The cell cycle, chromatin and cancer: mechanism-based therapeutics come of age

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Tumour cells grow and divide in an uncontrolled fashion. Recent advances in the cell cycle have uncovered new mechanisms that integrate growth and division with chromatin and gene expression control. Small-molecule drugs that target key enzyme classes involved in these pathways, the cyclin-dependent kinases (Cdk) in the cell cycle and histone deacetylases (HDAC) in chromatin control, have entered clinical studies, with emerging clinical efficacy. These new mechanism-based approaches could provide significant improvements over many current chemotherapeutics.

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▼ In normal cells the cell cycle is a tightly regulated and carefully balanced process through which one cell divides into two. The four phases, G1, S, G2 and M phase, reflect stages in cell cycle progression where DNA synthesis and replication (S phase) and mitosis (M) occur in a temporally regulated fashion, separated by two gap phases (G1 and G2). The fidelity of cell cycle progression is maintained by an array of regulatory decision points, governed in part by cyclin-dependant kinases (Cdk), which determine whether it is appropriate, or otherwise, for a cell to divide [1]. For example, the restriction (R) point, which is operationally defined within G1 and frequently abnormal in tumour cells, is a point of control that determines when a cell becomes committed to cell cycle progression [1,2]. Other control points act in either a positive or negative fashion, to delay or allow completion of the cycle [1]. In addition, a series of surveillance mechanisms, known as checkpoints, assess the integrity of cell cycle progression. The importance of checkpoints becomes apparent, for example, upon treating cells with DNA-damaging agents (which include many cancer chemotherapies) that activate the DNA damage checkpoint. This causes cell cycle arrest, allowing cells time to repair the resulting damage or apoptosis [3,4]. Checkpoints ensure the faithful completion of the division cycle, and provide the organism with the opportunity to repair or eliminate damaged cells. Checkpoints are often defective in tumour cells.

The regulation of the cell cycle is integrated with gene expression and chromatin control. Transcription of certain genes, such as those concerned with DNA synthesis and replication, is activated as cells approach S phase [5]. This provides a point of control in regulating the cell cycle, and is achieved through pathways that connect the cell cycle machinery with processes, such as chromatin control, that influence gene expression [5].

Recent developments in understanding the cancer cell cycle, particularly the interplay with chromatin control, are providing opportunities for developing a new range of cancer drugs. Our objective here is to highlight the connection between the cell cycle and chromatin control, and relate the mechanisms to abnormalities in tumour cells; we refer the reader to some excellent recent reviews covering the cell cycle and drug discovery [6–10]. We believe that therapies that target these abnormalities could offer new opportunities in mechanism-based drug design, yielding agents that act more specifically and with greater efficacy in the clinical setting. This has been the hope, and it is only now that we are beginning to see evidence emerging from clinical studies that supports this viewpoint. Long-term clinical studies will address whether the cancer cell cycle is sufficiently different from that of normal cells so as to provide clinical efficacy over and above that already provided by current cytotoxic drugs.

1359-6446/03/$ – see front matter PII: S1359-6446(03)02792-2

Table 1. Examples of conventional cancer chemotherapeutic agents

Drug	Category
Methotrexate	Anti-metabolite
Taxol	Spindle modulator
Cyclophosphamide	Alkylating agent
Cisplatin	Platinum-DNA complexes
Doxorubicin	DNA intercalating and topoisomerase inhibitor

Cancer remains clinically unmet

Statistics reveal that cancer will affect approximately one person in three, and in the Western World it is the cause of about a quarter of all mortality. Worldwide, cancer is the second largest cause of death, after cardiac disease, and the World Health Organization predicts that by 2020 there will be 20 million new cancer patients each year [11]. There are over 200 different types of cancer, but four (lung, breast, colon and prostate) account for over half of all new cases [12].

Currently, cancer chemotherapy is dominated by treatments that use cytotoxic agents (Table 1). Although advances have been made in cancer treatment, the impact on mortality rates has been modest. For example, lung cancer, the leading cause of cancer death among men and women worldwide, has a five-year survival rate of only 5%, a rate that is little different from 30 years ago [7]. Against this background, there are some notable improvements. Cures are achievable in certain childhood leukaemias and testicular cancer but, of the six most common cancers, only breast cancer has a five-year survival rate of greater than 50%, with survival rates increasing marginally [7].

