Molecular Pathways: Targeting ATR in Cancer Therapy
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Abstract

The human ATR gene encodes a kinase that is activated by DNA damage and replication stress as a central transducer of a checkpoint signaling pathway. Once activated, ATR phosphorylates multiple substrates, including the kinase Chk1, to regulate cell-cycle progression, replication fork stability, and DNA repair. These events promote cell survival during replication stress and in cells with DNA damage. Accordingly, there has been the tantalizing possibility that ATR inhibitors would be therapeutically useful, especially if they were more effective in tumor versus normal cells. Indeed, multiple studies have demonstrated that alterations that promote tumorigenesis, such as defects in the ATM-p53 pathway, constitutive oncogene activation, and acquisition of the alternative lengthening of telomeres pathway, render tumor cells sensitive to ATR inhibitor monotherapy and/or increase the synergy between ATR inhibitors and genotoxic chemotherapies. Now, nearly two decades after the discovery of ATR, two highly selective and potent ATR inhibitors, AZD6738 and VX-970, are in early-phase clinical trials either as monotherapies or paired with a variety of genotoxic chemotherapies. These trials will generate important insights into the effects of ATR inhibition in humans and the potential role of inhibiting this kinase in the treatment of human malignancies. Clin Cancer Res; 21(21): 1–6. © 2015 AACR.

Background

The ATR-Chk1 pathway

ATM (ataxia telangiectasia mutated) and ATR (ATM and rad3-related) kinases are two master regulators of DNA damage responses in human cells (1). These two PI3K-like protein kinases have overlapping and also distinct functions. Whereas ATM is primarily involved in the response to DNA double-stranded breaks (DSB), ATR responds to a wide range of DNA damage and DNA replication problems. When activated by DNA damage or replication stress, ATR phosphorylates and activates its effector kinase Chk1 (refs. 1, 2; Fig. 1). The ATR-Chk1 pathway protects the genome against DNA damage and replication stress by regulating and coordinating multiple cellular processes, which include but are not limited to cell-cycle arrest, inhibition of replication origin firing, protection of stressed replication forks, and DNA repair.

The activation of the ATR-Chk1 pathway is triggered by RPA-coated single-stranded DNA (ssDNA), a nucleoprotein structure commonly generated at sites of DNA damage and stressed replication forks (1, 2). ATRIP, the regulatory partner of ATR, directly binds RPA, thereby allowing the ATR–ATRIP complex to recognize the RPA-ssDNA at DNA damage sites or stressed replication forks (3). Several other regulators of ATR, including the Rad17 complex, the Rad9–Rad1–Hus1 (9-1-1) complex, and RIHNO, are recruited to junctions of RPA-ssDNA and double-stranded DNA (dsDNA; ref. 1). Together, these proteins enable TopBP1 to stimulate the kinase activity of ATR–ATRIP. With the help of mediator proteins such as Claspin, ATR recognizes and phosphorylates Chk1, leading to activation of the ATR-Chk1 pathway.

A growing list of DNA replication, DNA repair, and cell-cycle proteins have been shown to be substrates and effectors of ATR and Chk1. For example, the phosphorylation of Cdc25 phosphatases by Chk1 is important for DNA damage–induced cell-cycle arrest (4). The phosphorylation of WRN, SMARCAL1, and FANCI by ATR is important for proper DNA replication in cells under replication stress (5–7). ATR also regulates several DNA repair pathways, such as homologous recombination, DNA interstrand cross-link repair, and nucleotide excision repair (8–10). Importantly, the ATR-Chk1 pathway not only responds to extrinsic DNA damage and replication stress, but also to intrinsic problems such as those induced by oncogenic events (11). These properties of the ATR-Chk1 pathway have made it an attractive target for therapeutic intervention.

Role of the ATR-Chk1 pathway in cancer

ATR is important for cell survival; in mouse models, homozygous ATR inactivation is embryonically lethal, and mouse embryonic fibroblasts in which ATR is acutely genetically inactivated undergo one or two rounds of DNA replication before permanently exiting the cell cycle (12). The ATR pathway is likely essential due to the replication stress that is caused by spontaneous DNA damage and difficult-to-replicate regions of the genome such as fragile sites. Similarly, genetic inactivation of ATR in adult mouse tissues causes premature aging, defects in tissue homeostasis, and depletion of progenitor cells in rapidly proliferating tissues (13, 14). Notably, however, partially disabling ATR signaling is compatible with life. In humans with Seckel syndrome and in a mouse model of this syndrome, ATR levels are severely reduced due to a homozygous mutation that induces an mRNA splicing defect (15). Similarly, in a hypomorphic mouse model...
that reduces ATR levels to 10% of normal, these mice were remarkably normal, with no defects even in highly proliferative tissues (16).

