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Review

Cdc7 kinase – A new target for drug development

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ABSTRACT

The cell division cycle 7 (Cdc7) is a serine threonine kinase that is of critical importance in the regulation of normal cell cycle progression. Cdc7 kinase is highly conserved during evolution and much has been learned about its biological roles in humans through the study of lower eukaryotes, particularly yeasts. Two important regulator proteins, Dbf4 and Drf1, bind to and modulate the kinase activity of human Cdc7 which phosphorylates several sites on Mcm2 (minichromosome maintenance protein 2), one of the six subunits of the replicative DNA helicase needed for duplication of the genome. Through regulation of both DNA synthesis and DNA damage response, both key functions in the survival of tumour cells, Cdc7 becomes an attractive target for pharmacological inhibition. There are much data available on the pre-clinical anti-cancer effects of Cdc7 depletion and although there are no available Cdc7 inhibitors in clinical trials as yet, several lead compounds are being optimised for this purpose. In this review, we will address the current status of Cdc7 as an important target for new drug development.

1. Introduction

All major transitions through the cell cycle are tightly controlled by the cyclin-dependent kinases (Cdks) that signal at the cell cycle checkpoints. DNA damaging events modulate checkpoint signalling to facilitate DNA repair or programmed cell death depending on the damage incurred by the cells. Many cancers with proliferative mutations often have abnormal checkpoint responses allowing tumour cells to remain viable and for a survival advantage to be passed on when the genome is duplicated. Much work has been done to elucidate the mechanisms that govern cell cycle regulation and how these mechanisms are impaired in tumourigenesis. Mutations in checkpoint regulators cause a variety of human tumours.[1,2] Potential targets for anti-cancer therapy have emerged as a result of improved understanding of these mechanisms and of checkpoint signalling events. One of these targets, the highly conserved Cdc7 (cell division cycle 7) kinase, is critical for normal cell cycle progression and has several structure/function relationships with the Cdks, making it an important target for pharmacological inhibition.

2. Functions of Cdc7 kinase

2.1. *Role of Cdc7 in DNA replication*

DNA replication begins from the origin firing points which consist of pre-replicative complexes formed during the previous G1 phase of the cell cycle[3–5] (Fig. 1). The minichromosome maintenance proteins (Mcm 2–7) within these complexes retain helicase activity, and following phosphorylation lead to local unwinding of DNA. This is followed by

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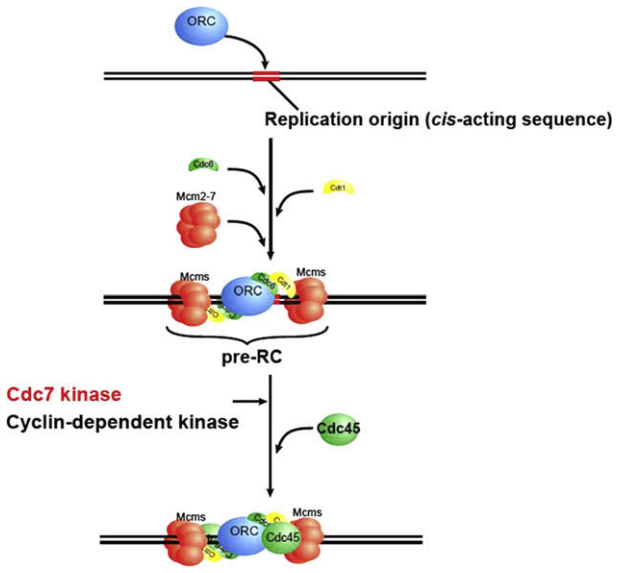

Fig. 1 – DNA replication in eukaryotic DNA and the role of Cdc7. DNA replication is first initiated by the binding of ORC (origin recognition complex) to replication origins. With the co-operation of both Cdc6 and Cdt1, Mcm (minichromosome maintenance complex) is delivered to the origins of replication and a pre-RC (pre-replicative complex) is generated. Both Cdc7 and Cdk2 phosphorylate elements of the Mcm complex (N-terminal tails of Mcm2, Mcm4 and Mcm6) which lead to loading of other accessory factors such as Cdc45 and the GINS complex. Active replication forks are then generated by association of 3 DNA polymerases that ultimately lead to the semiconservative replication of DNA.[73]

loading of DNA polymerases and accessory factors that participate in the semi-conservative synthesis of new DNA strands during chain elongation.[6] The S phase-promoting kinase Cdc7, together with Cdk2, is critical for G1/S phase transition through phosphorylation and activation of the Mcm2 helicase.

