from paraffin wax embedded tissues from these cases for the expression of oestrogen receptor (ER), preogesterone receptor (PR), human epidermal growth receptor 2(c-erbB-2 or HER2/neu), p53 and cyclin D1 (CCND1) was carried out using the avidin biotin complex (ABC) procedure. This procedure is briefly as follows:
Sections were mounted on adhesive coated glass slides and deparaffinised in xylene. They were rehydrated in graded alcohol and placed in 0.5% hydrogen peroxide to quench endogenous peroxidase. Antigen retrieval was achieved by microwave oven incubation in citrated buffer (0.01M: pH 6.0) for ER, PR, c-erbB-2 and p53 oncoproteins and in 1mM EDTA (pH 8.0) for Cyclin D1. Antigen localisation was achieved by incubating sections with primary antibodies (polyclonal rabbit antihuman erbB-2 and monoclonal mouse antihuman ER, PR, p53 and CyclinD1) at various dilutions. The indirect avidin-biotin complex (ABC) procedure using 3,3-diaminobenzidine tetrahydrochloric (DAB) as the substrate chromogene was applied for detection of bound antibody.
The Chi square test was used for statistical analysis.
Results: Invasive ductal carcinoma made up 92.7% of the Nigerian patients compared to 77% seen in the British patients. Significant difference in clinical stage but not tumour grades was also observed.
The expression of ER and Cyclin D1 was significantly higher in the British patients than the Nigerian patients (X*=6.9143 P=0.0086, X*=4.9281 P=0.0234 respectively). Other markers showed no statistical difference.
Conclusion: The differences in prognosis of breast cancer between Nigeria and Britain may be partly explained by differences in hormone receptors and cell cycle regulation in addition to the obvious differences in stage at presentation

Telomerase-targeting agents

434 POSTER

The interactions of acyclic nucleotide analogues with human telomerase

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Background: Purine acyclic nucleoside phosphonates (ANP) type PME {9-[2-(phosphonomethoxy)ethyl] derivatives of purines} and PMP {9-[2-(phosphonomethoxy)p ropyl] derivatives of purines} were shown as very potent antivirals active against DNA viruses and retroviruses. Moreover, these antimetabolites exhibit cytostatic activity *in vitro* and *in vivo*. Antiviral and cytostatic effects of these nucleotide analogues are the result of DNA polymerase and/or reverse transcriptase inhibition by their diphosphates (dNTP analogues). These findings led us to the idea to investigate their interactions with human telomerase, the reverse transcriptase capable of replacing the telomeric portion of the chromosome lost during DNA replication.
Material and Methods: ANP diphosphates were evaluated in telomeric repeat amplification protocol (TRAP) for their ability to inhibit the extension of telomeres by human telomerase, using extracts from human leukemia HL-60 cells as a source of the enzyme. Inhibition of telomerase was measured in the presence of various concentrations of studied ANPpp as inhibitors, and the natural dNTPs (125 $\mu mol \cdot l^{-1}$ each). The extent of the enzyme inhibition was expressed as IC_{50} values.
Results: Our data show that the most effective compound studied was the guanine derivative PMEGpp ($^{Tel}IC_{50}$ 12.7±0.5 $\mu mol \cdot l^{-1}$). The inhibitory effects of other PME and PMP diphosphates on telomerase reverse transcriptase decreased in the order: (*R*)-PMPGpp > PMEDAPpp > (*S*)-PMPGpp > (*S*)-HPMPApp > DAPympp > 6-cypr-PMPDAPpp > (*R*)-PMPApp > PMEApp > (*R*)-PMPDAPpp > (*S*)-PMPApp ≈ 6-Me_2-PMEDAPpp.
Conclusions: These results are consistent with the observed antineoplastic activities of the paternal PMEG and PMEDAP compounds. Moreover, structure-activity relationship indicates enantio-selectivity some of these human telomerase inhibitors. (*R*)-Isomers of the PMP-derivatives possess stronger affinity towards the enzyme than (*S*)-isomers. In accordance with human telomeric sequence, the adenine derivatives are less effective inhibitors than the guanine derivatives. The data contribute to the rational design of telomerase inhibitors based on the structure of acyclic nucleotide analogues.

