

## DNA-Checkpoint Kinase, ATR is a Promising Target for Synthetic Lethality-Aimed Anti-Tumor Therapy

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### Abstract

Cancer cells constitutively undergo DNA damages, in response to oxidant stresses, or during chemo- or radio-therapy. DNA checkpoint kinases, such as ATR, are important for cancer cells to overcome DNA damages, resulting in acquiring resistance. In this process, ATR kinase plays a key role in protecting cancer cells through DNA replication. In other words, ATR inhibition can be a reasonable strategy to release chemo- and radio-resistance. Now, clinical trials using ATR chemical inhibitors (*i.e.*, phase-I/II) are ongoing worldwide, with a focus on its synergic effect on DNA-damaging drugs or irradiation.

**Keywords:** ATR; CHK1; DNA checkpoint; Homologous recombination; Radio-resistance

### Introduction

DNA damages occur in living bodies, as a result of external exposure to UV, smoking, etc. or of intrinsic stress, such as oxidative stimuli. To reverse DNA damage, molecular network of checkpoint kinases, such as ATR, are involved in maintenance of “genome stability” via a faithful replication of DNA. For example, DNA-PK plays a crucial role in DNA replication post-DNA double-strand break (DSB) through a non-homologous end-join (NHEJ) system [1]. ATM acts as a master regulator, in response to DSBs [2]. Indeed, ATM induces DNA repair via utilizing homologous recombination (HR) in S- or G2/M-phase (and NHEJ in G1-phase). ATR signaling is triggered by any lesions exposed to single strand (ss) DNA, including resected ends of DSBs, ssDNA gaps during DNA repair and stalled or collapsed replication fork [2]. Notably, a loss in DNA checkpoint systems leads to a clonal selection of tumor cells with malignant phenotypes [3]. Based on this background, some researchers have proposed DNA checkpoint inhibition as a novel anti-cancer strategy. In this article, we will discuss the potential use of DNA checkpoint kinase inhibitors, with a major focus on ATR axis inhibition during conventional anti-cancer treatments, including chemotherapy or irradiation therapy.

### Synthetic Lethality-Based Monotherapy

Cancer cells with “a single mutation of critical checkpoint molecules” are still active, possibly via a redundant activation of another alternative checkpoint pathway(s). In this case, compensatory molecule(s) can be a pharmacological target for cancer death induction and this concept is defined as “synthetic lethal therapy”. With regard to this, there may be a crosstalk reaction between ATM and ATR for maintenance of genome stability and homeostasis. Indeed, ATR serine-1989 phosphorylation levels become much higher in ATM-deficient cancer cells than in ATM-preserved cells, suggesting a compensation of ATR for loss in ATM [4]. Notably, ATR chemical inhibitor, VE-821 selectively induced the apoptotic death in ATM-deficient cells. These findings clearly indicate that compensatory activation of ATR is responsible for genome homeostasis, while ATR inhibition is reasonable for killing ATM-mutant cancer cells, possibly as monotherapy. This result is reproducible in p53-, BRCA2- or Arid1A-deficient cells [4-6], thus convincing a redundant role of ATR under such a checkpoint molecule-deficient condition. In other words, ATR inhibitor alone may be not enough to efficiently kill cancer cells, if other DNA checkpoint cascade is well preserved.

### Chemotherapy Sensitization

Cisplatin-based DNA damaging chemotherapy is a standard choice for solid tumor-bearing patients. ATR is a key regulator of repair network during the cisplatin-induced DNA damage and ATR inhibition is known to sensitize cancer cells, but not normal cells, to cisplatin-induced death *in vitro*. Indeed, a recent report described that VX-970, a new drug of ATR inhibition, markedly enhanced the cancer regression, in a mouse model of primary lung xenograft [7]. ATR-CHK1 axis is known critical to prevent or minimize DNA damage and apoptosis. This provides a rationale why ATR inhibition produces the synergic anti-cancer effect on cisplatin treatment *in vivo*. Another key significance of ATR is to reverse chemo-resistance. Actually, ATR inhibitor (*i.e.*, VE-821) could re-sensitize cisplatin-resistant breast cancer cells (*i.e.*, MDA MB 468CR) to cisplatin [8]. Thus, ATR is now shown to have a large part in releasing chemo-resistance, a common serious problem in clinical cancer medicine.

Topoisomerase I (Top-I) inhibitor (such as camptothecin analogue, LMP-400) is also used for delaying tumor progression. However, malignant cancer cells sometimes acquire resistant to Top-I inhibitory drugs. In this process, phosphorylation levels of ATR (and its downstream effector, CHK1) become obvious, in response to Top-I inhibitors. Notably, ATR inhibitor (*i.e.*, VE-821) markedly enhanced the DNA damage, as evidenced by  $\gamma$ -H2AX accumulation and this result was associated with the losses in both ATR and CHK1 phosphorylations [9]. Overall, activation of ATR-CHK1 by chemo-drug was shown necessary for acquiring chemo-resistance. Thus, ATR inhibition is a principle-based strategy for chemotherapy sensitization, such as cisplatin, Top-I inhibitors and other DNA-damaging agents, as reviewed elsewhere [10].

