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Antitumor activity of the new aureolic acid derivatives mithramycin SDK and SK in human ovarian cancer xenografts

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Aureolic acid antibiotics, like mithramycin (MTM), are natural polyketides that interact with DNA at GC-rich sites and inhibit binding of GC-rich DNA binding proteins, like Sp1 transcription factors. Targeted gene inactivation in the *S. argillaceus* producer strain has recently yielded two new analogues, MTM SDK (SDK) and SK (SK), with improved activity as transcriptional repressors and anticancer agents compared to MTM (*NAR 34:1721, 2006*). SDK inhibited transcription of multiple Sp1-regulated genes controlling cell proliferation, apoptosis, migration, invasion and angiogenesis, consistent with the pleiotropic role of Sp1 transcription factors in cancer development and progression. SDK inhibited proliferation and induced apoptosis of several human cancer cell lines, including ovarian cancer cells, with minimal effects in normal cells. To extend these observations to an *in vivo* model, we established human ovarian cancer xenografts by intraperitoneal (i.p., orthotopic model) or subcutaneous injection of A2780 cells in female CD-1 nude mice. In the orthotopic model, SDK, given by i.p. injections for 11 consecutive days at the daily dose of 400 μg/Kg, prolonged survival compared to vehicle-treated control mice. Median survival for SDK was 80.5 versus 55 days for the control group (P = 0.00368). There was also a significant delay in the appearance of ascites in SDK treated mice versus control mice (P = 0.0135). SDK was more effective than MTM that gave a median survival of 58 days (P = 0.28 vs. control and P = 0.0474 vs. SDK), confirming the greater activity of the new analogue seen *in vitro*. SK gave marginal benefits in survival compared to the control group (median survival 68 days and P = 0.0657 vs. control mice). Both SDK and SK were well tolerated in mice without signs of toxicity. In the subcutaneous tumor model, SDK and SK, given by i.p. injections q2d × 10 at a dose of 600 g/kg, induced similar tumor growth delay. These results suggest that SDK and SK could be effective agents for treatment of ovarian cancer, blocking expression of genes involved in cancer cell proliferation and intraperitoneal dissemination.

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A systems biology approach to the analysis of DNA-interactive antitumour agents

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Background: We are developing a systems biology approach to the analysis and integration of the varied biological data from investigations of the molecular pharmacology of DNA-interactive agents. A computational model of the tumour cell is being developed that models the relationship between drug treatment, cell cycle distribution, growth inhibition, and senescence. The parameterisation of this model requires robust data from a well-characterised cell line. Here we describe the approach to the selection of this cell line, and initial data from it. The process involves optimisation against a variety of goals, including cell growth characteristics, sensitivity to DNA-interactive agents, tractability in a variety of assays, and not least clinical relevance.
Materials and Methods: Four colorectal cell lines have been chosen for initial study: HCT116, HT-29, HCC 2998 and KM12. Flow cytometry combining propidium iodide, BrdU incorporation and immunofluorescence for Ki-67 and histone H3 are used for cell cycle analysis alongside MTT assays and x-Gal senescence staining. Our trial DNA-interactive agent is 3,11-difluoro-6,8,13-trimethyl-8H-quino[4,3,2-kl]acridinium methosulphate (RHPS4), a potent inhibitor of telomerase linked to its selectivity for G-quadruplex DNA. However, recent studies suggest a more complex mechanism of antitumour action at the telomere (Leonetti et al. Mol. Pharmacol., 2004, 66, 1138−46).
Results: MTT assays reveal that GI_{50} values can be time dependent: HT-29 cells become 8-fold more sensitive to RHPS4 between 24 and 72h, while KM12 become 4-fold resistant. Cell cycle analysis shows a dose- and time-dependent effect in all cell lines, which computational modelling analyses as dose- and time-dependent blocks at two transitions: a) either the G1->S transition, or the G1/G0 equilibrium, plus b) the G2->M transition. The model predicts that analysis of senescence data will allow these two possibilities to be differentiated.
Conclusions: This data is being used to develop a computational model of the cell of sufficient complexity to reproduce these effects, yet of sufficient simplicity to yield useful predictive information and biological insights. Already it has proven useful in guiding optimal experimental design.

Drug screening

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New assays for histone methyltransferases

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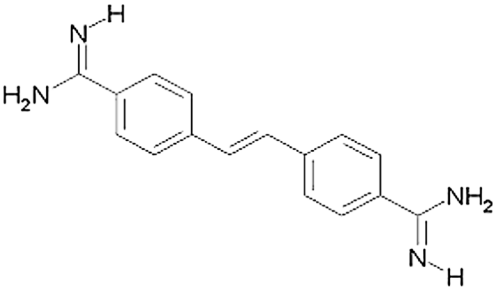
Background: Transcriptional regulation is maintained by posttranslational modifications of histone proteins such as methylation of lysines and arginines. Whereas inhibitors of histone deacetylases are already in clinical trials as anticancer agents very little is known about inhibitors of histone methyltransferases. We wanted to combine virtual screening and enzyme assays in the search for new inhibitors of arginine methyltransferase PRMT1.
Material and Methods: Biotinylated histone peptide fragments that are immobilized on streptavidine microplates are able to serve as substrates for fungal arginine methyltransferase PRMT1 and human PRMT1. The methylation is detected by a primary anti-dimethyl arginine antibody. The readout is performed with a europium labelled secondary antibody and subsequent time-resolved fluorescence analysis. We created a homology model of PRMT1 using the COMPOSER module of the program SYBYL 7.0. The virtual screening was carried out with the NCI Diversity SET using the program GOLD.
Recombinant aspergillus nidulans PRMT1 was used as the source of enzyme. The validation of the most potent compounds was carried out with commercial available human PRMT1. Furthermore, the hits were checked for their cellular activity in an estrogen-reportergen assay and in an antibody based cellular assay for the methylation level on histone H4 arginine3.
Results: The stilbene derivative stilbamidine showed an inhibition of PRMT1 with an IC_{50} of 60 μM and did not inhibit the lysine methyltransferase SET7/9. Additionally, a strong inhibition of the receptor activation by estradiol at 150 μM and a dose dependent inhibition of methylation between 50 and 150 μM in the cellular assay were discovered.



Stilbamidine.

Conclusions: A new inhibitor of PRMT1 was discovered that shows potential for the treatment of hormone dependent cancers.

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Screening for mitotic kinesin KSP inhibitors: implication of the microtubule binding regions of KSP motor domain as drug target

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Small chemical molecules that cause cell cycle arrest in mitosis might be useful for treatment of malignant tumors, since most cells in human body are not actively dividing in comparison to tumor cells. Discovery of monastrol, the first small chemical inhibitor against human kinesin protein KSP, reveals that KSP is one of attractive targets as anticancer drugs to perturb mitotic progression, to activate the spindle checkpoint and to trigger apoptosis. Until now, a number of KSP inhibitors have been identified, including HR22C16, CK0106023, terpendole E, K and S-trityl-L-cysteine.