Molecular Pathways

Targeting Cell Division Cycle 7 Kinase: A New Approach for Cancer Therapy

Alessia Montagnoli, Jürgen Moll, and Francesco Colotta

Abstract

The cell division cycle 7 (Cdc7) is a serine-threonine kinase, originally discovered in budding yeast, required to initiate DNA replication. Human Cdc7 phosphorylates the minichromosome maintenance protein 2 (Mcm2), a component of the DNA replicative helicase needed for genome duplication. Inhibition of Cdc7 in cancer cells impairs progression through S phase, inducing a p53-independent apoptotic cell death, whereas in normal cells, it does not affect cell viability. Small molecule compounds able to interfere with Cdc7 activity have been identified and shown to be effective in controlling tumor growth in animal models. Two Cdc7 inhibitors are currently in phase I clinical development. Inhibition of Cdc7 kinase activity in cancer cells restricts DNA replication and induces apoptotic cell death by an unprecedented molecular mechanism of action. Clin Cancer Res; 16(18); 4503–8. ©2010 AACR.

Background

DNA Replication

Eukaryotic DNA replication is a tightly regulated process, which ensures that no origins fire more than once per cell cycle, in order to maintain genomic stability. It consists of two major steps: initiation of DNA replication followed by elongation of nascent DNA strands (1).

DNA replication initiation requires the assembly of the prereplicative complex (pre-RC) at sites in the genome called origins, the activation of the pre-RC, and the formation of the replisome, which is ultimately responsible for the duplication of DNA that occurs during the elongation step (2).

The origin recognition complex (ORC) marks the origin and initiates the sequential loading of the components of the pre-RC, including cell division cycle 6 (Cdc6; ref. 3), Cdt1, and the six minichromosome maintenance proteins (Mcm2-7) in late mitosis and early G1 (4). Mcm2-7 is the catalytic core of the replicative helicase that is specifically recruited by Cdc6 and Cdt1 through direct protein-protein interactions and ATP hydrolysis (5, 6). In cells, the formation of an active helicase at replication origins requires MCM phosphorylation and the loading of additional factors, including Cdc45 and the GINS complex. Once activated, Mcm2-7 unwinds double-stranded DNA at the origin to generate the single-stranded DNA (ssDNA) template required to recruit the remaining DNA synthesis machinery (i.e., RPA, DNA polymerases) and to start chain elongation. The activation process is triggered by the S phase–promoting kinase Cdc7 that, together with cyclin-dependent kinase 2 (Cdk2), phosphorylates components of the MCM complex and stimulates the helicase activity by allowing the interaction between Cdc45, Mcm2-7, and Go-Ichi-Nii-San (GINS; Fig. 1; refs. 7, 8).

Cell division cycle 7 kinase

The gene encoding Cdc7 kinase was first isolated in budding yeast (9), whereas the human homolog was identified almost two decades later (10). In vertebrates, Cdc7 activation requires the interaction with either one of the regulatory subunits Dbf4 (11, 12) or Drf1 (13), whose binding to the kinase is mutually exclusive. In Xenopus, Drf1 seems to be the predominant activator of Cdc7 during early embryogenesis, whereas Dbf4 plays a more prominent role in somatic cells (14). In human cells, Dbf4 and Drf1 accumulate in S phase and regulate Cdc7 activity during the cell cycle, although the specific role of the two regulators has not yet been fully elucidated (15). Other proteins regulate formation of the complex at the transcriptional level; whereas Dbf4 is an E2F-regulated gene, Cdc7 gene expression is directly regulated by the transcription factor Myb (16) and is repressed by Cdc7 expression repressor (CR)/periphilin (17).

The major target of Cdc7 activity in mammalian cells is the MCM complex. Cdc7 phosphorylates chromatin-bound Mcm2 at the G1-S transition, and this phosphorylation is required for the initiation of DNA replication but not for Mcm2 loading on DNA (18–20). Another cellular target of Cdc7 activity is LEDGF, the immunodeficiency virus cofactor known to be functionally associated...
with transcriptionally active genomic loci (21). The interaction with LEDGF strongly stimulates Cdc7 activity and may provide a link between transcription and DNA replication.

In addition to its well-established role in S phase, Cdc7 controls chromosomal segregation in mitosis by phosphorylating the heterochromatin protein 1 (Hp1). Hp1 promotes cohesion of sister chromatids in mitosis specifically at centromeres (22). When sister chromatids are trapped into the cohesin ring, mitosis is blocked until DNA replication is completed (23).

