Minireview

G_2 checkpoint abrogators as anticancer drugs

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Abstract

Many conventional anticancer treatments kill cells irrespective of whether they are normal or cancerous, so patients suffer from adverse side effects due to the loss of healthy cells. Anticancer insights derived from cell cycle research has given birth to the idea of cell cycle G_2 checkpoint abrogation as a cancer cell specific therapy, based on the discovery that many cancer cells have a defective G_1 checkpoint resulting in a dependence on the G_2 checkpoint during cell replication. Damaged DNA in humans is detected by sensor proteins (such as hHUS1, hRAD1, hRAD9, hRAD17, and hRAD26) that transmit a signal via ATR to CHK1, or by another sensor complex (that may include γH2AX, 53BP1, BRCA1, NBS1, hMRE11, and hRAD50), the signal of which is relayed by ATM to CHK2. Most of the damage signals originated by the sensor complexes for the G_2 checkpoint are conducted to CDC25C, the activity of which is modulated by 14-3-3. There are also less extensively explored pathways involving p53, p38, PCNA, HDAC, PP2A, PLK1, WEE1, CDC25B, and CDC25A. This review will examine the available inhibitors of CHK1 (Staurosporin, UCN-01, Go6976, SB-218078, ICP-1, and CEP-3891), both CHK1 and CHK2 (TAT-S216A and debromohymenialdisine), CHK2 (CEP-6367), WEE1 (PD0166285), and PP2A (okadaic acid and fostriecin), as well as the unknown checkpoint inhibitors 13-hydroxy-15-ozoapathin and the isogranulatimides. Among these targets, CHK1 seems to be the most suitable target for therapeutic G_2 abrogation to date, although an unexplored target such as 14-3-3 or the strategy of targeting multiple proteins at once may be of interest in the future. [Mol Cancer Ther. 2004; 3(4):513–519]

Introduction

Many of the conventional anticancer treatments (including: ionizing radiation, hyperthermia, pyrimidine and purine antimetabolites, alkylating agents, DNA topoisomerase inhibitors, and platinum compounds) at least partly damage the DNA of cells. Because these treatments are not specifically selective for cancer cells, patients have suffered from adverse side effects when taking these drugs.

Efforts have been made to sensitize cancer cells specifically to these treatments since the late 1960s with compounds such as caffeine (1), which at the time was thought to directly inhibit the repair machinery. The suppression of UV damage repair by caffeine was reported using bacteria (2); this suppression was not thought to effect any checkpoints because it was presumed that the checkpoint signal cascades were significantly different between bacteria and eukaryotes. It would be many years before the concept of the cell cycle checkpoint would be fully realized (3), not until the molecular mechanisms of cell cycle checkpoints were first elucidated in the late 1980s using yeasts, fungi, and the oocytes of amphibians. When, concurrently, oncologists began examining the mechanisms of oncogenesis in higher eukaryotes using the same molecular biology techniques as their cell cycle counterparts, it was revealed that many cancer cells have defective G_1 checkpoint mechanisms and that cancer cells depend on G_2 checkpoint far more than normal cells (4, 5). Combining these two streams of research gave rise to the concept of ''cell cycle G_2 checkpoint abrogation'' as a tactic for the development of cancer cell specific medicines.

The original attempts using caffeine to disrupt the G_2 checkpoint to sensitize G_1-defective cancer cells were published in 1995 (6, 7). Because caffeine is apparently not a specific G_2 checkpoint abrogating agent (abrogator), the real outcome with regard to G_2 checkpoint abrogation was rather ambiguous (8, 9). Thus, research continued to obtain better selective G_2 checkpoint abrogators for use as clinical compounds. This review is intended to examine the theoretical background of G_2 checkpoint abrogation as a tactic for cancer specific therapy and the status of G_2 checkpoint abrogators as clinical candidates.