In many tumor cells, however, ATR appears to be even more important than it is in normal cells. First, the dysregulated signaling induced by some oncoproteins, such as the Ras isoforms, Myc, and Cyclin E, disrupts normal cell-cycle regulation and causes replication stress (17). Indeed, in such tumor cells, the ATR pathway is critical for survival, and multiple studies have shown that inhibiting this pathway is selectively toxic in cells with high levels of oncogene-induced replication stress (16, 18–24). Second, ATM deficiency renders cells far more sensitive to ATR inhibition in cell culture and in animal models (25–27), a finding that has led to clinical trials of an ATR inhibitor in tumors characterized by ATM deficiency or ATM mutations (see below). Third, loss of specific DNA repair proteins (e.g., XRCC1, ERCC1) also causes tumor cell lines to be more sensitive to ATR inhibition, at least in cell line models (28–31); however, these findings have not yet been extended to animal models. Third, hypoxic cells, which are typically resistant to chemo- and radiotherapy (32), are sensitive to ATR inhibition (33–35), likely because hypoxia causes replication stress (36). Fourth, tumor cells that rely on the alternative lengthening of telomeres (ALT) pathway are also more sensitive to ATR disruption due to the role of ATR in the homologous recombination reactions that maintain these telomeres (37). Taken together, these observations indicate that multiple events that drive tumorigenesis may create a synthetic lethality for ATR inhibition similar to the finding that PARP inhibition is synthetically lethal in BRCA1/2-deficient tumors (38).

Notably, however, the potential for ATR inhibitors extends well beyond tumors with these phenotypes. ATR promotes cell survival by blocking cell-cycle progression, stabilizing stalled replication forks, and facilitating DNA repair, including homologous recombination. As such, even in cells in which disabling/inhibiting ATR has limited (or no) cytotoxic activity, ATR inhibitors robustly sensitize to or synergize with nearly all genotoxic therapies that have been tested. One major concern with any agent that increases the cytotoxic activity of a genotoxin is the potential for similar sensitization or synergy in normal cells. That concern has been eased by the findings to date. Multiple studies have shown that ATR inhibitors more effectively synergize with genotoxins in cell with defects in the p53 pathway than in p53-proficient cells (20, 25, 27, 31, 39), possibly due to the loss of the G1 checkpoint and/or increased replication stress that occurs due to relaxed S-phase entry (17). These findings thus suggest that there may be a therapeutic index for ATR inhibitors in the clinic in tumors with defects in the p53 pathway.

**Clinical–Translational Advances**

**ATR inhibitors**

ATR is a member of the phosphatidylinositol 3-kinase (PI3K)–related kinase (PIKK) family, which also includes ATM, mTOR, DNA-PKcs, SMG1, and PI3K. The first identified ATR inhibitor was caffeine, which disrupts DNA damage–induced cell-cycle arrest and sensitizes cells to DNA damage (40, 41). However, this agent also inhibits ATM and other members of the PI3K family, and cannot be used clinically because concentrations that inhibit ATR and ATM are toxic.

Due to the atypical nature of the PIKKs, identification of potent and selective inhibitors for this family of kinases lagged behind the development of inhibitors for more conventional serine, threonine, and tyrosine kinases. Early screening studies identified schisandrin B as a weak ATR inhibitor with more specificity than caffeine. More recently, Nu6027, NVP-BEZ235, torin 2, and ETP-46464 were reported as more potent ATR inhibitors that sensitized tumor cells to a variety of genotoxic chemotherapies, but they also inhibited other kinases such as CDK2, PI3K, mTOR, and ATM (42).

