 (XRCC4), and DNA Ligase IV. Ligase IV is a flexible ligase that joins incompatible DNA ends, thus making NHEJ error-prone (for a review see ref. 20).

Although NHEJ is the preferred pathway for repairing DSBs in G1 phase, in S and G2 phases sister chromatids are available and DSBs are repaired by slower but error free ATM-dependent HR using the intact sister chromatid as a template. Upon recruitment to the DSBs, ATM phosphorylates CtBP interacting protein (CtIP) and increases its 5′-3′ exonuclease activity. Together with the exonucleases Exol and Mre11, CtIP resects DSB ends to generate 3′ single-strand overhangs. Replication protein A (RPA) initially binds these ssDNA ends but is later replaced by Rad51 to form nuclear filaments. Rad51 filaments initiate homology search and strand invasion on the intact sister chromatid, core steps in HR repair. The HR process is completed by formation...
and subsequent resolution of Holliday junctions. The breast cancer susceptibility gene 2 (BRCA2) protein binds and facilitates Rad51 function in HR, whereas ATM-phosphorylated BRCA1 participates in various steps of HR from DSB signaling to Rad51 filament formation. Activation of either the HR or the NHEJ repair pathway represses the other pathway: DNA-PKcs blocks the necessary recruitment of Exol for HR repair, whereas the MRN complex involved in HR repair inhibits DNA ligase/ XRCC4-mediated end joining. Moreover, ATM phosphorylates DNA-PKcs to promote the dissociation of DNA-PKcs from the DSB termini, thus improving Exol recruitment. In addition, ATM phosphorylates factors required for chromatin remodeling such as KAP1, RNF-20/RNF40, NuRD, and CHD4 to open up highly compacted chromatin around lesions, thus improving accessibility for signaling and repair proteins (for a review see ref. 4).

When DNA damage in a cell is beyond repair, ATM phosphorylates p53 in a process called DSB-induced apoptosis. Upon its activation by phosphorylation, p53 induces the expression of pro-apoptotic target genes such as PUMA, Bax, BAK, and Noxa. These factors increase mitochondrial membrane permeability, leading to cytochrome c release and activation of caspases, the direct executors of apoptosis.

**ATM defects**

ATM is a malignancy susceptibility gene, and patients with ATM deficiency are predisposed to malignant tumors. Early in tumorigenesis (i.e., prior to genomic instability and malignant conversion), activated oncopogenes enhance Cdk activity and enable rapid and uncontrolled cell proliferation as a result of deregulated entry to the cell cycle. Such hyperproliferation of cells frequently results in replication stress and continuous formation of DNA DSBs, as observed in precancerous lesions and cancers. Cells possess two anticancer barriers to cope with constitutive DNA damage in precancerous lesions: the ATR/ATM-regulated DNA damage response network and oncogene-induced senescence, in which ATM also plays an important role. In the absence of functional ATM, critical barriers of tumorigenesis are lost. ATM deficiency contributes to the progression of precancerous cells to malignancy and, once established, ATM-deficient cancers are resistant to chemo- and radiotherapies. Defective ATM is an indicator of reduced disease-free survival, reduced cancer-specific survival and lower overall survival.

ATM is altered in approximately 10% of human tumors. These alterations include deletions and mutations that often vary between alleles, leading to ATM allelic heterozygotes. Detection of deletion of chromosome 11q (where ATM is located) by fluorescence in situ hybridization (FISH) was traditionally the standard method of diagnosing ATM loss; nowadays, FISH is often used in conjunction with next-generation sequencing to verify the status of the remaining allele. Single allelic ATM loss does not necessarily lead to cells with deficient ATM: in the case of a single allelic 11q deletion (wt/del), normal ATM activity is maintained by the remaining wild type allele and cells show deficient responses toward DNA damage only when the second ATM allele is also mutated or deleted (del/del or del/mut). Nonsense mutations in ATM with often result in a truncated ATM (ATM*trunc*), whereas missense mutations (ATM*mut*) result in full-length, albeit mutated, forms of ATM. ATM*trunc* and ATM*mut* are proposed to have different effects on ATM function, with ATM*mut* (and ATM*del*) having a recessive effect and ATM*mut* having a dominant negative effect on the other allele. ATM deficiency refers to its functional loss, which is attributed to inactivated ATM protein or low protein levels. Biallelic ATM mutations (del/del, del/mut, mut/mut), as well as single allelic missense mutations (ATM*mut*/wt), have been shown to inactivate ATM. ATM protein levels can be reduced by overexpression of tumor protein D52, an ATM interaction partner and negative regulator, via a currently unknown mechanism. Moreover, micro-RNAs (miRNAs) miR203, miR181a/b, and miR18 are negative post-transcriptional regulators of ATM expression by either mediating degradation or inhibiting translation of ATM mRNA. The level of these miRNAs inversely correlates with cellular ATM protein levels. High levels of miR203, miR181a/b, and miR18 have been detected in aggressive and drug-resistant cancer samples with reduced ATM level and reduced ATM function with regard to the DDR.