The G_1 Checkpoint and Oncogenesis

The ''usual suspects'' of human oncogenesis (oncogenic viruses, mutagens, and inherited factors) all primarily affect the G_1 checkpoint. They disrupt the G_1 checkpoint causing an increase in the mutational rate while favoring the types of mutation that are more likely to lead to cancer. Many of the oncogenic strains of DNA viruses such as polyomavirus, adenovirus, papillomavirus, and simian sarcoma virus 40 have proteins that impair the function of p53 and RB (10). Natural and chemical mutagens, the prime suspects of human oncogenesis (such as aflatoxin from infected peanuts and the benzopyrenes of tobacco) also mutate p53 gene (11). Mutations to Rb and p53 account

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for the tumor prone phenotypes of retinoblastoma and Li-Fraumeni syndrome (12). Overall, mutations to p53 and Rb have been implicated in more than half of all human oncogenesis (4).

Malfunctioning p53 or RB proteins impair the cell cycle G_1 checkpoint, which normally holds the progression of the cell cycle at the G_1 phase until DNA damage can be repaired before its replication (4, 13). If the damage is too extensive to repair, the cell commits suicide via apoptosis. Because the G_1 checkpoint arrests the cell cycle by inhibiting this G_1-S transition machinery, it can be thought of as a ''brake'' for the cell cycle engine. It usually arrests the cell cycle by inhibiting Cyclin-dependent kinases (cdks) such as CDK2, CDK4, and CDK6. Many of the tumor suppressor genes are therefore components of G_1 checkpoint, including $p16^{INK4a}$, $p19^{ARF}$, and ATM, as well as Rb and p53, with p53 being the key protein for coordination of a variety of G_1 checkpoint functions, including cell cycle arrest, DNA damage repair, and apoptosis. Some oncogene products, such as MDM-2, act by enhancing the degradation of p53. The malfunction of this G_1 checkpoint ''brake'' increases the mutational rate by increasing the likelihood of replicating damaged DNA.

On the other hand, the forced progression from G_1 to S phase (akin to pushing the ''accelerator'' of the cell cycle engine) causes the G_1 checkpoint function to be bypassed, which presumably increases the mutational rate because it is functionally similar to inhibition of the ''brake'' signal. In general, this ''accelerator'' works through growth factor signals stimulating specific receptors on the surface of the cell (13). These activated receptors transmit signals by phosphorylation reactions to ultimately increase the amount of Cyclin D. Increased Cyclin D binds to CDK4 and CDK6 and phosphorylates RB. Phosphorylated RB releases E2F/DP-1 transcription enhancer complexes to activate the transcription of the downstream genes that are required to initiate S phase, while also increasing the amount of Cyclin E to further augment the phosphorylation efficiency of RB by forming active kinase complexes of CDK2/Cyclin E. Many oncogene products have been implicated in this signal cascade. Mutations which activate oncogenes (such as Ras, Cyclin D, erbB, epidermal growth factor receptor [EGFR], etc.) increase the rate of the transition from G_1 to S phase (13). In summary, more than half of human cancer cells have been shown to have impaired cell cycle G_1 checkpoint function (either by blockage of the ''brake'' signal or enhancement of the ''accelerator'' signal) leading to the accumulation of the individual mutations necessary for a cell to become cancerous (Fig. 1).

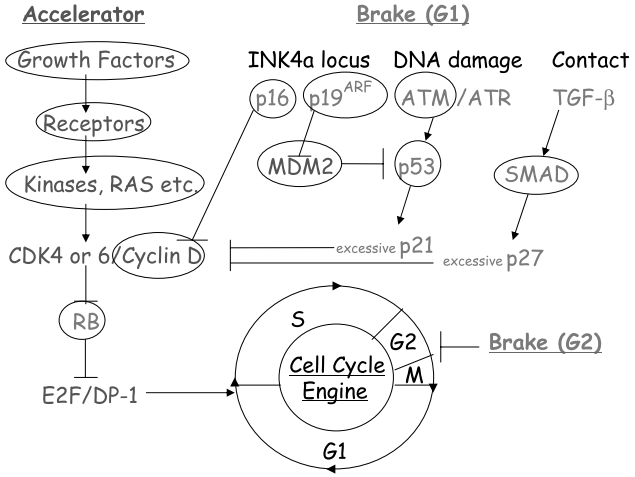

Figure 1. General concept of the G_1-S transition and the G_1 checkpoint machinery. Oncogenes and tumor suppressors are *encircled*.