**Cell division cycle 7 inhibition**

The cellular effects of Cdc7 depletion have been studied by antibody microinjection (24), and more recently by RNA silencing (25), showing that in human cells, Cdc7 is essential for DNA replication. In cancer cells, lack of Cdc7 results in progression through a defective S phase that leads to p53-independent apoptotic cell death. It has been shown that the lack of Cdc7 affects neither exit from mitosis, nor G1-S progression, but produces cells bearing incompletely and/or abnormally replicated DNA, which can result in cell death. Some cancer cells undergo a
that responds to Cdc7 depletion exists. Lack of Cdc7 causes a cell-cycle arrest at the G1-S boundary with unreplicated DNA, elevated p53 protein levels, and induction of the cyclin-dependent kinase inhibitor p21. As a consequence, Cdc7 depletion does not induce apoptotic cell death in normal human fibroblasts. Induction of p53 by Cdc7 depletion in normal cells has a key role in cell-cycle arrest and lack of cell death. By downregulating p53 in arrested cells, cell-cycle blockade can be completely bypassed, and the cells can progress through an aberrant S phase leading to apoptosis. The selective cytotoxic effect on cancer cells, which is a common and unique feature of inhibition of proteins involved in the initiation of DNA replication (32, 33), suggests that Cdc7 might be an attractive target for the development of drugs that kill proliferating malignant cells but spare actively replicating normal cells. The exact mechanisms accounting for the differential response on cancer cells have not yet been completely elucidated. We envisage that, in untransformed cells, inhibition of Cdc7 may trigger a "licensing checkpoint," leading to a block of DNA replication and reversible cell-cycle arrest that is lost in cancer cells. In particular, tumors that already harbor defects in G1-S checkpoint–related proteins might be more sensitive to the effects of a Cdc7 inhibitor. For example, tumors carrying defects in the p53 or Rb pathway, displaying low levels of tumor suppressors p27, p21, or p16 or overexpressing cyclins D or E, may be more sensitive to Cdc7 inhibition.

In summary, Cdc7 depletion impairs different cellular processes that cover regulation of DNA synthesis, chromosome segregation in mitosis, and the control of the DNA damage response.

**Cell division cycle 7 overexpression**

As reported for other proteins involved in the initiation of DNA replication, Cdc7 and its protein regulator Dbf4 are overexpressed in human cancer cell lines and in many primary tumors compared with matched normal tissues (34). Overexpression of Cdc7 in ovarian cancer correlates with tumor anaplasia, aneuploidy, and advanced clinical stage. The greatest discriminatory effect of Cdc7 expression was observed for stage 3 and 4 ovarian tumors, which make up the vast majority of epithelial ovarian carcinoma cases (35). Cdc7 and Dbf4 are overexpressed in malignant melanoma compared with benign melanocytic nevi, and Dbf4 overexpression has been associated with lower relapse-free survival (36, 37). In diffuse large B-cell lymphoma, increased Cdc7 activity is associated with poor clinical outcome (38). High Cdc7 levels were also found in breast cancers analyzed by tissue microarray. Here, high expression levels are significantly related to a medullary histotype, high tumor grade, negative estrogen receptor status, and the amplification of a number of genes including c-myc and HER2 (39). A role of Cdc7 protein in promoting gene amplification has been described in other organisms, but the underlying mechanism for this phenomenon still needs to be explored. A strong staining intensity and high percentage of positive cells showing...
Mcm2 phosphorylation, both indicators of high Cdc7 kinase activity, were found in particular in skin basalioma, laryngeal squamous cancer, serous ovarian cancer, in the majority of breast tumors, uterine tumors, urothelial bladder cancer, diffuse large B-cell non-Hodgkin’s and Hodgkin’s lymphomas, and in colon adenomas and adenocarcinomas.

Because Cdc7 is required for DNA replication, higher Cdc7 levels might be linked to the proliferative capacity of tumor cells. However, increased Cdc7 expression levels do not always correlate with the proliferative status of cells, whereas they correlate with the percentage of cells in S phase. Accordingly, Cdc7 and Dbf4 overexpression in cells causes cell-cycle arrest in S phase (40). An intriguing hypothesis is that, with Cdc7-Dbf4 being implicated in the DNA damage response, increased Cdc7 activity may aid recovery or repair of stalled replication forks to enhance survival of certain tumor cells.