The highly conserved Cdc7 kinase was first isolated by Hartwell[7] in budding yeast and its human homologue was identified almost 2 decades later.[8] The important role of Cdc7 kinase in origin firing during S phase was first elucidated by Bousset and Diffley.[9] In yeast models, partial loss of Cdc7 function resulted in a slow progression through S phase rather than slow entry into S phase. In addition, Ccd7 was still required for the timely completion of S phase after elongation blockade with hydroxyurea. Therefore, Cdc7 is important for both the G1/S phase transition and S phase progression through activation of both early and late origin firing points.

2.2. *Role of Cdc7 in chromatin structure and chromosome segregation*

In addition to its important roles in the S phase of the cell cycle, Takahashi et al.[10] outlined the critical function of Cdc7 for normal chromosomal segregation in mitosis through loading of cohesins onto chromatin. Cohesins consist of four-subunit complexes which form ring-shaped structures that are required for bi-orientation of sister kinetochores and accurate chromosomal segregation during cytokinesis.[11–13] In Xenopus egg extract models,[10] DDK (Cdc7 and its regulatory subunit Drf1), physically associates with the Scc2–Scc4 complex which in turn binds cohesin. The DDK component of this ternary complex docks onto Mcm2–7 and brings Scc2–Scc4 and cohesin close to chromatin, thereby allowing cohesin deposition on DNA. Coupling of Scc–Scc4 loading to DDK and Mcm2–7 enables loading of cohesin onto chromatin prior to the initiation of DNA replication. As a result, cohesin is available for chromosomal segregation as the cell cycle progresses from S phase. Additionally, using DDK and Mcm2–7 for cohesin loading provides a convenient mechanism to achieve a regular distribution of cohesin-binding sites.

Following on from this, Matos et al.[14] explored the function of Cdc7 in meiosis. Unlike mitosis, DNA replication is followed by the segregation of homologous chromosomes and not of sister chromatids. This process depends on the formation of inter-homologue connections through crossover recombination as well as the attachment of sister kinetochores to microtubules emerging from the same spindle pole. In yeast models, DDK promotes double strand break formation, the first step of recombination and the recruitment of the monopoly complex to kinetochores. It is not clear, however, if these functions are conserved across species.

2.3. *Role in DNA damage and DNA replication stress*

Proper processing of stalled or damaged replication forks is essential for cell viability.[15] The cells with checkpoint regulation defects allow DNA damage to be carried through the cell cycle into mitosis leading to mitotic catastrophe and cell death. Checkpoint responses occur at critical time-points during the cell cycle and consist of mediator proteins that activate the checkpoint kinases and effector proteins which execute the checkpoint effect. The Cdc7 kinase has been implicated in both phases of the checkpoint response. Cdc7 interacts with and phosphorylates the mediator protein claspin, and is required for the activation of the ATR–Chk1 checkpoint.[16] In yeast models, phosphorylation of Mrc1 (the yeast equivalent of claspin) depends on the Cdc7 yeast homologue Hsk1, and Mrc1 is required for the activation of Cds1, a yeast effector checkpoint kinase.[17] In other yeast models the role of Cdc7 in the continuous activation of the Rad53 checkpoint in response to hydroxyurea has been shown. In xenopus egg extracts, Cdc7 is inactivated in response to the DNA-damaging agent etoposide[18] and is downregulated in human models in response to the same agent.[19] Therefore, in non-clinical models, Cdc7 is an important player in both mediating and effecting the checkpoint response. However, it is not clear that these functions for Cdc7 are as important in human models. In fact, it has been shown that Cdc7 activity is unchanged by replication fork arrest in human cells.[20] What is clear though is that loss of Cdc7 function leads to s-phase arrest, DNA fragmentation and cell death in the case of abrogated checkpoint response.