ATM kinase activity is used as the indicator of ATM function in DDR and can be assayed by detecting the phosphorylation level of ATM substrates (including ATM itself, p53, H2AX, Nbs1, SMC1, and Kap1) in response to DSB-inducing agents such as IR or topoisomerase II inhibitors. As ATM phosphorylates and upregulates p53 expression and consequently induces p21 expression, increased levels of p53 and p21 have also been used as readouts (for a review see ref. 41).

**Personalized therapy**

Conventional therapies based on chemotherapeutics and radiation aim to introduce DNA damage into rapidly dividing cells. Both target tissues (cancer cells) as well as normal tissues with rapid cell division (e.g., hematopoietic cells, hair cells, and mucosa) are affected, and the resulting severe cytotoxicity in normal tissues limits the dose and duration of such therapies. Targeted therapies using antibodies and their conjugates against tumor specific antigens are potentially more selective and have fewer side effects; the drawbacks, however, are high cost and in many cases insufficient efficacy.

As defects in ATM and other DNA damage response proteins (e.g., p53, ATM, BRCA1/2, Nbs1, ATR) are involved in malignant transformation of cells, these defects can also be exploited for the treatment of such malignancies. Although normal cells can arrest their cell cycle and repair DNA damage induced by radio- and chemotherapeutic treatment, cancer cells that lack certain functional checkpoints or repair pathways depend heavily on alternative pathways to ensure their survival. Targeting these remaining functional checkpoints and repair pathways offers a chance of developing personalized therapies that can complement conventional first-line therapies by increasing the susceptibility...
of tumor cells. When the remaining checkpoint pathways are inhibited by drugs, cancer cells progress into mitosis despite the presence of DSBs and then die as a result of mitotic catastrophe, a form of cell death caused by aberrant mitosis. If alternative repair pathways are inhibited, cells are unable to repair lesions in time and the persistent DNA lesions can activate apoptosis pathways. In both cases, cancer cells are killed selectively.44,45

This scenario of selective cancer cell death is based on the concept of synthetic lethality (SL), in which cell death occurs when two genes are mutated whereas cells with a single mutation in one of these genes remain viable. Genes involved in synthetic lethal interactions usually govern critical steps in parallel pathways required for cell survival and are called synthetic lethal interactors (SLIs).44 Exploiting synthetic lethality for the treatment of malignancies is a recent approach to personalized therapy as many cancer cells with mutations in genes participating in key events of the DDR are vulnerable to DNA damage-inducing therapies when their corresponding SLIs are inhibited. Inhibition of poly(adenosine diphosphate [ADP]–ribose) polymerase 1 (PARP1) in BRCA1/2-mutated cancers is a prime example of clinical application of the SL concept. PARP1 is involved in the repair of SSBs by addition of a polymer of ADP-ribose (PAR) onto acceptor proteins, thus enabling recruitment of the SSB repair scaffold protein XRCC1/Ligase III to the site of the nick. PAR-modified histones H1 and H2B mediate relaxation of the chromatin superstructure to facilitate repair.46 PARP1 inhibition blocks SSB repair, and unrepaired SSBs are converted to DSBs upon DNA replication. The DSBs converted from replicating SSBs have a one-ended structure, as opposed to two-ended DSBs arising from treatment with IR or topoisomerase II inhibitors in which a duplex DNA is fractured into two parts. Repair of one-ended DSBs using NHEJ to join ends from independent loci will inevitably result in large-scale sequence rearrangements and is thus deleterious.20 One-ended DSBs are therefore predominantly repaired by HR, in which BRCA1/2 plays a critical role.37 PARP1 and BRCA1/2 are thus SLIs with regard to the repair of SSBs. In addition to its role in SSB repair, PARP1 is involved in multiple other repair pathways (BER, NER, HR, NHEJ) as well as other critical cellular functions including cell survival (for a review see ref. 48). In addition to the synthetic lethality of PARP1/BRCA observed in SSBR/DSBR, PARP1 and BRCA2 synergistically protect stalled replication forks and promote replication restart.49,50 PARP1 inhibition was first shown to be selectively cytotoxic to BRCA-deficient breast and ovarian cancer cells.51,52 More recently, PARP1 inhibitors have been shown to selectively kill BRCA1/2-deficient cells from patients with other solid tumors.53