Unicellular Organisms, Cancer Cells, and the G_2 Checkpoint

While the mechanism of the cell cycle G_1 checkpoint was determined for the most part during the course of cancer research, the molecular mechanism of the G_2 checkpoint has been extensively studied in yeasts and amphibian oocytes since late 1980s because it is the major cell cycle checkpoint for unicellular organisms and early embryonic cells. It is reasonable for unicellular organisms to have less stringent G_1 checkpoints and more stringent G_2 checkpoints because it acts to enhance the rate of mutation by increasing the possibility of replicating any damaged DNA that may give rise to a potentially favorable mutation, which can boost the likelihood of survival and adaptation to new environmental circumstances. On the contrary, multicellular organisms require stringent G_1 checkpoints to avoid oncogenesis. This makes cancer cells more similar to unicellular organisms in their checkpoint dependence for DNA damage. Normal cells rely on the G_1 checkpoint to protect against DNA damage, while cancer cells and unicellular organisms rely on the G_2 checkpoint. Therefore, cell cycle G_2 checkpoint abrogation is more likely to affect cancer cells than normal cells.

The G_2 Checkpoint as a Therapeutic Target

Damaged DNA is detected by sensor proteins such as HUS1, RAD1, RAD9, RAD17, and RAD26 transducing their signal via RAD3 to CHK1 and CDS1 in fission yeast (14). The human homologues of these proteins appear to function similarly to their yeast counterparts (15–20). The main exit of signal from this sensor complex is via ATR to CHK1 in human cells (Fig. 2). This sensor complex seems to work primarily for the detection of UV-induced DNA damage and replication stress (21). There is another sensor protein complex that mainly detects γ-irradiation-induced DNA damage, the proteins that form this complex include: γH2AX, 53BP1, MDC1, BRCA1, NBS1, hMRE11, and hRAD50 in human cells, although there is some variability in the exact components of this complex (22–24). The signal sensed by this particular complex is relayed mainly by ATM and CHK2. Of these components, 53BP1, which appears to be the human version of budding yeast RAD9, appears to be a good target based on the fact that RAD9 is a key protein for both the sensing and the signal transduction of DNA damage in budding yeast. The disruption of rad9 abrogates the DNA damage-induced G_2 checkpoint and

increases the sensitivity of these cells to irradiation without affecting the repair machinery (3). While the rad9 negative phenotype of budding yeast looks like an ideal case of G_2 checkpoint abrogation, there is a fear of adversely effecting normal cells by impairing 53BP1 function, because the p53 binding ability of 53BP1 suggests the involvement of 53BP1 not only in the G_2 but also the G_1 checkpoint (25). In fact, the 53BP1 knock-out mice show a similar phenotype to ATM knock-out mice (23), therefore, the total inhibition of 53BP1 is not an ideal target for therapeutic G_2 checkpoint abrogation. A more detailed molecular dissection of the function of 53BP1 will hopefully find a way to selectively disrupt the G_2 checkpoint pathway but not interfere with 53BP1 function in the G_1 checkpoint.

It has been shown that hRAD1, hRAD9, and hHUS1 form PCNA-like ring structures (15, 16). Both of the damage sensor complexes presumably use this type of ring-like structures to encircle the DNA and then slide down the cell's genome while scanning for irregularities. There is supposed to be redundancy between these two sensor complexes for sensing DNA damage, and there also seems to be some interplay between ATM and ATR, and CHK1 and CHK2, although these activities are not absolutely complementary. The sensor proteins are also involved in the process of initiating damage repair (budding yeast RAD9 protein is an exception) so targeting this machinery indiscriminately may harm normal cells by increasing the mutational rate. Data supporting this notion are that the tumor prone phenotypes of Ataxia telangiectasia, Nijmegen breakage syndrome, and hereditary mammary carcinoma patients have mutations in ATM, NBS1, and BRCA1, respectively (12).

The sensor complexes can also communicate with PCNA, hMLH1, hMLH2, and hMSH6, which form a mismatch repair complex involved in the sensing of DNA damage that is also capable of initiating a signal for G_2 arrest (26). Two protein-protein interaction domains, BRCT (breast cancer susceptibility gene 1 carboxyl terminus) and FHA (forkhead-associated), are implicated in this form of DNA damage response, and if these protein-protein interactions are specific to the damage sensor complex, they may become a target for possible G_2 abrogation in the future (27).