3. Cellular effects of Cdc7 depletion

As outlined in various models, Cdc7 has been implicated in the regulation of DNA synthesis, chromatin structure and

assembly, chromosomal segregation in mitosis and meiosis, regulation of double strand break formation and in the DNA damage response. The cellular effects of Cdc7 depletion have been studied in siRNA knockdown models in various cancer cell lines. Following a delayed S phase, tumour cells accumulate nuclear damage (presumably induced by stalled replication forks) and undergo chromosomal fragmentation and die, independent of p53 expression.[16,21–24] *In vivo*, Kim et al.,[22] observed that in mice carrying a hypomorphic allele of Cdc7, over 75% of the Cdc7–/–transgene mice died within 3 days of life. Surviving mice had a normal life span but attained only half the bodyweight of their unaffected littermates. Mouse embryonic fibroblasts (MEFs) from Cdc7–/–tg mice showed delayed entry into S phase and reduced S phase progression after serum stimulation of resting cells. Male and female mutant mice were infertile. Male Cdc7–/–tg mice had testicular atrophy with reduced numbers of spermatogonia and degenerated primary spermatocytes. Female mice had small ovaries containing no oocytes or follicles. Consistent then with *in vitro* models, Cdc7 depletion appears to have profound effects on somatic and germ line cell cycle progression.

4. Regulation of Cdc7 kinase

In budding yeast the levels of Cdc7 remain constant throughout the cell cycle and regulation of kinase activity comes largely from binding of other proteins that peak and trough during cell cycle progression. The first of these protein regulators was discovered during studies of Cdc7 kinase in budding yeasts when the DBF4 gene was identified during screening for mutants arresting as dumbbell forms (indicative of defects in the initiation of DNA synthesis).[25] The transcribed protein, Dbf4 (dumb-bell forming factor 4), binds to and activates Cdc7, and is regulated by both post-transcriptional and post-translational modifications.[26] Levels of Dbf4 begin to increase during G1 and explain, in part, why Cdc7 kinase activity peaks at the G1/S boundary.[26]

In human cells the regulation of Cdc7 kinase activity has not been fully characterised: two papers published in quick succession went on to describe the human homologue of Dbf4 (HuDbf4 or ASK) and independently reported similar findings.[6,30] The promoter region of the huDbf4 gene carries multiple copies of E2F transcription factor-binding sites[28,29] allowing E2F-regulated transcription of huDbf4 at different time-points during the cell cycle. This leads to huDbf4-dependent control of Cdc7 kinase activity.[30] Localisation of both proteins to chromatin also occurs at different times during the cell cycle and may also serve to modulate Cdc7 function.[31] Sequence analysis and mutagenesis studies of Dbf4-like proteins from several species indicate a common structure. Three conserved amino acid motifs (N, M and C motifs) have been identified from the N- to the C-terminus of Dbf4.[32] The M and C motifs bind and activate the catalytic subunit of Dbf4, whilst the N motif may serve to aid in protein–protein interaction and help target replication origins for Cdc7–Dbf4 complexes, however, the exact role of this motif remains unclear.[33] Using a bioinformatic approach, Montagnoli et al.[33] set out to explore the possibility of additional Dbf4-like molecules in humans and a second protein regulator, Drf1, was uncovered. Characterisation of the human Cdc7–Drf1 complex demonstrates that Drf1 binds to and activates Cdc7 and similar to Dbf4 becomes a substrate for Cdc7, however, the functions of the phosphorylated protein regulators have not been fully elucidated.[33] Levels of Drf1 accumulate when cells are in S phase and fall when cells exit mitosis, similar to Dbf4. It may be that the presence of two nuclear regulator proteins play a role in the temporal activation of origin firing with early and late activations by Cdc7 being determined by the specific regulator protein that is bound.[33] Both Drf1 and Dbf4 modulate Cdc7 kinase activity directly through protein–protein interaction, other proteins such as CR (Cdc7 repressor) act as negative regulators of Cdc7 kinase through transcriptional repression of the Cdc7 gene.[34] Similarly the transcription factor Myb has been shown to directly regulate gene expression of Cdc7 through identification of Myb response elements (MREs) located in the Cdc 7 transcription start site.[35]

