Mini-Review

Novel anticancer compounds induce apoptosis in human tumor cells

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Conventional treatments for human cancer are often fraught with side effects and limited efficiency, and repeatedly fail to achieve long-term patients survival. Therefore, further identification of novel therapeutic strategies for the treatment of cancer is extremely important. In this article I will describe anticancer compounds that were recently identified in my laboratory by screening libraries of small molecules from National Cancer Institute (NCI).

Recent studies have demonstrated that CDK inhibitor p21 inhibits apoptosis.1-4 Therefore, inhibition of p21 could stimulate apoptosis and be beneficial for cancer therapy. To identify small molecule transcriptional inhibitors of p21, we generated LIM1215 colon cancer cells, carrying lacZ under the control of p21 promoter as a read-out system for screening compound libraries obtained from NCI. Using this system, we identified a nucleoside analog ARC (NSC-188491; 4-amino-6-hydrazino-7-beta-D-ribofuranosyl-7H-Pyrrolo[2,3-d]-pyrimidine-5-carboxamide), that was able to potently repress the expression of p53-targets, p21 and hdm2, while simultaneously increased p53 levels.5 Investigation of the mechanism of action of ARC revealed that it acts as a general transcriptional inhibitor. We showed that it inhibits the phosphorylation of RNA polymerase II by PTEF-b (positive transcription elongation factor b) possibly leading to a block in the transcriptional elongation step similar to other transcriptional inhibitors such as flavopiridol and DRB.5 Also, ARC was able to elicit p53-independent, intrinsic, caspase-mediated apoptosis in multiple cancer cell lines of different origin, but not in normal cells.5-8

Survivin and Mcl-1 are short-lived anti-apoptotic proteins that are often overexpressed in human tumors.9,10 We found that ARC induced loss of mitochondrial trans-membrane potential and downregulated Mcl-1 and survivin, but not another anti-apoptotic protein Bcl-2 in human cancer cells of different origin.5-7 (Bhat and Gartel, manuscript in preparation). Moreover, ARC induced much more efficient apoptosis in colon cancer and neuroblastoma cells than the similar nucleoside analog DRB.5-7 The inability of DRB to induce apoptosis in human cancer cells could be partially explained by its inability to downregulate the antiapoptotic proteins Mcl-1 and survivin.7 Akt is a major downstream target of PI3K and has been associated with a wide variety of pro-survival, anti-apoptotic functions.11 We found that overexpression of myristoylated Akt promoted resistance to ARC-induced apoptosis and ARC inhibited the phosphorylation of Akt in multiple cancer cell lines.6,7 In addition, ARC was able to suppress the expression of N-myc, a frequently amplified oncogene in neuroblastomas, while overexpression of myristoylated Akt protected N-myc from ARC-induced downregulation.8 These data suggest that ARC may antagonize different anti-apoptotic pathways and induce apoptosis in wide range of cancer cells via multiple mechanisms. Overall ARC could represent an attractive candidate for anti-cancer drug development.

We also used another approach to identify potential anticancer drugs. It has been shown that FoxM1, a transcription factor of the Forkhead family is one of the most upregulated genes in human solid tumors12 and is implicated in tumor invasion, angiogenesis and metastasis.13-15 Since FoxM1 is activated in the majority of cancers, but not in normal cells, we hypothesized that it could be an attractive target for anticancer therapy by small molecules.16-19 We designed a novel screening system for the identification of compounds that will inhibit transcriptional activation of genes by FoxM1. We created a cell line that stably expresses doxycycline-inducible FoxM1-GFP fusion protein, firefly luciferase under the control of the CMV promoter.16 We screened libraries of small molecules from NCI for inhibitors of FoxM1 transactivation utilizing this cell line as a read-out system. As a result of the screening we identified a thiopeptide, Siomycin A (NSC-285116) as an inhibitor of FoxM1 transcriptional activity.16 Later we identified another thiazole antibiotic, thistrepton, which is structurally very similar to Siomycin A and also inhibits FoxM1, but not the transcriptional activity of other transcription factors (Halasi et al., manuscript in preparation). Both of these thiazole antibiotics exert their antibacterial effects by inhibiting bacterial translation via interaction with the 23S ribosomal RNA, but they do not inhibit eukaryotic translation.20 We found that FoxM1 may be involved in a positive feedback loop where it activates its own expression,16,19 (Halasi et al., manuscript in preparation) and we showed that thiazole antibiotics could inhibit not only the transcriptional activity, but also the protein and mRNA levels of FoxM1,16,19 (Halasi et al., manuscript in preparation).
To examine the anticancer potential of the thiazole antibiotics we studied their effect on human cancer cell lines of different origin. In the beginning, we compared the effect of Siomycin A on wild-type and SV40-transformed human fetal lung fibroblasts (MRC-5) and we found that the transformed cells were much more sensitive to cell death than normal cells. Treatment of human cancer cells of different origin with thiopetides resulted in repression of FoxM1 and robust apoptosis (Halasi et al., manuscript in preparation). Activation of apoptosis by thiazole antibiotics correlated with suppression of FoxM1 protein, suggesting that thiopetides may induce apoptosis to a certain extent through the inhibition of FoxM1. Overall, our data suggest that potentially FoxM1 inhibitors/thiazole antibiotics could be useful for cancer treatment, but additional experiments are needed to determine if FoxM1 inhibitors are feasible alternative for cancer patients treatment.

Recently, we decided to test ARC and the thiazole antibiotics against metastatic melanoma cells in vitro. These cells have a short median survival and are resistant to multiple anticancer drugs. In order to quantitatively evaluate the level of sensitivity of melanoma the four studied melanoma cell lines in concentration of 100 μM, while DTIC failed to induce apoptosis in three out of four the studied melanoma cell lines in concentration of 100 μM. These data suggest that the melanoma cells are much more sensitive to ARCI to the thiazole antibiotics than to DTIC. To examine if these compounds may synergistically induce apoptosis in melanoma cells we treated melanoma cells individually or with the combination of ARC and Siomycin A and assessed the degree of apoptosis in treated melanoma cells by caspase-3 cleavage after immunoblotting and annexin staining. We found that ARC and Siomycin A induce apoptosis much more potently together, than each drug individually, suggesting that they may act synergistically in melanoma cells, likely because of simultaneous repression of two antiapoptotic proteins FoxM1 and Mcl-1. These data suggest that ARC and the thiazole antibiotics potentially may be used for combination treatment of cancer. Since we recently established that these compounds are not very toxic to animals, further animal experiments are needed to determine the efficacy of these drugs in vivo.

**References**