Conventional chemotherapy is relatively ineffective for many cancers, in part reflecting the mechanism of action of current anticancer drugs. In general, existing chemotherapies target mechanisms that are employed by proliferating cells, rather than specific abnormalities associated with tumour cells. Because of this, the therapeutic window between normal and diseased cells is often small, leading to the highly debilitating side-effects that are associated with chemotherapy, such as gastrointestinal toxicity, hair-loss and myelosuppression. Poor quality of life together with diminishing effectiveness in the patient, usually because of drug-resistant tumour cells, means that late-stage cancer treatments frequently have minimal benefit for the patient. Because of these problems, there remains a great need for better cancer medicines and, in the longer term, the redesign of cancer treatment.

The cell cycle and cancer

Cancer is the cell cycle disease *par excellence*. Tumour cells acquire the capacity for uncontrolled growth through a multi-step process that involves the gradual transformation of a normal cell to a tumour cell. Mutation and subsequent inactivation of pathways that act to restrain proliferation and, conversely, the activation of those which promote proliferation, are key events in the transformation process [13]. Many mutations occur in proto-oncogenes and tumour suppressor genes, resulting in the cancer cell becoming liberated from its tightly regulated division cycle.

The retinoblastoma tumour suppressor protein (pRb) provides a perfect example of how a crucial point of control becomes abnormal in tumour cells. This protein regulates progression through G1 into S phase by influencing passage through the R point (Fig. 1), where its primary role is to influence the activity of the E2F family of transcription factors [2,5]. E2F co-ordinates the transcription of genes involved in cell cycle progression, such as cyclin E, and DNA synthesis, and it is the progressive phosphorylation of pRb by cyclin-dependent kinases (Cdks) that prevents the physical interaction with E2F, thereby allowing cells to move into S phase [1,2,5].

Tumour cells have acquired diverse mechanisms that allow them to overcome the effects of pRb. Aberrant levels of upstream regulators of pRb, such as cyclin D-dependent kinase, cause the release of E2F [5,14]. Similarly, the *Rb* gene is mutated in certain tumours, preventing pRb binding to E2F [13]. Consequently, gene targets of E2F become activated and cells enter S phase. Because of the high frequency of abnormal pRb control, de-regulation of the pRb/E2F pathway appears to be a hallmark of tumour cells [5,13]. Although, E2F is an obvious target for therapeutic intervention [6], other factors involved in the pRb/E2F pathway, such as the upstream signalling Cdks (Fig. 1), together with the downstream control of chromatin (Fig. 2) are attracting attention as drug targets in their own right.

Modulating checkpoint control

Interest in the pharmacological manipulation of checkpoint control within the cell cycle is gathering increasing momentum (Fig. 1). Checkpoints become activated when dividing cells meet adverse conditions, such as DNA-damaging agents. The checkpoint pathway that is activated by DNA damage involves two closely related sensor kinases belonging to the phosphatidyinositol-3-kinase (PI3) family, known as ATM/ATR, which signal to the effector checkpoint kinases (Chk) 1 and 2 [3,4]. Chk1 and 2 function to inhibit the cell cycle by phosphorylating certain regulatory proteins. For example, Chk1 phosphorylates the Cdc25C phosphatase to create a binding site for the 14-3-3 proteins

(regulatory proteins involved in cell cycle control and apoptosis), which prevents the nuclear localization of Cdc25C and subsequent dephosphorylation and activation of the mitotic Cdk1 kinase [15]. Similarly Chk2 phosphorylates substrates, including p53 and E2F-1, resulting in a potent apoptotic signal [16,17].

Although Chk1 and Chk2 are both serine/threonine kinases, they are structurally unrelated to each other and have distinct biological roles [18]. This is well illustrated through the phenotypes of the knockout mice; *Chk1*$^{-/-}$ mice exhibit embryonic lethality, whereas *Chk2*$^{-/-}$ mice are viable but display subtle defects in cell cycle arrest in response to DNA damage [18]. Current evidence supports a role for Chk1 at various points in the cell cycle, particularly the G2/M DNA-damage checkpoint and in response to replication inhibition during S phase [15]. However, Chk2 appears to be mostly required for the G1/S phase checkpoint in response to ionizing radiation and radiomimetic drugs [16,17]. By modulating the activity of Chk1 it might be possible to chemo-sensitize tumour cells by maintaining progress through mitosis, and conversely, the inhibition of Chk2 activity could alleviate the apoptosis in normal healthy cells and thereby limit some of the debilitating side-effects of current cancer therapies.