**Therapeutic targets in ATM-deficient malignancies**

ATM-deficient cancer cells fail to enter DNA damage-induced apoptosis due to an impaired ATM/Chk2/p53 pathway. This dysfunctional apoptotic response is responsible for their resistance to chemo- and radiotherapeutic intervention as well as the relapses observed in patients.15 Surviving cancer cells rely on SLIs of ATM for effective checkpoint controls and DNA damage repair. Genes that cooperate with ATM either in checkpoint control or in DNA damage repair can be used as targets for personalized therapy of ATM-deficient malignancies.

More synthetic lethal interactors of ATM have been described than can be reviewed here. Some of them are not druggable and therefore cannot be used for therapies.53BP1 is one such non-druggable proteins that is critical for NHEJ in addition to DNA-PKcs. Loss of 53BP1 markedly decreases the survival of lymphomas in Atm−/− mice.34 Druggable targets can be specifically modulated by small molecules that are bioavailable and safe for human application.55 Protein kinases are one family of druggable proteins. Kinases bind ATP through their catalytic core structure and catalyze the transfer of the terminal phosphate of ATP to substrate residues. Most small-molecule kinase inhibitors are either ATP mimetic compounds that compete with the binding of ATP to the kinases or compounds that bind outside the ATP binding site and inhibit kinase activity in an allosteric manner. Kinase inhibitors are approved for cancer treatment (for a review see ref. 56).

In ATM-deficient cells, repair by HR is impaired as a result of insufficient signaling of DSBs and disturbed Rad51 nuclear filament formation.45,57 These cells rely on DNA-PKcs-dependent NHEJ for their survival. Activity of the NHEJ pathway is increased in these cells and can compensate for the HR defects in DSB repair.58 Upon inhibition of DNA-PKcs, DSB termini in ATM-deficient cells are resected to form ssDNA ends but remain unrepaired. RPA binds to the accumulated ssDNAs and recruits ATR through ATR-interacting protein (ATRIP).19,45 ATR then activates its downstream kinase Chk1 and further phosphorylates p53. The proapoptotic protein PUMA is induced by phosphorylated p53 to execute apoptosis. Although binding of RPA to ssDNA overhangs is also required for HR repair, the RPA foci in ATM-deficient cells are significantly larger than those formed during HR, suggesting the presence of longer stretches of RPA-ssDNA.45 ATR is not normally activated as part of the DSB response, and it is unclear whether these longer stretches of RPA-ssDNA are a specific signal to recruit ATR for induction of apoptosis. The activated ATR/Chk1/p53 substitutes for the defective ATM/Chk2/p53 apoptotic pathway and exerts cell death. The effectiveness of inhibiting DNA-PKcs in ATM-deficient cancers has been demonstrated in a mouse model of ATM-deficient lymphoma xenografts. Grafted lymphomas were resistant to the DSB-inducing topoisomerase II inhibitor etoposide. However, the lymphomas were highly sensitized to apoptotic death after treatment with the DNA-PKcs inhibitor KU-0060648 alone or combined with etoposide, demonstrating that DNA-PKcs is a possible therapeutic target for ATM-deficient malignancies.45

The clinical success of PARP1 inhibitors in the treatment of BRCA1/2-deficient tumors has encouraged searches for additional SLIs based on the synergistic function of SSB repair and DSB repair pathways. Single-strand breaks can either be caused directly by agents such as free radicals or indirectly as intermediates of BER. As lesions that are repaired by BRE affect only one DNA strand, BER is considered a sub-pathway of
BER is the main guardian against alteration of bases by endogenous products of cellular metabolism including reactive oxygen species (ROS), methylation, deamination, and hydroxylation. In one of the first steps of BER, DNA glycosylase removes damaged bases and generates an apurinic/apyrimidinic (AP) site. The sugar residue of an AP site is cleaved by AP endonuclease 1 (APE1), resulting in a nicked strand. PARP1 is responsible for signaling and subsequent repairing of the nick. If APE1 is inhibited, AP sites are not processed and the subsequent repair steps are blocked, leading to an accumulation of AP sites that stall replication forks. When replication forks are not re-initiated or bypassed for a long time, they eventually collapse and one-ended DSBs are generated. The result of inhibiting APE1 is similar to that of inhibiting PARP1: in both cases, one-ended DSBs are generated from unrepaired single strand lesions, and a functional HR pathway is required for complete DNA repair. ATM and BRCA1/2 are two of the non-abundant proteins critically required for HR, and it is tempting to assume that impairing SSBR by inhibitors of either PARP1 or APE1 would effectively kill cancer cells that are deficient for ATM or BCRA1/2. Indeed, data from cell lines show that an APE1 inhibitor specifically inhibits the growth of ATM- and