5. Similarities between the Cdks and Cdc7 kinase

5.1. *Functional similarities*

In the control of G1/S progression, three cyclin-dependent kinases (Cdks), have been identified as key regulators (Cyclin D/Cdk4, cyclin E/Cdk2 and cyclin A/Cdk2).[36] Sequential activation of these kinases leads to phosphorylation of the retinoblastoma protein (pRb) and its related family members, p107 and p130.[37–39] Following this, the E2F transcription factor is activated and it induces the expression of other genes required for DNA synthesis, including cyclin E.[40] Of these three Cdks, Cdk2 (also a serine threonine kinase) along with Cdc7 activates the origin replication complexes and initiates the process of DNA replication and fork progression through modulation of Mcm2. The Cdk2-interacting protein, CINP, was recently proposed as a functional link between Cdk2 and Cdc7 in origin firing and is likely to play a role in the regulation of both kinases in Mcm2 activation[36] (Fig. 2). *In vitro* phosphorylation of Mcm2 with Cyclin E/Cdk2 followed by

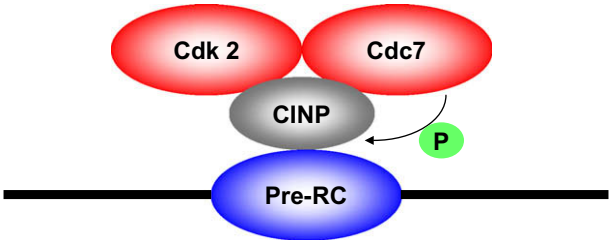

Fig. 2 – Functional relationship between Cdc7 and the cyclin-dependent kinases. CINP (Cdk2 interacting protein) is a component of the active cyclin A/Cdk2 complex. CINP also interacts with Cdc7 and is phosphorylated by Cdc7, but not by Cdk2. CINP binds to chromatin in a replication-dependent manner, and associates with the pre-replicative complex (pre-RC). CINP is a part of the Cdc7-dependent mechanism of origin firing and becomes a functional and physical link between Cdk2 and Cdc7 complexes at these sites on DNA.

the addition of a Cdk inhibitor (p27) increased Mcm2 phosphorylation by Cdc7 kinase by up to 2-fold, suggesting that both kinases work in tandem to generate phosphorylation sites on Mcm2.[41] In support of this observation, Cdc7 phosphorylation of Mcm2 at Ser40 only occurs if the nearby Ser41 was previously phosphorylated by Cdk.[42]

5.2. *Similarities in regulation*

The regulation and activation of Cdc7 kinase is similar to those of the Cdks by the cyclins. Both the Cdks and Cdc7 are dependent on the levels of their associated protein regulators which rise and fall during the cell cycle and correspond to the kinase activities of Cdc7–Dfb4/Drf1 and Cyclin-Cdk, respectively. Despite this, Dbf4 shows no sequence homology with the cyclins and Cdc7 is not a member of the cyclin-dependent kinase family.

5.3. *Potential structural similarities*

The structure of several Cdks has been solved, the structure of Cdc7 has yet to be fully characterised. It is possible, however, that Cdc7 and the Cdks share some similarities in their catalytic sites, as the first Cdc7 inhibitors were identified among compounds that were originally designed as Cdk2 inhibitors[43] and the best characterised Cdc7 inhibitor has strong cross reactivity with Cdk9.[44]

6. Rationales for a Cdc7 kinase inhibitor

Deregulation of normal cell cycle progression has been the hallmark of many traditional anti-cancer agents prior to the era of targeted therapies. Compounds that unbalance the pool of available deoxyribonucleotides and/or become incorporated into replication forks to act as chain terminators include the nucleoside analogues, 5-flourouracil metabolites and the alkylators among others. The clinical efficacy of these drugs is limited by toxicity and the rapid development of resistance. Given the importance of Cdc7 in the survival of tumours cells, it becomes an attractive target for pharmacological inhibition.