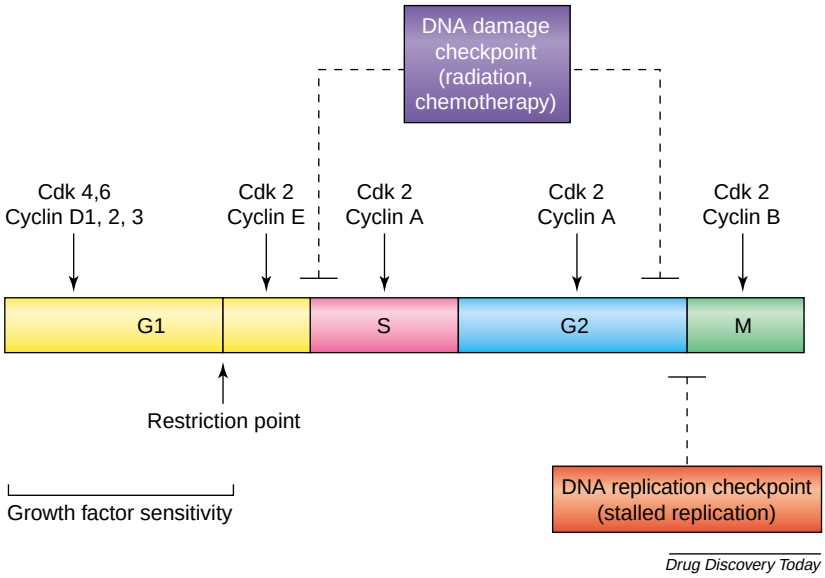


Figure 1. Regulation of the cell cycle. The four phases of the cell cycle are indicated, together with the nature and timing of the cyclin–Cdk complexes involved in cell cycle progression. The Restriction point, positioned in G1 (R), is the point at which the cells have become committed to cell cycle progression and no longer require growth factor stimulation. The position of DNA damage and replication checkpoints is also indicated, together with the stimuli (radiation and chemotherapy, and stalled replication forks respectively) that activate them and lead to cell cycle arrest or apoptosis.

Targeting chromatin control in cancer

Recent advances have highlighted a new and exciting development in anticancer drug discovery, namely the interplay between the cell cycle and chromatin control [19]. Chromatin is the DNA–proteinaceous material in which chromosomal DNA resides in the nucleus. The majority of chromatin protein is composed of histones, which assemble into nucleosomes, thereby assisting in DNA packaging but also providing an important regulatory role. The histone tail is subject to a variety of enzymatic modifications, including phosphorylation, acetylation and methylation, and many of the critical enzymes responsible for these modifications have recently come to light [20]. Histone deacetylases (HDACs), which are responsible for removing acetyl groups from lysine residues in histone tails, are involved in cell cycle regulatory processes [21]. Similarly, histone acetyl transferases (HATs), which act in the opposite fashion to HDACs, regulate the activity of a variety of cell cycle proteins (Fig. 2).

From the perspective of cancer therapy, HDACs are gaining increasing recognition as a relevant target which, in part, reflects the identification of proteins other than histones that are subject to acetylation control [19,22]. The activity of cell cycle regulators like E2F, p53 and pRb is influenced by acetylation, and pRb controls E2F activity through the recruitment of chromatin-modifying enzymes, such as HDACs (Fig. 2). Perhaps, not surprisingly, compounds that inhibit HDAC activity cause potent cell cycle effects and frequently induce apoptosis in tumour cells.

Mechanism-based cancer therapy

In this review, we have restricted ourselves to providing notable examples of small-molecule drugs that target key regulatory processes in the cancer cell cycle and chromatin control, rather than to catalogue the extensive list of compounds that have entered development. Our objective has been to focus on selective examples that illustrate the principle of the approach, and in some cases have provided encouraging clinical data.