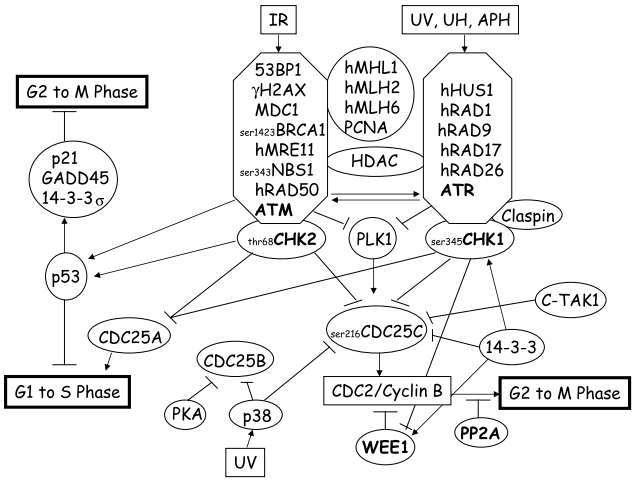

Figure 2. Pathway of the G_2 checkpoint. The targets of available G_2 checkpoint abrogators are indicated as *bold*.

The two sensor complexes discussed are at least partly used by all the checkpoints, G_1, S, and G_2. Accordingly, the ideal target for selective G_2 checkpoint abrogation may be downstream of this sensor machinery. Most of the damage signals from the sensor complexes are conducted via CHK1 and/or CHK2 to CDC25C, a main activator of the CDC2/Cyclin B master switch for the G_2-M phase transition at the G_2 checkpoint (21). The phosphorylation of CDC25C inhibits its phosphatase activity and/or maintains the binding of 14-3-3 to CDC25C. The importance of the localization of CDC25 in the cell has been controversial (28). Curiously, one of the main phosphorylation sites of CDC25C by CHK1 and CHK2, serine 216, is constitutively phosphorylated by kinases including C-TAK1 (14). Nonetheless, the G_2 checkpoint is disrupted by mutating this serine 216 to alanine (19) or by adding an artificial peptide consisting of the sequence around serine 216 of CDC25C (5), indicating the convergence of the G_2 checkpoint signal at CDC25C, specifically at serine 216, or the proteins (including kinases and non-kinase proteins such as 14-3-3) that directly bind to the sequence around this residue.

With regard to the CHK proteins themselves, the activation of CHK2 by phosphorylation of threonine 68 transmits signals to p53 and CDC25A to activate the G_1 checkpoint as well as to CDC25C to activate the G_2 checkpoint (21, 29). The redundancy seen for the G_1 checkpoint seems to imply that the inhibition of CHK2 may not affect the cell cycle at the G_1 checkpoint. However, the mutation of CHK2 is suspected to be the cause of Li-Fraumeni syndrome with wild-type p53 (30) and the disruption of CHK2 showed similar phenotype to p53 knock-out mice in thymus cells (31); this indicates that CHK2 is likely a non-redundant component of some aspect of the G_1 checkpoint and may not be a good candidate for therapeutic G_2 checkpoint abrogation. More promising is CHK1, while the gene knock-out of CHK1 was lethal at the embryonic stage (32, 33), the depletion of CHK1 by siRNA in somatic cells is not lethal, or even toxic; plus, it increases the sensitivity of human tumor cells to DNA damaging agents (34). This effect may be due to differences between embryonic cells and adult cells, or that the knock-out depletes all protein expression while siRNA leaves some residual expression. Anyway, these data showing sensitization to DNA damaging agents indicate that CHK1 may be a promising target for G_2 checkpoint abrogation. The potential downside of inhibiting CHK1 needs to be investigated further because there are a couple of reports suggesting the involvement of CHK1 ablation in oncogenesis and/or advancing tumor grade (35, 36).