Recently, Bonte et al.,[45] screened the NCI-60 cancer cell lines and an additional panel of five leukaemia cell lines for over-expression of Cdc7 and its partner Dbf4. They showed that both proteins had increased expression in 50% of the tumour cell lines examined. Expression was confirmed in primary patient samples also (breast, colon and lung). Importantly, Cdc7 expression in normal tissue did not appear to be increased. There was also a high correlation between mutated p53 and Cdc7 overexpression suggesting that Cdc7 may, in part, be directly suppressed by p53 or that p53 mutations lead to Cdc7 activation.

Mechanistically, the role of Cdc7 in tumourigenesis is not fully understood. In fact, there may be none and Cdc7 may simply be expressed and regulated as a function of the mechanisms responsible for the transformed character of the cells. Because of its pivotal role in S phase progression, increased levels may serve to drive the proliferative capacity of the tumour, however, this does not appear to be the case when proliferation indices are correlated with Cdc7 expression levels.[45] But, since phosphorylation of Mcm2 is an indication of Cdc7 activity, phospho-Mcm2 may be a more relevant indicator of proliferation than expression of Cdc7 alone, though this has yet to be shown.

Because of the role of Cdc7 in checkpoint signalling following DNA-damaging events,[18,46–48] perhaps overexpression may be an important step in the repair of stalled replication forks and in enhancing the survival of some tumour types. *In vitro*, down-regulation of Cdc7 expression using siRNA knockdown models produces an abortive S phase progression and cell death in HeLa cell lines independent of p53.[23] In tumour cells, therefore, inhibitory signals preventing further cell cycle progression are not generated. However, in normal fibroblasts, a p53-dependent system prevents progression through a lethal S phase and protects the cell from the effects of reduced Cdc7.[23] There may also be several other redundant checkpoint pathways in normal cells that may offer protection from Cdc7 depletion[21] in addition to p53, such as p38 dependent activation of MK2 which induces cell cycle arrest at the G1/S boundary.[49] Therefore, differential cell killing of tumour versus normal cells appears to be a unique feature of inhibition of origin firing and was also seen by blocking the activities of both Cdc6 and Cdt1 (which are involved with Mcm loading).[50,51] Taken together, these data validate Cdc7 kinase as an important target for new drug development. Tumour cells that already harbour defects in checkpoint related proteins may be more sensitive to the effects of a putative Cdc7 inhibitor. Such an inhibitor would also be expected to induce tumour specific cell death. Also, tumour cells primed by treatment with available DNA damaging agents may be more prone to the effects of Cdc7 depletion by producing increased mitotic catastrophe as a result of deregulated checkpoint control.

7. Available Cdc7 kinase inhibitors

Progress in pharmacogenetics, high through put screening (HTS) and homology models (Fig. 3), allowed for the identification of specific small molecule inhibitors of the Cdc7 kinase (Fig. 4).

Using HTS, Vanotti et al.[43] from Nerviano Medical Sciences developed a first in class group of Cdc7 kinase inhibitors, the 2-heteroaryl-pyrrolopyridones. The screening of 100 Cdk inhibitors produced a 65% successful hit rate. Of these, the pyrrolopyridinones had the most favourable characteristics by having low molecular weights, good solubility and low plasma protein binding. From these data, the lead compound PHA-767491 emerged as the first nanomolar, ATP-competitive, Cdc7 small molecule inhibitor described so far.[44] Following optimisation of the PHA-767491 scaffold, the important effects of Cdc7 depletion were reproduced in pre-clinical models. The compound impaired Mcm2 phosphorylation at Cdc7-dependent phosphorylation sites, origin firing blockade occurred without interfering with the progression of replication forks and a DNA damaging stress response was not observed. *In vitro* and in cell-based assays PHA-767491 off target effects on Cdk9 and RNA polymerase II phosphorylation were observed. Depletion of Cdk9, however, does not impact significantly on cell viability in yeast models[52] and