Cyclin-dependent kinase inhibitors

An important role of Cdks is in the phosphorylation of pRb which, as discussed, releases E2F to facilitate DNA replication

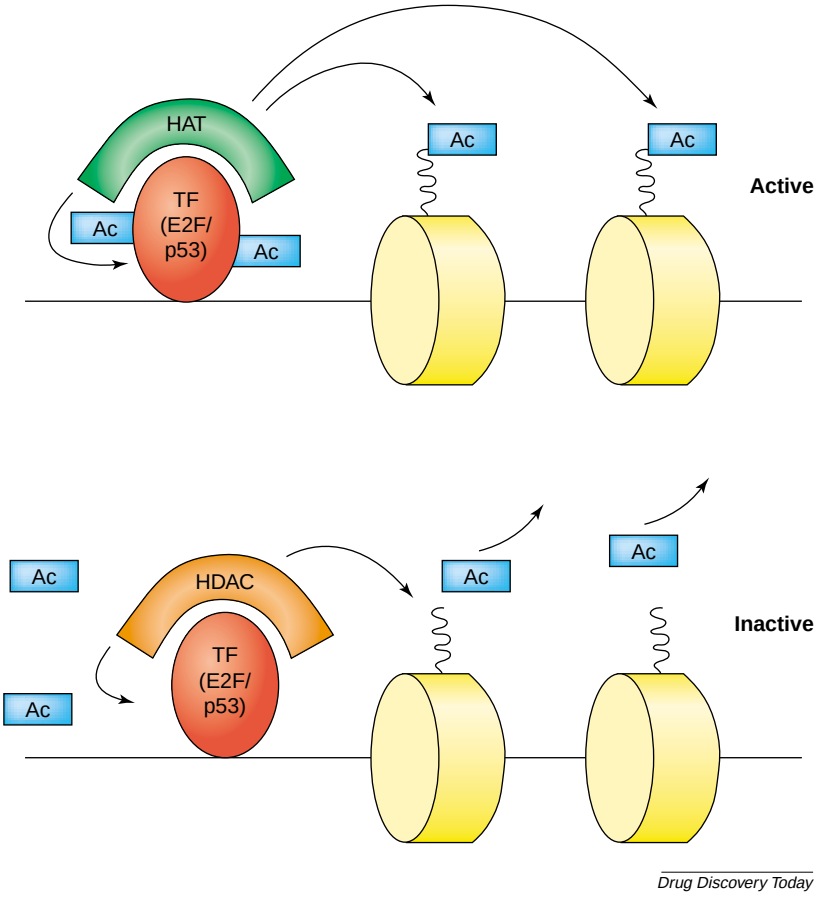


Figure 2. Acetylation control in the cell cycle. Acetylation (Ac, blue) mediated by acetyltransferases (HATs, green) can target histone tails in the form of nucleosomes (yellow) or transcription factors (TF, red) that are involved in cell cycle control, such as E2F and p53. In most cases so far studied, acetylation appears to activate transcription. In contrast, deacetylation mediated by histone deacetylases (HDAC) causes transcriptional inactivity by targeting histones in nucleosomes, leading to a more transcriptionally inert state, together with a decrease in the activity of transcription factors.

and progression through the cell cycle. Cdk4 and Cdk2 play a key role in pRb control [2,5], and both enzymes are being exploited for cancer therapy [23,24]. Compounds that act on diverse Cdk targets, together with those that act more specifically, have been taken into clinical development (Table 2).

UCN-01 (7-hydroxystaurosporine)

UCN-01 (Table 2) is an example of a general kinase inhibitor that is active against a variety of kinases, including Cdks, protein kinase C (PKC), Chk1 and thymidylate synthase. UCN-01 induces p21 expression, reduces cyclinA expression and leads to p53-independent apoptosis, potentially mediated via caspase-3 activation [25,26]. UCN-01 has completed Phase I clinical trials and is entering Phase II trials as a monotherapy. Gastrointestinal toxicity, as well as hyperglycaemia, pulmonary dysfunction and hypotension were observed in the Phase I study [27,28]. The long half-life (days) of the drug led to the revision of the dosing regimen (leaving a 3-day interval), which upon further investigation, significantly improved the side-effect profile.

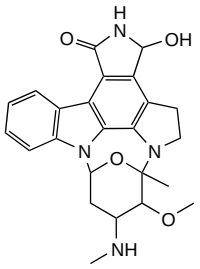
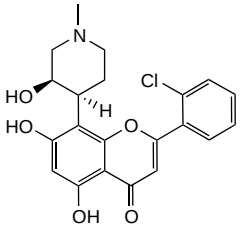
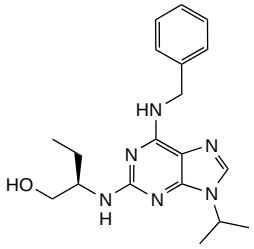
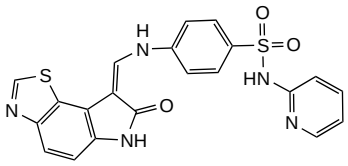
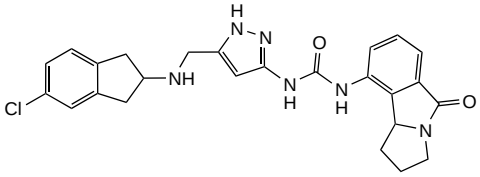
Flavopiridol