Figure 1. Model for synthetic lethal interactions between ATM and APE1 and between ATM and FANC. (A) Replication forks (RF) stall when they encounter base lesions. Inhibition of APE1 blocks the PARP1-mediated SSBR pathway (grayed out), leading to collapsed replication forks. The FA pathway is required for stabilization of sporadic stalled replication forks. Blocking the FA pathway and inhibiting the monoubiquitination of FANCD2 therefore destabilizes stalled replication forks (grayed out) and leads to fork collapse. The resulting DSB lesions are repaired predominantly by ATM-dependent HR. (B) When APE1 or FANC are inhibited in ATM-deficient cancer cells, the cells fail to repair DSBs caused by collapsed RFs. Accumulation of unrepaired DNA lesions causes cell death. APE1, AP endonuclease 1; ATM, ataxia telangiectasia mutated; DSB, double-strand break; FA, Fanconi anemia; FANC, FA complementation group; HR, homologous recombination; PARP1, Poly(adenosine diphosphate [ADP]-ribose) polymerase 1; RF, replication fork; SSBR, SSB repair.
BRCA1/2-deficient cells (Fig. 1). These cells show elevated levels of unrepaired DSBs and decreased viability, but it is unclear whether cell death is due to apoptosis or mitotic catastrophe. Furthermore, PARP1 has been suggested as an SLI for many HR proteins, including ATM. Cell lines that have either de novo mutated ATM or ATM deficiency due to siRNA expression are sensitive to PARP1 inhibitors. A PARP1 inhibitor selectively killed cells from ATM-deficient lymphoma patients or xenografts through mitotic catastrophe, further supporting the concept of SL of PARP1 and ATM.

ATM null cells show reduced checkpoints in S/G2/M phases and rely heavily on ATR for a functional cell cycle arrest in response to DSBs. Cells from A-T patients and ATM-deficient oral squamous cell carcinoma (OSCC) lines show decreased sensitivity to IR and upregulated ATR-Chk1 activity. The decreased sensitivity to IR is possibly due to a defective ATM/Chk2/p53 apoptosis pathway. Both ATR/Chk1 and ATM/Chk2 can phosphorylate Cdc25 and Wee1 to allow G2 progression to M phase. Increased activity of the ATR/Chk1 pathway allows the cells to regain the capacity for G2 arrest, allowing time for DNA damage repair. Activated ATR could potentially relay apoptotic signaling through p53 to compensate for the defective ATM/p53 apoptosis pathway; however, apoptotic cell death was not observed in these experiments. When ATR or Chk1 is depleted by siRNA, ATM-defective OSCC cells lose G2 arrest and die from mitotic catastrophe.

ATM and ATR thus act cooperatively for effective G2/M arrest after DNA damage (Fig. 2). The ATR inhibitor VE821 increases the sensitivity of ATM-defective cells toward cisplatin, the first line treatment for OSCC. Previous concerns regarding inhibition of ATR related to the fact that ATR is involved in normal DNA metabolism (e.g., for preventing replication fork collapse) and ATR knockout mice are embryonic lethal.