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## Radiation Sensitization

X-ray radiation induces double strand break of DNA, followed by apoptotic cell death in tumor cells. The network of DNA checkpoint kinases (such as ATM, DNA-PK and ATR) is involved in this process. Indeed, ATR-mediated cell cycle arrest and DNA damage repair are known to produce radio-resistance in cancer cells. Inversely, ATR inhibitors dramatically enhance radio-sensitivity of malignant tumors. For example, a new ATR-inhibitor, VE-822 (also known as VX-970 or MM6620) markedly enhanced X-ray (6 Gy) radiation-induced death in pancreatic duct-derived carcinoma cells *in vitro* [11]. In this model, VE-822 enhanced the DNA damages, as evidenced by nuclear deposition of  $\gamma$ H2AX and this was associated with the decrease in nuclear Rad51 foci, a HR marker. Furthermore, VE-822 repressed the downstream CHK1 phosphorylation, but not ATM- or DNA-PK cascade activation, hence suggesting the selective effect on ATR-CHK1 cascade during the radiation-induced DNA damage. Overall, suppression of HR-based DNA repair by ATR inhibitors was shown critical for radiation to produce synergic effect on tumor killing. The prognosis of pancreatic cancer is very poor with a lower 5-year survival rate (<5%), due to malignant mutations (such as KRAS- or p53-mutant). Thus, forced induction of synthetic lethality by ATR inhibitors is reasonable for prolonged survival periods, as shown in a mouse model of pancreas cancer xenograft [11].

## ATR Cascade Inhibition Strategy

ATR is now a promising target for controlling malignant cancer behavior. Thus, it is important to understand the molecular hierarchy of ATR cascades (including upstream or downstream target molecules). ATR serine-1989 phosphorylation is important for initiation of DNA repair, especially at a single strand DNA (ssDNA) region [12]. Replication protein A (RPA) is an initial sensor for recognizing ssDNA at the damaged sites, followed by the assembly of RPA to ATRIP, an anchor for trapping ATR. As a result, ATR can be recruited at ssDNA region via a RPA-ATRIP hetero-assembly and then ATR is auto-phosphorylated at serine-1989, along with the loader proteins, such as 9-1-1 complex, Rad17 and TopBP1 [12].

Since these molecular machineries are necessary for ATR to initiate HR-based DNA repair, upstream molecule can be a target for rapid ATR inhibitions. For instance, a new inhibitor of RPA might be available for this purpose: a naphthalene-derived chemical compound, HAMNO selectively inhibits RPA32 activation, resulting in prohibition of ATR auto-phosphorylation [13]. This agent creates DNA replication stress in cancer cells, but not normal cells. Of importance, HAMNO acts synergistically with Top-I inhibitor, etoposide to kill cancer cells *in vitro* and slow tumor growth *in vivo* [13]. Taken together, RPA inhibitor may also be taken into consideration as an alternative drug to inactivate ATR.

Growth factor-mediated signaling pathway may be critical for ATR activation as an upstream of ATR axis. HGF is known to induce chemo- and radio-resistance in many types of cancer cells [14]. Of interest, X-ray radiation rapidly induced the tyrosine phosphorylation of c-met, an HGF receptor in cancer cells, associated with the ATR and CHK1 over-activations [15], thus suggesting the contribution of HGF signaling pathways. Of note, c-met tyrosine kinase inhibitor (i.e., PH665752) inhibited ATR and CHK1 phosphorylations post-irradiation, hence identifying HGF-c-met as an upstream cascade of ATR-CHK1 axis. Consistently, PH665752 enhanced the radio-sensitivity of cancer cells through severe DNA damage [15]. Another report suggested the critical role of IGF1-IGF receptor for producing radio-resistance via

sequential activation of IGF→IGFR→PI3K→AKT→ATR pathways [16]. Thus, growth factors, such as HGF, secreted from cancer cells may be a trigger for activating ATR signaling cascades to reduce (or reverse) DNA damage in an early process of chemotherapy or X-ray irradiation.

Checkpoint kinase, CHK1 is known as a direct substrate of ATR. In fact, phosphorylation of CHK1 at serine-435 by phosphorylated ATR leads to aurora kinase-B (Auro-B) activation and this downstream effector directly contributes to DNA replication and repair in an HR-dependent manner. Thus, CHK1 inhibitors may also be promising to control tumor development. There is now growing evidence to show the usefulness of CHK1 inhibitors for enhancing chemo- or radio-sensitization in animal studies [17,18]. ATR has multiple functions, required for DNA repair and chromosome segregation [12], while CHK1 function is limited to ATR. It is still unclear which ATR- or CHK1-targeting treatment is better for anti-tumor therapy and future studies would shed more light on this notion.

## Summary and Remarks

So far, checkpoint kinases, ATM and DNA-PK have been defined as a key regulator for DNA replication and repair, especially in a site of DSB post-irradiation. In addition to these kinases, there is now emerging evidence to show a critical function of ATR to overcome DNA damage, caused by cisplatin, Top-I inhibitors and irradiation, all of which are used as standard anti-cancer drugs or tools. In basic research, ATR inhibitor is also available as a screening probe to identify mutant genes responsible for synthetic lethality among malignant cancers [4-6]. Now, numerous pharmaceutical companies have a great interest in development of ATR inhibitors. For example, safety and effectiveness of MM6620 (same as previous code name, VE-822 or VX-970) was carefully evaluated in a phase-I clinical study [19], with a focus on a combination with cisplatin or irradiation (clinical trial number: NCT-02567422, 02157792 and 02595931). Oral drug of ATR inhibitor, AZD6738 is useful for acquiring chemo- and/or radio-sensitive outcomes [20], possibly in advanced cancers with stage-III/IVa. Other candidates, such as EPT-46464, AZ20 and BAY-1895344, may also be available as ATR-inhibitory drugs in the near future. ATR maintains chromosomal integrity during neurogenesis [21]. Recently, ATR signaling pathway (i.e., ATR→CHK1→AuroB kinase) was shown necessary for faithful chromosome segregation, via recognizing ssDNA gap generated in an R-loop during a prometaphase of mitotic cells [22]. This DNA checkpoint kinase is now a hot topic not only in clinical medicine [19,20] but also in genomic biology [21,22].

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