Flavopiridol is an example of a compound that acts on diverse Cdk targets. Flavopiridol targets Cdks 1,2,4,7 (inhibiting Cdk2 enzymatic activity at nanomolar concentrations) by competitively binding to the ATP binding site, blocking cell cycle progression at the G1/S and G2/M phases. In addition, flavopiridol targets other Cdks, including Cdk9/cyclin T1, thereby inhibiting RNA polymerase II activity [29], and other classes of kinases, such as protein kinase A and C, albeit at low potency (145 μM and 6 μM, respectively). This broad-acting agent induces caspase-dependent and independent apoptosis that cannot be overcome by Bcl2 overexpression [27]. Flavopiridol is a synthetic small-molecule flavonoid-based compound (Table 3) that has reached Phase II clinical trials as a monotherapy for cancer and other proliferative diseases [24]. Phase I combination studies are underway using flavopiridol with docetaxol and irinotecan.

R-Roscovitine

Roscovitine (also known as CYC202) possesses specificity for Cdk2 (cyclin E/Cdk2, IC_{50} 0.1 μM), but also inhibits Cdk7 (IC_{50} 0.49 μM), ERK-2 (IC_{50} 1.17 μM) and Cdc2 at lower micromolar potency [30]. Structurally, roscovitine is a substituted purine analogue that acts as a competitive inhibitor for ATP binding, where the purine moiety binds to the adenine binding pocket of Cdk2 (Table 2; [30]). Roscovitine inhibits cell growth on a diverse panel of tumour cell lines and demonstrates efficacy in colorectal xenograft models [31]. The primary effect of roscovitine is thought to result from increased apoptosis rather than cell cycle arrest. Roscovitine entered Phase I clinical trials in 2002 and appears to be well-tolerated [32].

Table 2. Cdk inhibitors and molecular target selectivity

Compound	Structure	Target selectivity	Clinical trial status
UCN-01		Cdk2,4,6,7 PKC Chk1	Phase II
Flavopiridol		Cdk1,2,4,7	Phase II (monotherapy) and Phase I (combination)
R-Roscovitine (CYC202)		Cdk1,2,7 ERK-2	Phase II
Oxindole derivatives (GW491619)		Cdk2 (also Cdk1, 6)	Discovery
Diarylurea based inhibitors		Cdk4	Discovery

Abbreviations: Cdk, cyclin-dependent kinase; Chk, checkpoint kinases; ERK-2, extracellular signal-regulated protein kinase 2; PKC, protein kinase C.

Selective Cdk inhibitors in pre-clinical development

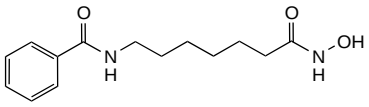
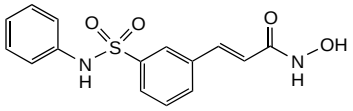
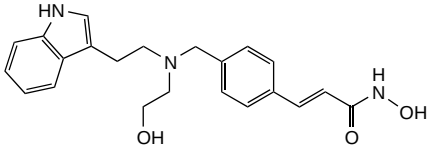
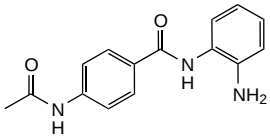
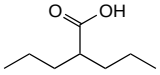
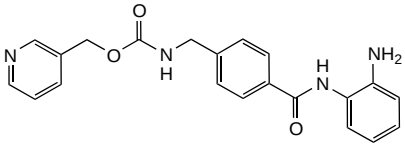
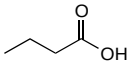
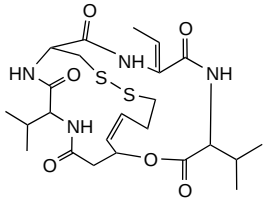
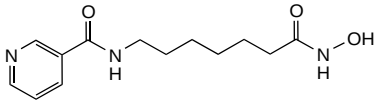
Although the early Cdk inhibitors were broad-spectrum molecules with a variety of potencies across the enzyme family, several advances have been made towards Cdk inhibitors with increased specificity. Oxindole-substituted Cdk inhibitors (Table 2) have tenfold selectivity for Cdk2 against a panel of other Cdks [33]. GW-491619 kills HCT116 colon cancer cells (IC_{50} 0.7 μM), and has a tenfold lower potency in normal human diploid fibroblasts. In addition, a series of diarylurea-based cyclin D/Cdk4-selective inhibitors (Table 2) are under investigation as potential cancer therapeutics [34]. The most active molecule in this series inhibits Cdk4 with an IC_{50} of 2 nM and with a 190-fold selectivity against Cdk2.

