alvocidib-mediated mRNA down-regulation of 64 nM, consistent with the IC50 determined for inhibition of global mRNA synthesis. Replacement of fetal calf by human serum did not significantly change the outcome. Results presented here on alvocidib-mediated down-regulation of *MCL1* in HCT116 cells are in agreement with previous data published for CLL.

References

[1] Clin Ca Res 11:4176 (2005).
[2] Blood 106:2513 (2005).

DNA-interactive agents

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Selective targeting of critical components of DNA replication by HKH40A (RTA 502)

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Mammalian replication is tightly regulated because it must occur at the right times and only once per cell cycle. The timing is carefully choreographed during the G1 and S phases of the cell cycle. Kinases that play important roles include the licensing kinase Cdc6, and Cdc7, that is required for replication inititation. Disruption in the timing of replication leads to premature entry into S phase. Consequently mammalian cells have checkpoint controls that prevent premature progression through the cell cycle. In contrast, cancer cells frequently exhibit mutated controls hence disruptions in the assembly of the replication complex can be lethal to these cells. Disruption in timing of replication in wt p53 tumor cells causes initiation of the p53-dependent apoptotic cascade. In absence of functional p53, tumors with disrupted timing of the replication enter into S phase that cannot be completed, which results in genomic instability, mitotic crisis and death. HKH40A is a synthetic anti-tumor agent with excellent in vitro and in vivo activity against a number of GI tumors. It binds to DNA but its biological activity is manifested only after the initial complex hijacks a protein that is involved in repair, transcription or replication. Sensitive wt p53 cancer cell lines such as colon HCT116 and RKO or hepatoma HepG2 are killed by p53-mediated apoptosis (IC_{50} < 1 nM; LC_{50} ~ 30 nM). Cancer cell lines with mutated p53 such as the colon HT29 and pancreas such as ASPC1 or 10.05 are also killed by the drug (IC_{50}~2 nM; LC_{50}~90 nM), but die as a result of a G2-M block and grossly dysregulated S-phase. The compound showed potent activity in orthotopic liver cancer in rats, as well as in HCT116 and pancreatic cancer BxPC3 and MIA Paca-2 xenografts in mice. It is a candidate for Phase 1 trials. The most downregulated genes in DNA micorarrays of drug-treated HCT116 cells are ribonucleotide reductase M2 (RRM2) and the kinase Cdc7. RRM2 is responsible for maintaining dNTP pools and is frequently up-regulated in cancer where it is a factor in chemoresistance of tumors to drugs. Cdc7 (with Dbf4) phosphorylates MCM2–7 proteins, which are obligatory components of the replication complex. RRM2 and Cdc7 are also downregulated at the protein level (Western analysis), maximally at 48 hrs. Cdc6 is not affected by the drug treatment at the RNA level, but is dramatically downregulated at the protein level after 3 hrs. Western analysis of mutated p53 lines revealed similar changes in these three critical components of replication, suggesting that the effect of the drug on the expression of RRM2, Cdc6 and Cdc7 is p53-independent. FACS analysis of treated cells is consistent with replicative arrest and premature entry into S-phase, that tumor cells unlike normal cells are not able to survive. Thus, agents that target critical components of replication control should be selectively toxic toward cancer cells whose checkpoint functions are impaired.

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Poly(ADP-ribose) polymerase-1 mediated transcriptional responses to genotoxic stress: a possible target for anticancer therapy

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PARP-1 plays a critical role in various cellular responses to genotoxic stress, albeit its function has been attributed primarily to DNA damage repair. We have previously demonstrated that (i) PARP-1 binds to undamaged DNA where it recognizes non-B DNA structures such as hairpins, cruciforms, and unwound regions that can form in the transcriptional regulatory elements [1−3], (ii) PARP-1 can bind to the promoter of its own gene both in vitro and in vivo [1,3], and (iii) PARP-1 interactions with non-B DNA structures lead to its catalytic activation [2]. Together, these data implicate PARP-1 in coordinating structural transitions in genomic DNA and chromatin during transcription. Here we investigated the PARP-1 function in activation of nuclear transcription factors, such as activator protein-1 (AP-1), which participates in adaptive responses of the genome to various oxidant and toxic stimuli. Genetic inhibition of PARP-1 resulted in a delayed activation of Jun transcription factors, and significantly diminished DNA-binding activity of the Fos family proteins. We show that PARP-1 proficient cells exposed to oxidative stress exhibited an early and marked induction of individual components of the AP-1 and that stimulation of the AP-1 expression by DNA damage was dramatically attenuated in cells with PARP-1 null genetic background. Thus, PARP-1 is a dynamic sensor of cellular stress playing important role not only in DNA damage repair but also in DNA damage-induced transcription. Therefore, strategies directed at targeting the DNA-binding properties of PARP-1 may alleviate cellular resistance to genotoxic stress because non-covalent contacts of the DNA-binding domain of PARP-1 with DNA, either containing strand breaks or undamaged, underlie the molecular basis for PARP-1 function. We tested the feasibility of this approach using an enforced expression of the DNA-binding domain of PARP-1. We show that the expression of a dominant-negative mutant of PARP-1 sensitized prostate carcinoma cells to DNA-damage in vitro, and significantly enhanced the radiation-induced inhibition of tumor growth in vivo. It is conceivable that the shared components of the DNA repair and transcription systems, such as PARP-1, allow both systems to control cellular survival in response to genotoxic stress.
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References

[1] JBC 2002, 277: 665.
[2] JBC 2005, 280:17076.
[3] JMB 2005, 348: 609.

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The cytotoxic activity of the monofunctional alkylator S23906 is mediated by generation of DNA double strand breaks that are repaired by homologous recombination in mammalian cells

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Background: S23906, a new monofunctional DNA alkylating agent is under evaluation in phase I clinical trials. We have recently shown (*Léonce et al., Cancer Res*, in press) that cellular exposure to S23906 is accompanied by the formation of DNA double strand breaks (DSBs) as detected by single cell electrophoresis and gamma-H2AX formation. In this work we wished to further characterize the DSBs and establish their role in the antiproliferative activities of S23906.
Results: Exposure of tumor cells to S23906 is accompanied by a dose-dependent formation of DSBs that can be detected within minutes by single cell electrophoresis. Incubation with S23906 for 1 h followed by post-incubation in drug-free media revealed that the even short exposure to S23906 is accompanied by long-lasting DNA damage that is difficult to repair. Co-incubation with aphidicolin, a specific inhibitor of DNA synthesis was accompanied by decreased DSBs formation suggesting that the DSBs may be a result of collision between the DNA-S23906 adducts and the advancing replication fork. Importantly, co-exposure to aphidicolin and S23906 followed by post-incubation in drug-free media was accompanied by a gradual formation of DSBs, that after 4 h post-incubation had reached the same levels as after 1 h in the presence of S23906 alone. Therefore, inhibition of ongoing DNA synthesis does not interfere with the initial formation of the S23906 adducts which retain their ability to induce DNA damage for hours after the drug has been removed. Cells deficient in homologous recombination repair showed up to 16-fold increased sensitivity to S23906 compared to parental cell lines whereas no differences were observed for cells deficient in end-joining. The sensitivity of recombination-deficient cells was associated with an inability to repair the S23906-induced DSBs. In contrast, the formation of DSBs was not affected.
Conclusions: Our results indicate that the cytotoxic activity of S23906 is mediated by the formation of DSBs that, at least in part, are replication dependent. They further suggest that expression levels of proteins involved in recombination repair may serve to identify patients particularly likely to respond to this interesting agent.