There are other pathways that could arrest cells at the S-G_2 phase transition, such as the inhibition of CDC25B by kinases like p38 (37) and PKA (38) and the inhibition and degradation of CDC25A by CHK1 (39) and CHK2 (29). Because CDC25A is the activating phosphatase of G_1 Cyclins such as CDK2/Cyclin E, CDK4/Cyclin D, and

CDK6/Cyclin D, the activation of CDC25A may disrupt not only the S-G_2 checkpoint but also the G_1 checkpoint in G_1 checkpoint-intact cells. This may mean that CDC25A is not be a suitable target for G_2 checkpoint abrogation, especially as CDC25A and CDC25B are even suspected of being oncogenes (40); nevertheless, the feasibility of CDC25B as a target for G_2 checkpoint abrogation still needs to be investigated. PLK1 has also been shown to be involved in the G_2 checkpoint (41). However, because PLK1 is involved in the initiation and progression of M phase, and the disruption of PLK1 has been reported to interfere with M phase progression, it is not a suitable target for selective G_2 checkpoint abrogation. (This does not eliminate the possibility that PLK1 remains a valid anticancer target using other approaches.) WEE1 may also not be a suitable candidate because the total inhibition of WEE1 would impair the normal cell cycle progression at G_2-M, because tyrosine 15 phosphorylation on CDC2 by WEE1 is a prerequisite for the G_2 phase in fission yeast (42). There is a thought that PCNA may directly be involved in the cell cycle arrest at G_2, coordinating the interaction between Fen1, DNA polymerase, p21, CDC2/Cyclin B, and CDC25C (43, 44). If this is the case, the induction or augmentation of a particular protein-protein interaction on PCNA can be an option for disruption of the G_2 checkpoint.

Inhibition of the rather abundant and less-specialized proteins such as PP2A and 14-3-3 would make it difficult to show specific effects unless one could target the specific regulatory subunit or specific isotype, or control the spatial or temporal effect of the inhibitors. However, because 14-3-3, especially sigma isotype, is reported to be a player in p53-mediated G_2 arrest (45), the hypermethylation of this locus is found in the course of breast cancer development (46), and antisense treatment of overexpressed 14-3-3 in lung carcinoma cells decreases the G_2 arrested population and sensitizes cells to ionizing radiation (47), 14-3-3 can be a good target for the future development of G_2 specific abrogators. Another potential target for selective G_2 checkpoint abrogation is histone deacetylase (48, 49).

The main player of the G_1 checkpoint, p53, is also a primary component of the G_2 checkpoint (50). DNA damage signals are conducted to p53 via ATM, ATR, and CHK2, which induces p53 to activate the transcription of GADD45, p21, and 14-3-3 sigma, all of which can suppress G_2-M transition. This suggests that normal cells have two independent G_2 checkpoint pathways in which the key players are p53 and CDC25C, indicating that a selective G_2 checkpoint abrogation disrupting a signal pathway not involved with p53 should not harm normal cells.

In summary, to achieve selective G_2 checkpoint abrogation with minimal adverse effects on normal cells, the target molecule should not be involved in other cellular processes, such as G_1 and S phase checkpoints, DNA repair or with the regular cell cycle progression of normal cells as a non-redundant component. Even if the protein targeted is used by other pathways as a non-redundant component, it still can be a valid target if any side effects caused by a transient and/or weak inhibition were minimal compared to the therapeutic effect.

Candidate G_2 Abrogators

(The targets of available G_2 checkpoint abrogators and any known irrelevant targets are summarized from published data in Table 1.)

Caffeine has been used as a therapeutic for more than a hundred years. The expected biological actions include: inhibition of cyclic nucleotide phosphodiesterase, monoamine oxidase, and cyclooxygenese; calcium mobilization; and effects on the uptake of neuromodulators (51). Caffeine action on the cyclic AMP accumulation occurs at a dose