Cdk/cyclin antagonists and the RXL motif

The agents described above have an acceptable therapeutic window but are not directly targeted to tumour cells. It is possible to target tumour cells that have deregulated components of the cell cycle machinery. This has been demonstrated in proof-of-principle studies using peptides that antagonise the interaction of cyclin A/Cdk2 with proteins like E2F-1 [35].

Cyclin–Cdk complexes phosphorylate E2F in S phase, resulting in decreased transcriptional activity. The RXL motif (where the motif X is a basic residue) in E2F-1 is responsible for the physical interaction between the cyclin–Cdk complex and E2F-1. By targeting and disrupting this interaction between cyclin A–Cdk2 and E2F-1, transformed cells

Table 3. HDAC inhibitors currently in clinical trials

Molecule	Structure	Clinical trial status
SAHA		Phase II
PXD101		Phase I
LAQ-824		Phase I
CI-994		Phase I
Valproic acid		Phase I
MS-275		Phase II
Butyrate		Phase I/II
Depsipeptide		Phase II
Pyroxamide		Phase I

Abbreviations: HDAC, histone deacetylase; SAHA, suberoylanilide hydroxamic acid.

undergo apoptosis, whereas their normal counterparts remain viable [35]. In addition, these same non-transformed cells with an inducible E2F-1 protein become susceptible to apoptosis induced by the RXL inhibitor peptide. Recent results extend these experiments to cells that have inactivated Rb, demonstrating selective cell killing *in vitro* and induction of apoptosis *in vivo* [36] Although these experiments use peptides that are unlikely drug candidates, there is the potential to use a peptidomimetic approach to design antagonists to selectively target tumour cells. Such antagonists of the E2F-1–cyclin A interaction have been developed with moderate activity (IC_{50} 30 μM) [37].

Checkpoint control

It might be possible to abrogate DNA-damage-dependent cell cycle arrest, such as in response to radiation or chemo-therapeutics (Fig. 1), so that the therapeutic window between normal and cancer cells is increased.

Control through Chk1 inhibition

It is interesting to note that the classic radiosensitising effects of methylxanthines, such as caffeine, might be exerted through modulating the DNA-damage response pathway. At pharmacologically relevant concentrations, caffeine inhibits the PI3 ATM/ATR kinases, both of which lie upstream of Chk1/2 [38,39].

The staurosporine analogue UCN-01 (Table 2) inhibits Chk1 (IC_{50} of 5–11 nM) but not Chk2 (IC_{50} >1 μM; [40,41]). In different cell-types, UCN-01 reduces the level of G2 cells seen after DNA-damage, and UCN-01 can act additively in cells treated with DNA-damaging agents, including ionizing radiation, 5-fluorouracil (5-FU) and camptothecin [42].

Another molecule, the indolocarbazole SB-218078, which is related to UCN-01 [43], is a potent inhibitor of Chk1 (15 nM). It prevents camptothecin and radiation-induced cell cycle arrest, and enhances camptothecin-induced cytotoxicity [43]. Studies such as these support the importance of Chk1 as a potential drug target.

Control through Chk2

The potential of modulating Chk2 to alleviate the side-effects of chemotherapy treatments is attracting interest. The activation of the p53 response and the resulting apoptosis is involved in these side-effects; for example, $p53^{-/-}$ knockout mice survive higher doses of ionizing radiation compared to their wild-type counterparts [44]. A compound, pifithrin α, regulates the p53 response by interfering with apoptosis, although the molecular target in the p53 pathway is not known [45]. Accordingly, pifithrin α can rescue mice from 60% killing doses of gamma-radiation, but fails to have any effect in $p53^{-/-}$ knockout mice.

