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Checkpoint kinase 1 inhibitors for potentiating systemic anticancer therapy

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ABSTRACT

The checkpoint kinase 1 (Chk1) is a key component of the DNA damage response, a molecular network deputed to maintain genome integrity. Nevertheless, cancer cells aberrantly exploit these circuits to overcome chemotherapy-induced cytotoxicity. Chk1 inhibitors have been developed as a chemopotentiating strategy and different molecular mechanisms underlying the synergism with chemotherapeutics have been uncovered. The monotherapy with Chk1 inhibitors seems to be endowed with antitumor activity against cancer cells characterized by specific defects in the DNA damage machinery or characterized by elevated levels of oncogene-induced replication stress. In this biological framework Chk1 neutralization represents a synthetic lethality-based therapeutic approach. Moreover, a dual targeting of the DNA damage machinery has been proposed envisioning the association of Chk1 abrogation with poly-ADP ribose polymerase inhibitors. The spectrum of antitumor properties of Chk1 antagonists is completed by the activity against cancer stem cells, the prominent tumorigenic population that is equipped to survive stressful conditions through multiple and interconnected mechanisms. Although the clinical development of the first generation of Chk1 antagonists was hindered by off-target effects and an unfavorable pharmacokinetic profile, a new wave of early clinical trials with more selective compounds are currently being carried out. To this end, the identification of predictive biomarkers and an in-depth characterization of molecular circuits governed by Chk1 are issues that need to be addressed for sharpening the therapeutic potential of Chk1 inhibitors.

Introduction

The DNA damage response (DDR) is a molecular network consisting of sensors, transducers, mediators and effectors assigned to maintain genome integrity through an integrated activity of multiple pathways.[1] Schematically, the DDR encompasses cell cycle checkpoints, DNA repair and apoptotic pathways. The detection of DNA damage evokes a cellular response that consists in activating cell cycle checkpoints, which delay cell cycle progression via the negative regulation of cyclin-dependent kinases. This event allows the recruitment of DNA repair pathways, encompassing multiple and partially overlapping mechanisms deputed to correct genetic lesions. If repair fails or the entity of the damage exceeds repair capacity, apoptotic signals are activated leading to the self-elimination of damaged cells.[2] Therefore, the DDR ensures a faithful transmission of undamaged DNA to the progeny. Eukaryotic cells exploit distinct, albeit cooperating, checkpoints that regulate cell cycle progression. When DNA lesions occur, the G1/S-phase checkpoint blocks S-phase entry, the intra-S-phase checkpoint delays S-phase progression while the G2/M-phase checkpoint prevents mitotic entry. In such a manner each step of the cell cycle is dependent on successful completion of the previous event. The checkpoint-mediated cell cycle arrest coordinates the activation of phase-specific repair mechanisms. For instance, double-strand breaks (DSBs) are repaired via the nonhomologous end-joining (NHEJ) in G1-phase, or through the homologous recombination repair (HRR) in dividing cells. Whether the original form of the genome cannot be restored, checkpoints trigger p53-dependent or -independent apoptosis. DNA damage are sensed by multiprotein complexes that recruit the proximal transducers ataxia telangiectasia mutated (ATM) and ataxia telangiectasia and Rad3-related protein (ATR). Upon amplification of the signal, ATR and ATM activate checkpoint kinases 1 and 2 (Chk1 and Chk2), respectively, despite an existing extensive crosstalk between the ATR-Chk1 and ATM-Chk2 pathways. Chk1 and Chk2 are structurally unrelated kinases that mediate different activities in the context of the DDR network.[3] Chk1 is a serine/threonine kinase that regulates the G2/M checkpoint by preventing aberrant mitotic entry of damaged cells, albeit its activity is not restricted to the above mentioned checkpoint. Conversely, Chk2 is thought to be an amplifier of checkpoint responses.[4] While these mechanisms

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protect mammalian cells from mutagenic insults by avoiding non-lethal mutations to be passed down to the progeny, cancer cells aberrantly exploit the DDR machinery to overcome chemo- and radiotherapy-induced cytotoxicity. Given the multifaceted involvement of Chk1 in the DDR, Chk1 abrogation has been extensively explored as a chemotherapy-potentiating strategy.[5] Furthermore, many tumors are characterized by defects in the G1/S-phase checkpoint coming from mutational events involving the retinoblastoma protein (Rb) or p53. As a result, cancer cells mainly depend on the G2/M checkpoint for halting the cell cycle and repairing genetic lesions. Therefore, it is expected that the pharmacological inhibition of Chk1 increases the therapeutic activity of many chemotherapeutic agents by abrogating the remaining intact checkpoint. This raised the possibility that Chk1 neutralization represents a synthetic lethality-based therapeutic approach,[6] similar to Poly-(ADP ribose) polymerase (PARP) inhibition in the BRCA-mutated background, while somatic cells should mostly be spared by the chemosensitizing effects of Chk1 antagonists. In this review, we discuss the biological background underlying the development of Chk1 inhibitors and preliminary data deriving from early clinical trials.