Table 1. Published G2 checkpoint abrogators

	G2-Specific Target (IC_{50} in nM)	Other Targets (IC_{50} in nM)
Caffeine (52, 54)	ATM (200,000), ATR (1,100,000)	Adenosine receptor (21,000) etc.
Pentoxifylline (73)	ATM, ATR	
Staurosporin (60)	CHK1 (8)	PKC (5), CDC2 (6)
UCN-01 (60, 66, 74, 75)	CHK1 (7), CHK2 (>1,000) (10)[a]	PKC (4), PDK1 (33)
Go6976 (59)	CHK1 (similar UCN-01, cell[b]	PKC (20-fold less UCN-01, cell[b])
SB-218078 (60)	CHK1 (15)	CDC2 (250)
ICP-1 (61)	CHK1 (5-fold less UCN-01)	
CEP-3891 (64)	CHK1 (4), CHK2 (300,000)	TrkA (9)
Debromohimenialdesine (65)	CHK1 (3000), CHK2 (3,500)	
TAT-S216A (5)	CHK1 (~30,000), CHK2 (~30,000)	
CEP-6367 (64)	CHK2 (20), CHK1 (300)	MLK3 (19)
PD0166285 (68)	Wee1 (24), Myt1 (72)	
Okadaic acid (76)	PP2A (0.5)	PP1 (60–500)
Fostriecin (70, 77)	PP2A (40) (3.2)	PP1 (4,000) (131,000)
13-Hydroxy-15-ozoapatlin (72)	unknown	
Isogranulatimide (67)	unknown	

[a]IC_{50} for immunoprecipitated CHK2 from HCT116 (66).
[b]MDA-MD-231.

10 times less than that which causes G_2 checkpoint abrogation (52). The inhibition of ATM and ATR is reported to be the reason of G_2 abrogation by caffeine (53); however, there is evidence that implicates the involvement of other pathways (54). Pentoxifylline is a derivative of caffeine that has also been used in humans for a variety of reasons. It shows as much variety in activities as caffeine does (55). Due to this broad range of effects, it is fair to say that caffeine and pentoxifylline are only non-specific G_2 checkpoint abrogators. Furthermore, because caffeine (2), pentoxifylline, and related methylxanthine derivatives directly impair DNA damage repair, they are not ideal candidates for therapeutic G_2 checkpoint abrogation *per se* (56).

Originally identified as PKC inhibitors, Staurosporin (57), UCN-01 (58), and Go6976 (59) are indolocarbazole-type inhibitors with some CHK1 inhibitory activity, although Go6976 shows much higher specificity toward CHK1. SB-218078 (60) and ICP-1 (61) are also CHK1 inhibitors with indolocarbazole structures but they show little significant activity against PKC compared to that shown for CHK1. Among these, UCN-01 is the most clinically advanced molecule and is in Phase I/II clinical trials for cancer indications. The expected mechanism of action for UCN-01 in the clinical study is inhibition of PKC activity, promotion of apoptosis, arrest of the cell cycle at G_1-S, and abrogation of the DNA damage checkpoint; to date, the dose-limiting toxicities of UCN-01 include nausea/vomiting, hypoxemia, and insulin-resistant hyperglycemia (62). While the final results of these UCN-01 Phase II clinical studies are eagerly awaited, the specific effects on G_2 checkpoint abrogation may be difficult to assess. Also, because UCN-01 has an apparent downside resulting from a property where it tightly binds a human serum protein, α-1-acid glycoprotein (63), it may also be of interest to see how well SB-218078, Go6976, or ICP-1 acts in humans. All of these show much less inhibitory activity against PKC, and Go6976 and ICP-1 have been reported not to have the human serum binding problem. A new potent and orally available inhibitor of CHK1, CEP-3891, which has similarly potent activity against Trk A, has been reported in the supplemental data for a paper (64), but the structure and the details of this molecule have yet to be published. There will undoubtedly be even more CHK1 inhibitors available in the near future. Because small molecules with different structures are expected to have different spectrums of activity against the various kinases, it will be worth investigating these new CHK1 inhibitors in the clinic.