The Fanconi anemia (FA)-pathway might also be a target for the treatment of ATM-deficient malignancies. Cells from FA patients have a defective response to DNA interstrand crosslinking (ICL) agents and are therefore very sensitive to mitomycin, cisplatin, and other platinum-based chemotherapeutics. Fourteen FA complementation (FANC) groups have been described—FANCA, B, C, D1, D2, E, F, G, I, J, L, M, N, P—together with accessory proteins, including FAAP20, FAAP24, and FAAP100. The key event in the FA pathway is monoubiquitination of FANC-I/FANC-D2 dimers. Monoubiquitinated FANCD2(FANCD2-Ub) is also recruited to stalled replication forks in an ATR/Chk1-dependent manner. Replication forks...
stall in S phase when they encounter DNA lesions or when dNTP levels do not match the replication speed, which can occur during normal cellular metabolism.69 This type of sporadic S-phase DNA damage is common in dividing cells, but more frequent in cancerous cells due to their hyperproliferation. Although it is known that cells with defects in the FA pathway are sensitive to stalled replication forks,70 the function of FANC-D2-Ub at stalled replication forks is unclear. It has been proposed that FANC-D2-Ub contributes to the role of BRCA2 in stabilizing and protecting stalled replication forks from degradation through interaction with BRCA2.68,71 Stabilized replication forks are resumed after bypass of the lesions or after the fork is reinitiated.69 After prolonged stalls, however, the replication forks collapse and replication proteins dissociate from the DNA. The resulting structure makes chromosomes vulnerable to breakage and generation of deleterious DSBs. Cells therefore rely on a functional HR pathway for the repair of secondary DSBs resulting from collapsed replication forks. Indeed, FANC-deficient cells have elevated DSB levels and require constitutive activation of ATM to prevent accumulation of spontaneous DNA breakage. These cells die after treatment with ATM inhibitors as a result of dramatically increased frequencies of chromosomal breaks, an indicator of unrepaired DSB termini,72 suggesting parallel and compensatory roles of ATM and FANC in coping with sporadic DNA damage (Fig. 1). The concept of ATM and FANCs as SLIs is supported by observations that Fancg Atm−/− mice exhibit embryonic lethality, whereas mice with individual gene knockouts are viable.72 ATM-deficient cells are selectively killed by EF24, a derivative of curcumin that inhibits FANC-D2-Ub via a poorly-defined mechanism,73 further supporting a role of FANC proteins as targets for ATM-deficient cancers.

**Perspective**

Malignancies with genetic defects in the DDR frequently rely on remaining DNA damage response pathways to repair the DNA damage induced by radio- and chemotherapies. Synthetic lethality exploits the reliance of cancer cells on these remaining pathways to develop personalized therapies that block these pathways and thus sensitize cancer cells to DNA damage. Identifying these critical survival pathways is the first step toward developing personalized therapies. A combination of functional genomics and high-throughput screening with RNAi could produce a candidate list of SLIs for malignancies with a particular mutation. To date, no such systematic screen of SLIs has been reported for human ATM; the SLIs of ATM known so far (PARP1, ATR, p53, 53BP1, DNA-PKcs, FANC, and APE1) were identified either in individual experiments or by screening SLIs of other mutations. Once identified in a screen, SLIs of ATM need to be validated in cell lines and animal models to determine their usefulness as targets for the treatment of ATM-deficient cancers.

When inhibitors of DNA repair proteins are used in combination with genotoxic drugs, DNA damage can also accumulate in normal tissues with rapid cell division and eventually lead to harmful DNA lesions, as observed after long-term application of PARP1 inhibitor.74 To avoid these problems, treatment regimens with appropriate intervals to allow regeneration of normal tissues or use of inhibitors as single agents can be considered. As an example, PARP1 inhibitor alone can effectively kill BRCA1/2-deficient cell lines.71,72 Similarly, DNA-PKcs inhibitor alone prevents ATM-deficient lymphoma growth in a mouse model.45 This effect might be because cancer cells produce higher levels of ROS, which attack DNA molecules, and have higher replication stress due to uncontrolled hyperproliferation, both of which result in endogenous DNA damage that could substitute for DNA damage induced by chemotherapeutic drugs or radiotherapy.

Several selective inhibitors of ATM kinase activity have been developed (e.g., KU60019, KU59403, KU55933, CP466722). ATM kinase inhibitors sensitize tumor cells and xenografts toward IR and DNA damage-inducing chemicals; however, no clinical trials have been reported (for a review see ref. 74). It is tempting to speculate that ATM kinase inhibitors would be effective for treating cancers that are deficient in known synthetic lethal interactors of ATM. For example, the ATM inhibitor KU59403 selectively inhibited the growth of p53-defective tumor xenografts in a mouse model.75 The combination of low doses of ATM kinase inhibitors and Chk1 or PARP1 inhibitors could offer a novel perspective to the treatment of cancers with other deficiencies in the DNA damage response. As an example, combined treatment with PARP1 and ATM kinase inhibitors preferentially sensitizes cancer cell lines deficient in p53,76,77 whereas combined treatment with an ATM kinase inhibitor and a Chk1 inhibitor increases the specific cytotoxicity to FA-deficient tumor cells compared with treatment with individual inhibitors.78

In the case of complex malignancies such as chronic lymphocytic leukemia with clonal evolution and the resulting heterogeneity, targeted therapies relying on ATM deficiency would exert a selective pressure. Inhibiting SLIs of ATM can decrease the number of ATM-defective cells, however subcolonies with other mutations will eventually proliferate and dominate during later disease stages.79,80 Therefore the fight against malignancy is a long-term battle that requires ever-changing strategies.

**Disclosure of Potential Conflicts of Interest**

The authors declare no potential conflicts of interest.

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