Biological functions of Chk1

The cell cycle is driven by cyclin-dependent kinases that are negatively regulated by checkpoints when defects occur. G1 phase arrest relies on p53 activation and the consequent accumulation of its target genes, such as the cyclin-dependent kinase inhibitor p21. Entry into mitosis is governed by the G2/M checkpoint, a molecular cascade involving a large set of proteins that ensures all damage is repaired before cells continue to divide. This process culminates in the activation of Chk1 that in turn regulates the activity of distal effectors that ultimately produces the checkpoint-mediated arrest.[7] The G2/M checkpoint is initiated by single-strand DNA (ssDNA) breaks coming from either a lesion hindering the progression of the replication fork or the exonuclease-mediated processing of DSBs. The replication protein A (RPA) rapidly binds ssDNA, and the resulting RPA-coated ssDNA serves as a "landing pad" for the recruitment of several checkpoint proteins. Two independent sensor complexes are engaged: the 9-1-1clamp (Rad9, Rad1 and Hus1proteins) is loaded onto RPA-coated ssDNA by the loading complex Rad17/RFC, while ATR is recruited through its binding partner ATRIP. Once engaged, ATR phosphorilates both its binding partner and components of the 9-1-1 complex, leading to the activation of mediator proteins (TopBP1, clapsin, MDC1, Timeless, Tipin and BRCA1). This amplification of the signal allows Chk1 to be recruited to the assembled checkpoint complex, where it is activated through the ATR-mediated phosphorylation. The last step of this cascade is the Chk1-mediated regulation of the kinase Wee1 and the phosphatase Cdc25, the distal controllers of the checkpoint. Activation of Wee1 and inhibition of Cdc25 culminates in the inhibition of the cyclin-dependent kinase Cdc2. This determines both the maintenance in an inactive state of pre-existing Cdc2-B-type cyclin complexes and the inhibition of newly formed complexes, therefore delaying mitotic entry (Fig. 1). Nevertheless, Chk1 exerts multiple biological activities that are not limited to the G2/M checkpoint.[3] When DNA damage occurs during the S-phase, the ATR-mediated Chk1 activation is essential for activating the S-phase checkpoint which leads to reducing DNA synthesis, and stabilization and recovery of stalled replication forks. It is known that Chk1 is a negative regulator of Cdc25A phosphatase stability. This event prevents activating the Cdk2–CyclinE complex and loading Cdc45 onto origins, ultimately inhibiting origin firing.[8,9] A similar biological outcome is elicited by the Chk1-mediated phosphorylation and consequent inhibition of Cdc7, an essential S-phase kinase whose activation is required

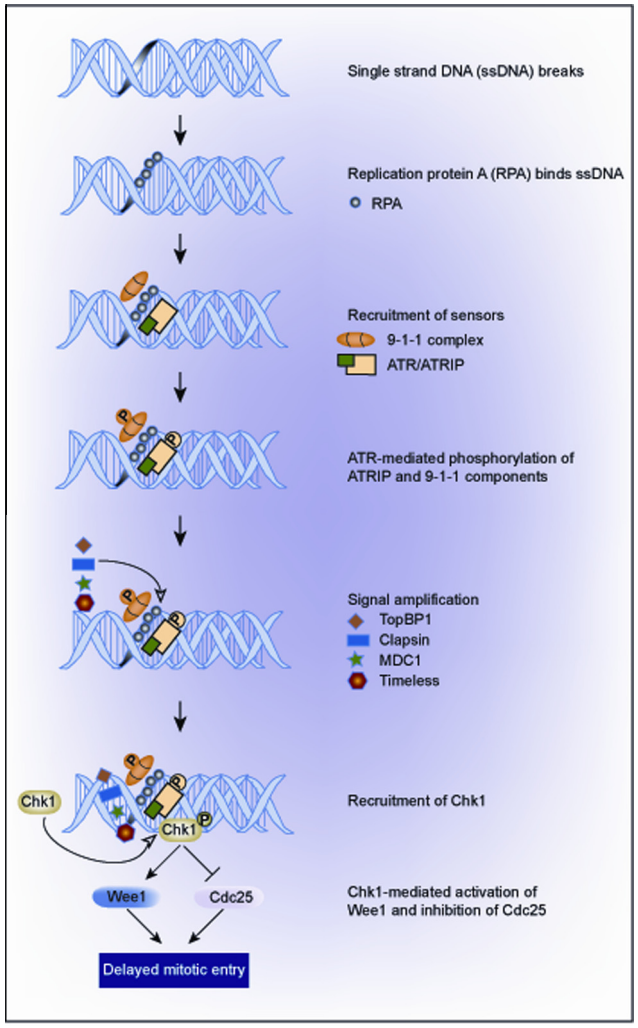

Fig. 1. The cascade of molecular events required for assembling the G2/M checkpoint. Single-strand DNA (ssDNA) breaks (step 1) are coated by the replication protein A (step 2). This initial event allows the recruitment of the two independent sensor complexes 9-1-1 and ATR/ATRIP (step 3). The subsequent ATR-mediated phosphorylation of ATRIP and 9-1-1 complex components (step 4) leads to the engagement of several mediator proteins (step 5). The cascade is completed with the recruitment of Chk1 to the assembled checkpoint complex where it is phosphorylated and activated (step 6). Once activated Chk1 operates a dual control on the distal effectors Wee1 and Cdc25 (step 7) that ultimately delay mitotic entry (step 8).

for initiating DNA replication.[10] Furthermore, Chk1 phosphorylates and inhibits Tousled-like kinases 1 (Tlk1), a serine/threonine kinase activated during S phase of the cell cycle and required for chromatin assembly,[11] and it has been involved in the translesion DNA synthesis through the ubiquitination of proliferating cell nuclear antigen (PCNA).[12] This bypass process allows the replication of some DNA lesions, thus avoiding that damages escaping the repair activity block the replication machinery. More recently, Chk1 activation has been associated with the mitotic spindle checkpoint, the molecular network that delays the metaphase–anaphase transition when erroneous chromosome-spindle interactions occur. In particular, it has been demonstrated that Chk1 activates Aurora-B, a key component of the chromosomal passenger complex controlling correct chromosome alignment and segregation.[13] Furthermore, Chk1 negatively regulates polo-like kinase 1, a crucial mitotic substrate, leading to the activation of the spindle checkpoint.[14] When considering DNA damage repair pathways, evidence indicates that Chk1 is required for HRR, NHEJ and the Fanconi Anemia/BRCA pathway owing to its