In 2011, the first potent and selective inhibitor, VE-821, was reported by Vertex Pharmaceuticals. VE-821 has >100-fold selectivity for ATR versus ATM, PI3K, DNA-PK, and mTOR (43), and its analogue VE-822 (VE-970) has been further improved with increased solubility, potency, selectivity, and pharmacodynamic...
properties (25, 44). Preclinical studies have shown that these agents robustly sensitize multiple tumor cell lines to cisplatin, ionizing radiation, gemcitabine, PARP inhibitors, topoisomerase I poisons, etoposide, and oxaliplatin in vitro (34, 35, 45–50). In xenograft studies, VE-821 and VX-970 have shown impressive results. VE-821 and VX-970 synergized with radiotherapy and gemcitabine in pancreatic cancer xenograft models (35, 45) and with irinotecan in a colorectal cancer model (48). Similarly, VX-970 sensitized six of seven patient-derived primary lung tumor xenograft models to cisplatin, even sensitizing cisplatin-resistant tumors to cisplatin (49). Importantly, the addition of VE-821 or VX970 did not exacerbate the general toxicity of the genotoxic therapies.

AstraZeneca has also developed highly selective and potent ATR inhibitors. AZ20 was first reported in 2103 (26). AZ20’s analogue, AZD6738, an orally available agent with better solubility and pharmacodynamic properties, was reported in 2013 (51). AZD6738 has excellent selectivity for ATR versus the other PIKKs and other kinases (52). Both compounds show single-agent activity in p53- and ATM-deficient tumor models and sensitize to or synergize with gemcitabine, cisplatin, ionizing radiation, and PARP inhibition in vitro (29, 30, 51–53). In xenograft models, AZD6738 has activity as a monotherapy in xenografts of ATM- and p53-deficient mantle cell lymphoma cell lines (54) and in primary chronic lymphocytic leukemia (CLL) patient-derived xenografts with 11q deletion (ATM-deficient) and 17p deletion (p53-deficient; ref. 53). AZD6738 also synergizes with carboplatin, bendamustine, and cyclophosphamide in an ATM-deficient diffuse large B-cell lymphoma model (52), and with a PARP inhibitor in a primary triple-negative breast cancer explant where neither AZD6738 nor the PARP inhibitor had antitumor activity as single agents (52).

**ATR inhibitors in clinical trials**

Two selective and potent ATR inhibitors are currently in phase I/II clinical trials. The first to enter the clinic was VX-970. This agent, which is administered intravenously, is being studied in combination with cisplatin, gemcitabine/cisplatin, and etoposide/cisplatin in advanced solid tumors to identify MTD and determine optimal dosing schedules (clinicaltrials.gov NCT01085568 for clinicaltrialsregister.eu 2013-005100-34), two tumors that frequently have defects in homologous recombination due to mutations in BRCA1/2 and other genes that encode homologous recombination passengers (58). Interestingly, another part of this trial will also identify the MTD of AZD6738 in combination with the PARP inhibitor olaparib and then examine this combination in gastric cancers with low ATM expression, a frequent occurrence due to ATM mutations in this malignancy (59). This trial is particularly interesting based on the observations that (i) ATM is frequently mutated in this cancer, (ii) disabling ATM sensitizes tumor cells to ATR and PARP inhibition, and (iii) ATR inhibition sensitizes to PARP inhibition even in cells with intact homologous recombination (30, 31, 46).

**Open Questions**

Despite the exciting preclinical data and progress in moving ATR inhibitors into the clinic, significant questions remain.

**ATR versus Chk1 inhibition**

Because ATR and Chk1 are in the same pathway, it is possible that the antitumor activities of ATR inhibitors may not differ significantly from that of Chk1 inhibitors, which are currently in clinical trials. While this is possible, it seems unlikely because ATR has many substrates other than Chk1, thus suggesting that it controls additional cell responses. Consistent with this idea are recent studies showing that ATR inhibition sensitizes to a wider range of chemotherapy agents in most cell lines (46, 60, 61). Accordingly, ATR inhibitors may have broader clinical utility than Chk1 inhibitors.