There are two compounds that inhibit both CHK1 and CHK2 equally, a synthetic peptide TAT-S216A (5) and a marine sponge-derived debromohymenialdisine (65). Although their potency for inhibiting purified kinases *in vitro* are much less compared to the small molecules mentioned above, the differences between IC_{50} for the *in vitro* kinase inhibition and ED_{50} for the G_2 abrogation in live cells tend to be less with these compounds. It will be interesting to determine the reasons for these observations, and if the differences are due to the distribution of the target molecules or the spectrum of inhibiting kinases, and how effectively this inhibit the activities of both CHK1 and CHK2. The marked difference seen in the IC_{50}s of UCN-01 when used against recombinant CHK-2 *versus* immunoprecipitated CHK-2 may indicate a potential difference between the *in vitro* and *in vivo* conditions of this protein (66). It also will be interesting to see if there are consequences of such a difference in discovering new G_2 checkpoint abrogators using high-throughput screening with recombinant proteins in opposition to the relatively low-throughput screening with live cells (67). A CHK2 specific inhibitor, CEP-6367, has also been reported (51), but the potency of it as a G_2 checkpoint abrogator and sensitizer to DNA damaging treatment has not been published.

A novel pyridopyrimidine class WEE1-inhibitor, PD0166285, was obtained using a new *in vitro* screening protocol (68). The effect of WEE1 inhibitors on normal cells needs to be investigated as research has indicated that this inhibitor seems to affect p53-defective cancer cell lines more than p53 wild-type lines.

PP2A inhibitors such as okadaic acid (69) and fostriecin (70) have been shown to abrogate the G_2 checkpoint; however, the treated cells arrest at M phase rather than passing through it. Okadaic acid is considered a tumor promoter and a food poison, so it may not be an ideal therapeutic candidate. Fostriecin is an anticancer drug originally thought to act on topoisomerase II and is already in the market. Its G_2 abrogating activity was found much later and is the first published paper which shows that G_2 checkpoint abrogation is an effective mechanism of action for anticancer medicines (70), although the S-G_2 checkpoint disruption by staurosporine had been reported before (57). Because inhibition of PP2A by fostriecin occurs at a lower dose than the Topoisomerase II inhibition, it could be the main mechanism of action for this medicine. There are reports of additional PP2A inhibitors obtained using novel cell-based screening protocols (71); however, as PP2A is involved in a variety of signal cascades, it may be difficult to make specific G_2 checkpoint abrogation occur by using these inhibitors unless they target a specific substrate recognition or regulatory site.

Two newer G_2 abrogators, 13-hydroxy-15-ozoapathin (72) and isogranulatimides (67), both with unknown mechanisms of action, were reported with a cell-based screening method. The effect on the cell cycle to cells treated with 13-hydroxy-15-ozoapathin is somewhat similar to cells treated with PP2A inhibitors, passage through the G_2 checkpoint, and arrest at early M phase. As of now, there are no reports of compounds targeting 14-3-3.

As discussed, CHK1 seems to be the most suitable candidate to date for selective G_2 checkpoint abrogation if one has to pick a single protein as a target. In fact, to date, most of the novel checkpoint inhibitors reported target CHK1, with a wide range of specific activities. It also has to be taken into account, considering the redundancy of the checkpoint pathways, that targeting multiple cascades at once might provide the most effective means of G_2 checkpoint abrogation, although it will likely be difficult to obtain a single molecule capable of this task.

Concluding Remarks

If selective G_2 checkpoint abrogation, which does not affect p53-dependent G_2 arrest or the G_2 phase of normal cells, was achieved, it could be used to minimize the adverse effects on noncancerous cells. The G_2 checkpoint of many cancer cells is activated by the increased DNA damage that results from a defective G_1 checkpoint; therefore, G_2 checkpoint abrogators should kill cancer cells by reducing the already prolonged G_2 phase and inducing apoptosis. In the case of a combination therapy that adds DNA damaging agents to G_2 checkpoint abrogators, the G_2 checkpoint abrogators are expected to show, depending on the dose of DNA damaging agent used, either increased efficacy with the same level of side effects seen with conventional treatment or an equivalent efficacy with decreased side effects. The G_2 checkpoint is rather unique because intact checkpoints at G_1 and M sensitize cells to anticancer medicines such as cisplatin and taxol, respectively, while the G_2 checkpoint functions primarily to decrease sensitivity to G_2 checkpoint activating drugs. The most promising target to date seems to be CHK1 and there will be a growing number of selective inhibitors of CHK1 available in the future with a variety of activities that promise to have potential G_2 checkpoint abrogation qualities. Although one of these compounds, UCN-01, is currently undergoing clinical trials, the development of further G_2 abrogators with the same or different mechanisms of action is also eagerly awaited.

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