That a primary target of Chk2 is activation of the p53 response argues that modulation of Chk2 could be chemoprotective. Unlike $p53^{-/-}$ mice, $Chk2^{-/-}$ knockout mice do not develop tumours [18], although, as a cautionary note, it remains unclear whether pharmacological intervention of Chk2 would compromise DNA-based anticancer treatments.

Histone deacetylase inhibitors in clinical development

The chromatin control HDAC enzymes are classified into three families depending on protein sequence similarity: HDACs 1,2,3 and 8 representing class I, HDACs 4–7, 9–11 are class II and class III are NAD-dependent deacetylases, with similarity to the yeast Sir2 proteins [46]. Encouraging results of HDAC inhibitors in clinical trials have begun to validate HDAC enzymes as drug targets [47,48].

Suberoylanilide hydroxamic acid

Suberoylanilide hydroxamic acid (SAHA) is a hydroxamate-containing small-molecule HDAC inhibitor that directly interacts with the hydrophobic catalytic site of HDACs [49,50,51]. The aliphatic group of SAHA fits into the pocket that co-ordinates the Zn^{2+} ion that is necessary for catalytic activity. Interestingly, SAHA has reached Phase II clinical trials for the treatment of both solid tumours and haematological malignancy.

In vitro and *in vivo* studies demonstrated the potential for synergy using combinations of HDAC inhibitors with several mechanistically different antitumour agents. For example, SAHA, in combination with radiotherapy, produces an additive effect in human prostate cancer spheroids [52]. In mice bearing androgen-independent DU-145 tumours, treatment with SAHA and retinoic acid resulted in a 58% reduction in tumour volume, a significant increase above either treatment alone. Further combinations of SAHA with 5-FU, raltitrexed and flavopiridol demonstrated the ability to target tumour cells using drug combinations with distinct mechanisms of action [53,54].

In breast cancer cells, SAHA induces growth inhibition, cell cycle arrest and apoptosis by regulating genes such as p21, p27, Rb and gelsolin, contrasting with the growth arrest seen in prostate cells *in vitro* and *in vivo* [50,54,55]. These effects could be achieved through the regulation of p53 and E2F-1, both of which have properties associated with cell cycle arrest and apoptosis and which exhibit increased activity upon acetylation [22,56].

Furthermore, several oncogenic proteins recruit HDACs, which leads to aberrant gene transcription [57,58]. This is exemplified by the fusion protein promyelocytic leukemia-retinoic acid receptor alpha (PML-RARα), which arises through chromosomal translocation in acute promyelocytic leukaemia (APL). The PML-RARα fusion protein recruits HDAC, which represses transcription, causing a block to differentiation and promoting the oncogenic phenotype seen in APL [59]. Because HDACs play a key role in leukaemic cell differentiation, molecules such as SAHA have been used to induce differentiation of leukaemic cells *in vitro* and *in vivo*, with some success [60,61].

Although HDAC inhibitors have yet to be completely clinically validated and shown to be efficacious agents, they do nevertheless represent a promising new therapeutic approach to cancer treatment by intervening in cell cycle progression. The enthusiasm for targeting HDAC is borne out by the large number of molecules in early clinical trials or pre-clinical development (Table 3).

PXD101

Although SAHA has provided clinical validation of HDAC, a range of other hydroxamate-containing compounds against HDAC are gaining clinical acceptance. PXD101 is a highly potent HDAC inhibitor that blocks proliferation of diverse tumour cell lines at low micromolar potency (IC_{50} 0.08–2.43 μM) and HDAC enzyme activity (IC_{50} 9–110 nM) [62]. In xenograft models, PXD101 slows tumour growth in a dose-dependent manner and is particularly active in leukaemic mouse models. As with other HDAC inhibitors, PXD101 causes cell cycle arrest and apoptosis in rapidly proliferating cells, and could have widespread applications in diseases other than cancer that are marked by aberrant proliferation.

Remarkably, as a general class of new agents, HDAC inhibitors do not appear to have restrictive toxicological side-effects associated with their use at pharmacologically relevant concentrations. Indeed, PXD101 at doses up to 100mg ml^{-1} has insignificant toxicological effects in rodents [62]. In addition to the anti-proliferative effects, PXD101 regulates expression of many genes that contribute to tumour cell growth and metastases. For example, expression of the pro-inflammatory cytokine tumour necrosis factor α (TNF-α) and secretion of the pro-angiogenic factor VEGF is reduced *in vitro* by PXD101.