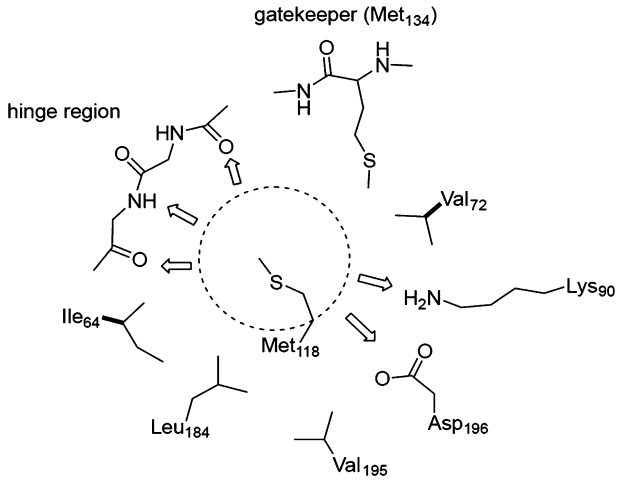

Fig. 3 – Mapping of important residues within the ATP-binding pocket of Cdc7 kinase based on a homology model. The dotted circle area marks the hydrophobic area (adenine-binding region) formed by a hydrophobic sandwich with two residues, Met 118 and Ile64. Possible hydrogen bonds between a Cdc7 inhibitor and the ATP-blinding site of Cdc7 are indicated by the arrows.[73]

Drosophila melanogaster embryos are capable of prolonged survival without Cdk9 expression.[53] Importantly, depletion of Cdk9 in human cells did not alter growth rates or rates of cellular transcription.[54] Importantly, inhibition of Cdk9 may in fact augment the effects of Cdc7 depletion through down-regulation of the anti-apoptotic proteins Mcl-1 and Xiap,[55,56] both of which are critical for the survival of many tumour types. Activity of PHA-767491 has been shown in several cell lines and its dual inhibition of both Cdc7 and Cdk9 may be important for this to occur.[44] Anti-tumour activity of PHA-767491 has been confirmed in AML, colon and breast xenograft models[44] as well as in rodents with induced mammary tumours.[57,58] Selectivity for tumour cells was confirmed by showing preserved cell viability in drug-treated normal human dermal fibroblasts. PHA-767491 efficacy was confirmed in a wide variety of pre-clinical tumour models irrespective of p53 in addition to activity in treatment resistant models.

NMS-354, also a Nerviano compound, demonstrated p53-independent anti-proliferative activity and induction of apoptosis in a broad panel of cancer cell lines (including 5-fluouro-uracil resistant cells). In xenograft tumour models (ovarian, colon, mammary and leukaemia) and also in a rat mammary carcinogenic-induced tumour models (DMBA model), tumour regression of up to 80% was observed. Pathway inhibition in