**Predictive biomarkers**

Preclinical studies have identified a series of tumor-specific alterations that affect sensitivity to ATR inhibition. As such, these alterations may be potential biomarkers to predict tumor responses to ATR inhibitor monotherapy and combination therapies. The best-validated biomarkers are defects in ATM and p53 pathways. Strong preclinical data, including animal models using ATM-deficient primary patient-derived CLL neoplasms (53), show that ATM deficiency is synthetically lethal with ATR inhibition. Similarly, p53-deficient cell lines are much more sensitive to combinations of an ATR inhibitor plus genotoxin than are p53-proficient cell lines (20), although ATR inhibitor sensitizes to genotoxins even in some primary tumors with wild-type p53 (49). Other potential predictive biomarkers include the genes that encode Myc, the Ras isoforms, and Cyclin E. Overexpression or constitutive activation of these oncogenes dysregulates cell-cycle progression and generates replication stress, thus making these tumors hyper-reliant on the ATR-Chk1 pathway (11).
tumor hypoxia, XRCC1 and ERCC1 deficiencies, and reliance on the ALT pathway also render cells more sensitive to ATR inhibition (28–31, 34, 37), although these potential biomarkers have not yet been as extensively examined and whether they affect responses in animal models is not known.

Toxicity of ATR inhibition

Because ATR is critical for the survival of normal cells in some contexts, it was initially unclear whether ATR would be a viable therapeutic target. However, the observations that severely reduced ATR levels in mice were compatible with viability suggested that ATR inhibition might be tolerated. Indeed, multiple mouse studies have now established that ATR inhibitors are not severely toxic, likely because inhibition is neither continuous nor complete. Importantly, these studies showed that ATR inhibition did not worsen the toxic effects of multiple genotoxic agents (similar to the findings with the potent and selective Chk1 inhibitor MK-8776 in human clinical trials), while still synergizing with genotoxic therapies. Accordingly, these findings suggest that there is a therapeutic window, likely due to the increased reliance of tumor cells on the ATR pathway because of onco-gene-induced replication stress and/or disabled ATM-p53 signaling. Nonetheless, despite these positive outcomes, toxicity issues remain a concern. For example, Clack and colleagues observed that AZD6738 dosing before carboplatin was tolerable in an animal model, whereas the reverse sequence was not (52). Furthermore, there have been no assessments of long-term toxicities. Because the DNA damage pathway is a barrier to tumorigenesis (62, 63), it is possible that disabling this pathway while administering genotoxins may increase the risk of second cancers due to the potential genome destabilizing effects of such a combination. However, it is also possible that reduced ATR signaling may actually prevent tumorigenesis; indeed, reduced ATR levels limit tumorigenesis in several mouse models of cancer (16, 19, 64). The clinical trials that are currently under way will determine whether ATR inhibition in monotherapy and combination therapy settings has acceptable short-term toxicity.

Timing of ATR inhibition with respect to genotoxin delivery

A major unanswered question is how to sequence the delivery of an ATR inhibitor with respect to a genotoxic chemother-


dapeutic during combination therapy. On the one hand, it seems logical to have ATR suppressed during and after genotoxin exposure, and strong synergy is observed in cultured cells treated in this way. However, studies with a Chk1 inhibitor in cultured cells and in an animal model showed that adding the inhibitor 24 hours after exposure to gemcitabine led to more synergism than other sequencing strategies (e.g., simultaneous addition, or adding the Chk1 inhibitor first), thus suggesting that delayed inhibition might increase synergy (65, 66). We are not aware of similar sequencing studies with ATR inhibitors in cell lines. The problem becomes even more complex when considering the timing of ATR inhibitor dosing with respect to the genotoxin in animal models and humans. Under these conditions, it is unclear when maximal genotoxin-induced DNA damage in the tumor has occurred and whether the ATR inhibitor can be delivered over long or short intervals with acceptable toxicity.

Since the discovery that the ATR-Chk1 pathway promoted the survival of tumor cells exposed to DNA-damaging agents and replication stress, there has been hope that small-molecule inhibitors of this pathway would increase the efficacy of chemotherapy agents that can, in some cases, cure human cancers. With the development of highly specific ATR inhibitors, we are now poised to determine if disabling ATR will have therapeutic benefit. We look forward to the results of these trials and the additional questions they will almost certainly raise.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

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Grant Support

Research reported in this publication was supported in part by the NIH under award numbers P50CA136393 (to L.M. Karnitz) and GM076388 (to L. Zou). L. Zou is a Jim & Ann Orr Massachusetts General Hospital Research Scholar.

Received July 15, 2015; accepted August 12, 2015; published OnlineFirst September 11, 2015.

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Clin Cancer Res  Published OnlineFirst September 11, 2015.