interaction with GSH/GST pathway. Interestingly, its sequence-specificity is completely different from that of tallimustine whose DNA interaction is not affected by the presence of GSH/GST. The DNA interaction and the sequence-specificity of PNU-571077 are superimposable to that of brostallicin. These findings further support the role of GSH in the mechanism of action of brostallicin.

544 POSTER

Atomic force microscopy study of structural transitions of supercoiled DNA in response to Poly(ADP-ribose)polymerase-1 protein binding

S. Chasovskikh. *Georgetown University Medical Center, Radiation Medicine, Washington, DC, USA*

Background: Poly(ADP-ribose) polymerase (PARP-1) is a multifunctional nuclear DNA-binding protein that interacts with single and double stranded DNA breaks as well as with secondary structures in undamaged, supercoiled DNA. Secondary structures of DNA, such as cruciforms, can play a role in transcription by creating new protein binding sites, to which various factors bind and restrict movement of the transcription-elongation complex. Modulating the level of DNA supercoiling has been proposed as a possible mechanism for regulating gene expression at a distance. PARP-1 has been shown to affect transcriptional regulation of specific genes.

Materials and methods: Supercoiled topoisomers of the cruciform structure containing plasmid, pUC8F14C, were used as substrate recombinant human PARP-1 binding. Atomic force microscopy (AFM) images were obtained using the NanoScope IIIa instrument equipped with an E-scanner (Digital Instruments, Santa Barbara, CA) and analyzed by using the computer program accompaning the imaging module.

Results: We observed that PARP-1 binds to the ends of the hairpin arms of the topoisomers of pUC8F14C DNA. This DNA contains a 106-bp F14C inverted repeat with predicted cruciform arm length of 53 bp. Analysis of the volume distributions of PARP-1 molecules in DNA-PARP-1 complexes revealed that PARP-1 forms a few dimers on interaction with cruciform structure. We determined that when PARP-1 binds to one segment of the supercoiled plasmid DNA in these complexes, it appears partially relaxed. Whereas, when PARP-1 interacts with nodes, it makes a DNA but, the level of supercoiling in the surrounding plasmid does not decrease.

Conclusions: Proteins that bind to enhancer elements and interact with the transcriptional machinery regulate transcription. Protein binding may alter local DNA structure through the change of DNA superhelicity. In previous work, we found that PARP-1 repressed transcription when it binds to the PARP promoter. This suggests that the affinity of PARP-1 to secondary DNA structures and the changes in the topology of supercoiled DNA generated by PARP-1 binding across poly(ADP-ribosyl)ation reactions can play a role in the regulation of gene expression. Accordingly, PARP inhibitors may be therapeutic agents capable of gene regulation.

545 POSTER

Biological effects of G-Quadruplex binding agents in various human cancer cell lines

M. Gunaratnam[1], O. Greciano[1], C. Martins[1], C.M. Schultes[1], S. Neidle[1], L.R. Kelland[2]. [1]*CRUK Biomolecular Structure group, Biological and Pharmaceutical Chemistry, London, UK;* [2]*Antisoma Research Laboratories, St George's Hospital Medical School, London, UK*

Findings that expression of telomerase and the maintenance of telomere length in the overwhelming majority of tumours together with the absence of such features in normal somatic cells, have created much interest in targeting the enzyme and telomeres, as a new cancer drug discovery strategy. An approach developed by this laboratory involves targeting the 3′-single stranded overhang telomeric DNA substrate, by inducing it to fold into a four stranded guanine-quadruplex structure (G4) that is incompatible with telomerase extension, and which itself may serve as a signal for DNA damage responses.

A series of small molecules that have been designed and synthesised to stabilise G4 structures, have been previously reported by us. These compounds have shown inhibitory effects against telomerase, detected by the TRAP assay. Many of these compounds have demonstrated selective potency against human carcinoma cell lines in short-term cytotoxicity studies while presenting low toxicity against normal human cells. Further, an initial lead compound, the 3,6,9-trisubstituted acridine BRACO-19, has displayed long term growth arrest in carcinoma cell lines and replicative senescence in vitro as well as in vivo activity in a tumour xenograft model. These in vitro cellular effects are both dose and time dependent. The loss of chromosomal integrity by generating end-to-end chromosomal fusions produced by BRACO-19 is consistent with its rapid induction of telomere uncapping.