LAQ-824

The clinical candidate LAQ-824 is a hydroxymate-based HDAC inhibitor. *In vitro*, LAQ-824 inhibits HDAC enzyme activity (IC_{50} 10 nM), inhibits tumour cell growth at submicromolar concentrations and induces apoptosis [63]. *In vivo*, the molecule possesses antitumour activity in several xenograft models, including breast (MDA-MA-435), colon (HCT116) and lung (A549). LAQ-824 is in Phase I trials for solid tumours [63].

Depsipeptide (FR-901228)

This natural product HDAC inhibitor is currently progressing through Phase II clinical trials for cutaneous T cell lymphoma [64]. It is a natural product purified from *Chromobacterium violceum* that undergoes intracellular reduction to generate an active HDAC inhibitor. Initial toxicity (cardiac and inflammatory responses) have been overcome by using intermittent dosing schedules as opposed to daily dosing, allowing higher drug administration with reduced side-effects [65]. As with other HDAC inhibitors, this cyclic peptide is pro-apoptotic, anti-proliferative and anti-angiogenic. Despite being a substrate for P-glycoprotein, FR-901228 has potent antitumour activity, and results from clinical trials against T cell lymphoma have demonstrated encouraging activity [64].

Hydroxamate-replacements

The majority of the small-molecule HDAC inhibitors in development are hydroxamate containing and in most cases the hydroxamate moiety, which chelates the zinc ion, is necessary to retain potency *in vitro*. The most potent hydroxamate-replacement compound reported to date has an IC_{50} of 9 nM against HDAC enzyme and an IC_{50} of 170 nM and 120 nM in MDA435 breast and HT1080 fibrosarcoma tumour cell lines, respectively [66]. This is an exciting area of HDAC drug discovery and could well contribute to second-generation HDAC inhibitor programmes.

Conclusions and perspectives

It is becoming apparent that exploiting mechanism-based approaches in the cancer cell cycle and chromatin control will yield cancer medicines that are improved compared with many current chemotherapeutics. In both Cdk and HDAC inhibitor programmes, we can find examples of emerging clinical efficacy. While we await the results of Phase II and Phase III clinical trials, it seems likely that HDAC could well provide a compelling target. Nevertheless, many more questions remain to be answered.

Of importance for HDAC inhibitors is the potential future need to engineer HDAC subunit specificity into inhibitory compounds. This has been an underlying theme in Cdk-inhibitor programmes where, for example, targeting aberrant levels of the G1 Cdk complexes has been a clear priority. Although at the current time there is limited information on the pathological significance of HDAC subunits in clinical disease, it is likely that subunit specificity will be a desirable property of inhibitors and will help to improve the clinical benefit and side-effect profiles of HDAC inhibitors. Some progress is being made in this area [67].

Of greater significance, perhaps, will be to understand the clinical practice of Cdk and HDAC inhibitors. Will these compounds be progressed as single agents or, as seems more likely, will they be applied in combination therapies? Addressing this question remains a significant challenge, and further provides the scientific rationale for considering the array of possible combination options that could be studied. A related question concerns the scheduling regime for administering the drug, particularly in combination therapies, which will reflect knowledge of the critical targets through which Cdk and HDAC inhibitors regulate cancer cell growth.

The possibility of resistance to mechanism-based drugs like Cdk and HDAC inhibitors remains to be determined. Tumour cells are adept at acquiring drug resistance phenotypes through diverse mechanisms. However, resistance to HDAC inhibitors could be a rather rare event, at least *in vitro*, but further information is needed before this can be implied to the clinical setting. Of course, if drug resistance does appear as a clinical hurdle, then it might be possible to modulate its progression through a combination therapy approach.

Furthermore, as our knowledge of DNA-damage signalling pathways has increased, evidence has accumulated that suggests that therapeutic manipulation of different components of the pathways can either chemosensitize (Chk1) or chemoprotect (Chk2/p53). Drugs that modulate Chk1 and Chk2 could, therefore, have a potential role in widening the therapeutic window of many cancer drugs and thus have significant utility in the clinical setting, most likely as a combination therapy.

It is clear that many questions remain to be answered. Nevertheless, we are witnessing an exciting era in cancer drug discovery. Our views and anticipations, which are shared by many others, are that by translating and applying knowledge about the cell cycle and chromatin control, improved and more efficacious drugs for treating the cancer patient will be developed.

Acknowledgements

We thank Marie Caldwell for assistance with the manuscript and acknowledge support from the Medical Research Council, Leukemia Research Fund and Cancer Research UK.

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