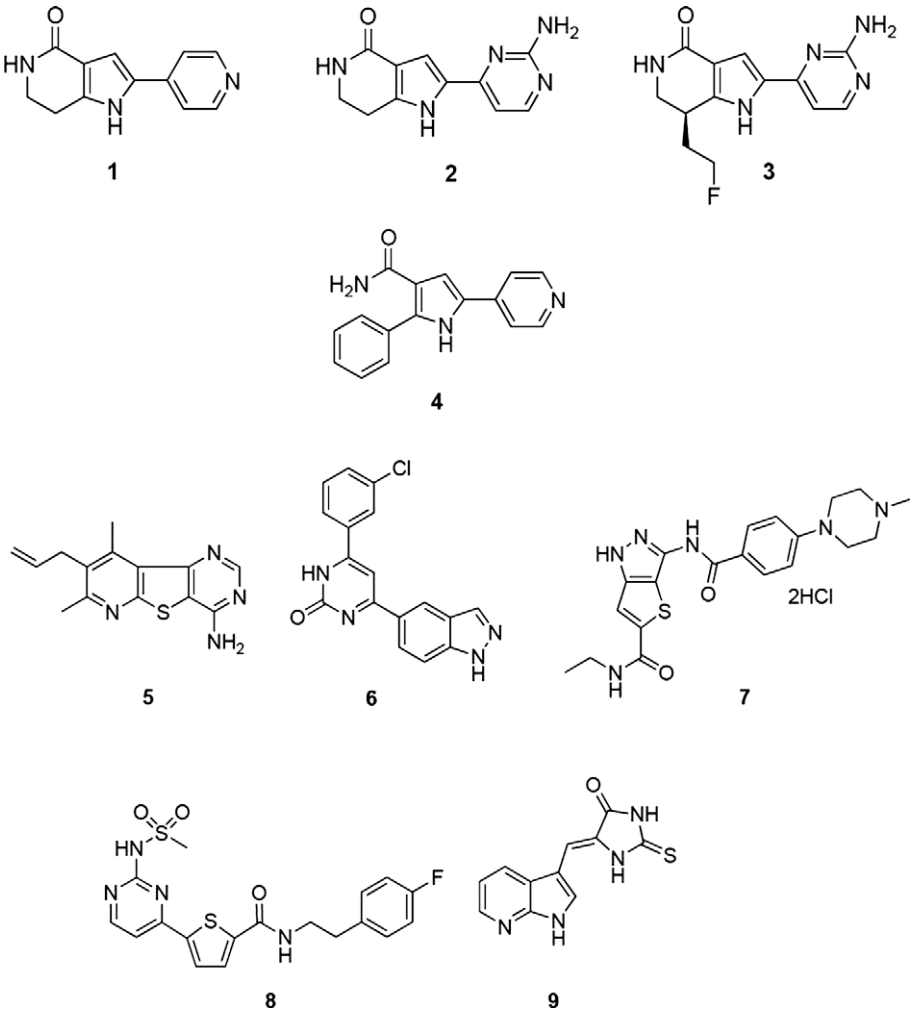

Fig. 4 – Available Cdc7 inhibitors.[73]

tissue culture cells and in tumour models was confirmed with reduced Mcm2 phosphorylation[74]. The pre-clinical profiles of both PHA-767491 and NMS-354 hold promise for the development of these agents in clinical trials.

In addition to the Nerviano Cdc7 programme several other compounds are in development. Roche, Novartis, Pfizer and Sanofi-Aventis have all generated potent Cdc7 inhibitors reporting low-nanomolar activity across a broad range of histologies.[59–62] In collaboration with Exelixis, Bristol Myer Squibb (BMS) appears to be closest to developing their Cdc7 inhibitor (BMS-863233) in the clinic. Few published data are available on the pre-clinical development of this molecule. A phase 1 protocol for BMS-863233 (CA198-001), an orally available Cdc7 inhibitor, is now open for patients with refractory haematological malignancies.

8. Conclusions and future directions

The critical importance of Cdc7 in the survival of tumour cells and the tumour-specific cell death observed with Cdc7 depletion makes this a very promising target for clinical development. Cell death is likely to be due to the combined effects of destabilisation of replication forks, uncoupling of S phase with mitosis, impaired DNA damage responses and the aberrant cell cycle progression that results. Recent reports also implicate activation of p38 MAP kinase with initiation of the caspases in Cdc7-depleted tumour cells[21] as an additional mechanism. In normal cells, however, preserved checkpoint mechanisms and activation of other redundant pathways may be sufficient enough to withstand the effects of Cdc7 depletion, however, the exact mechanisms accounting for the differential effect on cancer cells are not yet clear. The hypothesis proposed to explain the preferential killing observed relies on the idea that non-functional checkpoints allow cancer cells to undergo mitosis in spite of aberrant/incomplete DNA replication, resulting in non-viable daughter cells; normal cells, on the other hand, do not mitose until the DNA has been fully and correctly replicated. This explanation is attractive but probably does not tell the whole story. Aberrant checkpoints of cancer cells are (partly) responsible for the frequent alterations of their genetic material and accelerated 'darwinian' evolution, and inhibition of Cdc7 may not have a long-lasting therapeutic effect. At the same time, stalled DNA replication forks in normal cells may well eventually drive them to apoptosis, so that Cdc7 inhibition would not spare them (published studies with normal fibroblasts were limited to short-term; what is also pertinent are normal, fast-dividing cells such as leucocytes). It would be indeed remarkable if Cdc7 inhibitors really made a clear distinction between normal and cancer cells (and not merely between cells which proliferate fast and those that do not).