Somatic mutations in CDC7 have been identified in colorectal and gastric carcinomas through comprehensive kinase screens of human tumors (41), although the biological consequences of these mutations have not yet been evaluated. These findings suggest that alterations in Cdc7/Dbf4 protein abundance or activity during tumorigenesis may have important consequences for cell survival, underlining the potential of Cdc7 as an anticancer target with a new mechanism of action.

Clinical-Translational Advances

The first Cdc7 kinase ATP-competitive small-molecule inhibitors, belonging to the 2-heteroaryl-pyrrolopyridones chemical class, were identified at Nerviano Medical Sciences Srl by high-throughput screening using a biochemical kinase assay (42–44).

Further lead optimization resulted in the selection of NMS-1116354, which entered phase 1 clinical trials in 2009 for solid tumors with a treatment schedule of 7 or 14 consecutive days every 2 or 3 weeks with oral administration (45). NMS-1116354 causes inhibition of DNA replication and p53-independent apoptosis during S phase in cancer cells. Additionally, the compound is able to reduce the expression of the anti-apoptotic proteins Mcl-1 and XIAP. This latter finding could be considered as an additional opportunity to increase the apoptotic response in particular tumor settings (46–48). The compound shows significant antitumor single-agent activity, with tumor regression in different preclinical models of solid tumors after oral administration without overt toxic effects. Mcm2 phosphorylation is also modulated in vivo, supporting its use as a pharmacodynamic biomarker in the tumors and/or surrogate tissues of patients.

In collaboration with Exelixis, Bristol Myers Squibb (BMS) is developing a benzo[f]uracipyrimidinone Cdc7 inhibitor (XL-413/BMS-863233), which entered phase I-II clinical trials in 2009 in solid tumors and hematologic malignancies, with an oral treatment schedule of 7 or 14 days per 21- or 28-day cycle. BMS-863233 is a potent ATP-competitive inhibitor of Cdc7 kinase with some cross-reactivity against PIM-1 kinase. The mechanism of action of the compound was confirmed by following the effects on DNA replication and on Mcm2 phosphorylation. The compound is orally available and produces antitumor activity in a colon cancer xenograft model, when given daily for 2 weeks (49).

In addition to Nerviano and BMS, other companies, such as Novartis (50) and Sanofi-Aventis (51), have described low nanomolar Cdc7 inhibitors, but no further data are available for these compounds. A potent Cdc7 inhibitor with antitumor activity in a model of Philadelphia chromosome-positive acute leukemia was identified by high-throughput screening at the Memorial Sloan-Kettering Cancer Center and is reported to be in preclinical development (52).

Even if the toxicologic data for these compounds are not disclosed yet, the nature of the dose-limiting toxicity for many cell-cycle inhibitors is hematologic. However, preclinical data suggest that Cdc7 inhibition induces tumor-specific effects. These data might be explained by the combined effect of replication fork arrest and deficient checkpoint responses, which induce aberrant cell-cycle progression and death in tumor cells, whereas normal cells can survive through execution of back-up checkpoint mechanisms.

As a consequence, differently from other compounds that inhibit cell proliferation, a Cdc7 inhibitor is expected to have a wide therapeutic window. Data derived from animal models strongly support this hypothesis because an excellent antitumor efficacy as single agent is achieved without overt toxicity, using an optimized schedule of treatment. The fact that sustained inhibition of Cdc7, in the presence of compounds inducing DNA damage, increases cell death in vitro might suggest the use of Cdc7 inhibitors not only as single agents, but also in combination with other chemotherapeutics such as topoisomerase inhibitors and alkylating agents.

In conclusion, preclinical data show that Cdc7 is a novel and promising target for tumor-cell killing, as has been shown with different inhibitors. Ongoing clinical trials will reveal whether inhibition of this kinase represents a successful strategy that will bring benefits to cancer patients in terms of superior activity and/or better tolerability compared with the currently approved agents that affect DNA replication.

Disclosure of Potential Conflicts of Interest

The authors have no conflicts of interest to declare.

Received 05/03/2010; revised 06/23/2010; accepted 06/30/2010; published OnlineFirst 07/20/2010.
Cdc7 Kinase Inhibition in Cancer

References


Clinical Cancer Research

Targeting Cell Division Cycle 7 Kinase: A New Approach for Cancer Therapy

Alessia Montagnoli, Jürgen Moll and Francesco Colotta


Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-10-0185

Cited articles
This article cites 49 articles, 23 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/16/18/4503.full#ref-list-1

Citing articles
This article has been cited by 4 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/16/18/4503.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.