We report here on detailed cellular and molecular studies for a new set of 3,6,9-trisubstituted acridines, with the goal of establishing structure-activity relationships and identifying optimal candidate telomere maintenance inhibitor molecules for subsequent in vivo studies. A panel of carcinoma cell lines, representing prostate, breast, non-small-cell lung and ovarian cancers, has been established, together with a series of evaluation criteria. Possible reasons for the observed differences in responses will be presented, together with details of the structure-activity relationships. The evaluations involve comparison of quadruplex affinity, telomerase inhibition, with potency in long-term inhibition-of-proliferation studies and measurements of apoptosis and senescence.

546 POSTER

A novel aureolic acid antibiotic analogue has potent anti-proliferative activity and induces multiple changes in gene expression in ovarian cancer cells

V. Albertini[1], S. Vignati[1], A. Rinaldi[1], F. Bertoni[1], J. Rohr[2], G. Carbone[1], C. Catapano[1]. [1]*Oncology Institute of Southern Switzerland, Laboratory of Experimental Oncology, Bellinzona, Switzerland;* [2]*University of Kentucky, Division of Pharmaceutical Sciences, Lexington, USA*

Aureolic acid antibiotics are interesting lead compounds for drug development because of their ability to bind to GC-rich sequences in DNA, block binding of Sp1-family transcription factors and inhibit transcription of Sp1-regulated genes preferentially. Aureolic acid antibiotics, such as mithramycin and chromomycin, are active against several types of cancer but their clinical use is limited by severe side effects. In the attempt to identify compounds with improved activity and therapeutic index, we have evaluated the activity of a new aureolic acid analogue, SDK, which had been generated by genetic manipulation of the mithramycin biosynthetic pathway in *S. Argillaceus*. SDK was an effective inhibitor of proliferation of several ovarian cancer cell lines with IC$_{50}$ concentrations ranging between 50 and 250 nM. Flow cytometry analysis of A2780 ovarian cancer cells showed cell cycle alterations and induction of massive apoptotic cell death as indicated by the appearance of a prominent sub-G1 peak after 24 and 48 hours of drug treatment. To determine the mechanisms involved in the response of ovarian cancer cells to SDK, we evaluated its effects on gene expression after 6 hours of incubation of A2780 cells using Affymetrix U133 GeneChips. Multiple genes involved in transcription regulation, DNA repair, cell cycle, proliferation, apoptosis and angiogenesis, were n egatively modulated by SDK. Gene expression analysis by RT-PCR and Western blotting confirmed that SDK induced down-regulation of genes, such as c-myc, c-src, hTERT, Bcl-XL, Ets2 and VEGF at low concentrations (50–100 nM) and early time points (<24 hours). The ability of SDK to inhibit cell proliferation and modulate expression of critical cancer promoting genes is an important feature for further development of this compound as a cancer therapeutic agent.

547 POSTER

DNA adduct formation by C-1748, a potent antitumor 4-methyl-1-nitroacridine of lowered toxicity

J. Grzeskowiak[1], A. Bartoszek[1], B. Wysocka-Skrzela[1], J. Konopa[1], R. Tiwari[2]. [1]*Gdansk University of Technology, Dept. of Pharmaceutical Technology and Biochemistry, Gdansk, Poland;* [2]*New York Medical College, Valhalla, NY, USA*

4-Substituted 1-nitroacridines represent a new group of acridine derivatives synthesized at Gdansk University of Technology. In contrast to parent 1-nitroacridines, these compounds exhibit low toxicity and enhanced antitumor efficacy. The leading derivative 4-methyl-1-nitroacridine denoted C-1748 is being prepared for phase I clinical evaluation. The introduction of an electron donating methyl group into position 4 (para to 1-nitro) decreased the susceptibility of 1-nitro substituent to reduction. 1-Nitro group is crucial for biological activity and also for the ability of 1-nitroacridines to form DNA adducts. In the present study, we investigated DNA binding properties of C-1748 in comparison to the parent 4-unsubstituted analogue – C-857. Two methods were used: the elaborate [32P]-post-labelling technique and a newly developed by us simple and rapid method exploiting restriction enzymes to the detection of covalent modification of PCR-amplified DNA fragment. The latter approach enabled us to demonstrate covalent binding of C-1748 to DNA in different activating systems and to study kinetics of this reaction. The one involving DTT as a reducing agent required long exposures of DNA to C-1748 (17 h), while C-857 modified DNA within 1 h. In the presence of microsomes, short incubation times (1–3 h) were required for both compounds. For both acridines, the level of binding was concentration- and time-dependent. Another difference revealed by this method was the base pairs preference; C-857 was clearly GC specific, while C-1748 seemed to bind with similar efficiency to both GC and AT base pairs. In parallel, the detection of DNA adducts was carried out by [32P]-post-labelling technique. The maps of [32P]-labelled adducts formed by C-1748 displayed more chromatographic