Deregulated cell cycle progression is a hallmark feature of several tumour types. Over 90% of patients with mantle cell lymphoma overexpress cyclin D1, both cyclins D and E are overexpressed in many carcinomas, p53 is deficient in 50% of all cancers and p27 expression is lost in a subset of aggressive carcinomas of breast, prostate and ovary.[63,64] These patient populations may be particularly sensitive to the effects of Cdc7 depletion by having prior checkpoint signalling defects. Sustained inhibition of Cdc7 in the presence of both hydroxyurea and etoposide increases cell death *in vitro*,[20] therefore, combining the effects of a DNA damaging agent with a Cdc7 inhibitor may have additive or even synergistic value and it would be interesting to evaluate the combination approaches in pre-clinical models to optimise for development in patient sensitive populations.

Overexpression of Cdc7 and its protein regulator Dbf4 in human cancer cell lines has been described,[45] however, the prognostic significance of this finding has not been thoroughly explored. Loss of p53, generally a marker of poor prognosis, and high levels of Cdc7 and Dbf4 have been described in breast cancer and other cell lines *in vitro*.[45] Also, melanomas over-expressing Dbf4 are associated with lower relapse-free survival.[65] By comparison, the case for Mcm2 overexpression as a prognostic marker is better established, particularly for solid tumours.[66–70] In diffuse large B-cell lymphoma, an increased Mcm2 labelling index, as defined by immunohistochemistry of lymph node specimens, was associated with inferior survival on univariate analysis, although on multi-variate analysis this finding lost clinical significance.[71] Other microarray data are available in acute leukaemia where Mcm2 upregulation correlated with treatment refractoriness.[72] Based on these observations, clinical correlative studies pursuing the Cdc7/Mcm2 pathway as a potential biomarker for response and prognosis is an important avenue worth exploring.

Finally, it is interesting to note the structural similarity between the inhibitors of Cdk and Cdc7. As a matter of fact, the problem with Cdk inhibitors as anticancer agents may well be the fact that they prevent mitosis (by blocking Cdk-1), and allow cells to survive until the inhibition wears out. Thus, the molecules of choice should inhibit Cdc7 but not Cdk 1.

In conclusion, the pre-clinical data available on Cdc7 as a target for drug development are exciting and novel. Well designed and innovative protocols are now required to maximise the chances of the Cdc7 inhibitors in making a significant impact in anticancer therapy.

Company	Compound class	IC50
1. Nerviano	PHA-767491	10 nM
2. Nerviano	3-Aminopyrimidine analogue	7 nM
3. Nerviano	(S)-2-(2-aminopyrimidiny-4-yl)-7-(2-fluoroethyl)-1,5,6,7-tetrahydropyrrolo[3,2-c]pyridinione	2 nM
4. Nerviano	Pyrrolopyridinone analogue	3 nM
5. Roche	Tricyclic Cdc7 inhibitor	NA
6. Novartis	Indazolylpyrimidin-2(1H)-one inhibitor	5 nM
7. Pfizer	Thienopyrazole-based inhibitor	<1000 nM
8. Novartis	2-Pyrimidyl-5-amidothiophene analogue	NA
9. Sanofi-Aventis	Imidazole-based inhibitor	NA

Conflict of interest statement

None declared.

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