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Biophysical, biological, and in silico investigation of 3,6,9-trisubstituted acridines targeting human telomeric G-quadruplex DNA

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The activation of the holoenzyme telomerase is one of the key events in the oncological transformation of human cells (Hahn et al., 1999) and inhibition of telomerase has thus become established as a promising potential strategy for anti-cancer drug development in recent years. The single-stranded, G-rich telomeric DNA substrate of telomerase can form higher-order G-quadruplex (GQ) structures (Wang and Patel, 1997; Parkinson *et al.*, 2002), the detailed characterisation of which has allowed the application of rational drug design approaches to the development of small molecules that will have specificity for the unique features of this human GQ. Work in our group and others has thus resulted in the development of compounds that inhibit normal telomerase function in the nanomolar range by the targeting of telomeric GQ-DNA (Read *et al.*, 2001; Mergny *et al.*, 2002; Harrison *et al.*, 2003).
We shall report on the biophysical characterisation and initial biological evaluation of a set of 3,6,9-trisubstituted acridine derivatives as potent new telomerase inhibitors. The development of a number of compound series has allowed the establishment of clear structure-activity relationships (SARs) describing quadruplex-drug interactions, and current work is focused on elucidating the biochemical pathways activated upon cellular exposure to compounds selected in this manner. Screening of compounds for their ability to bind and stabilise the human telomeric GQ structure was carried out using a series of fluorescence resonance energy transfer (FRET)-based DNA melting experiments. Significant differences in the abilities of compounds to increase the melting temperature of GQ-DNA can be compared to enzyme inhibition data obtained from an *in vitro* cell-based assay of telomerase activity (TRAP assay), and this can then be translated directly into SARs that have enabled us to gain insights into the extent to which our *in vitro* assays allow the prediction of enzyme inhibition. A direct correlation between the GQ-stabilising ability of a compound and its ability to inhibit telomerase *in vitro* has been established in this way, both allowing the rapid screening of novel compounds with a high-throughput fluorescence method and lending support to the proposed mechanism of telomerase inhibition *via* GQ-stabilisation.
On-going studies aiming to elucidate in more detail the ligand-DNA interactions and the cellular response to compound exposure will also be presented. Molecular modeling approaches, including molecular dynamics simulations methods, are being used to rationalise the results from the biophysical assays on a molecular level, while changes in the cellular levels of telomerase and specific other telomere-associated and DNA-damage-response proteins are being investigated at the DNA expression level following exposure to our lead compounds.
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436 POSTER

The novel compound KML001 induces telomere attrition, senescence and chromosomal instability in cell lines with short telomeres

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Arsenic trioxide is experiencing a revival in cancer medicine since it has proven effective in the treatment of acute promyelocytic leukemia. Its mechanisms of action are currently being revisited to enable rational use of inorganic arsenic. Induction of apoptosis and reactive oxygen species, as well as striking effects on telomeres and telomerase have been described. The novel arsenic compound KML001 has shown preclinical activity in solid tumors and has just entered clinical trials. This study was initiated in order to investigate whether KML001 can target telomeres and telomerase. MCF-7 (6 kb), a human breast cancer cell line with longer telomeres, PC3 (3,5 kb) and UXF 1138L (2.5 kb), prostate and uterus cancer cell lines respectively with shorter telomeres, were chosen for *in vitro* experiments. The TRAP assay (telomeric repeat amplification protocol) was used to measure telomerase activity, ß-galactosidase staining for detection of cellular senescence, the sulforhodamine B assay for proliferation tests, Southern blotting to determine mean telomere fragment length (TRF), and fluorescence *in situ* hybridization (FISH) with human centromere and telomere probes to study chromosomal integrity. The IC_{50} for KML001 in PC3 cells was 1 μM, in MCF-7=4 μM, and in UXF 1138L=5 μM. KML001 treatment at doses around the IC_{50} potently shortened telomeres in PC3 and UXF 1138L, but not MCF-7 cells under continuous exposure.

The most dramatic telomere shortening was seen in PC3, the TRF length (3.5 kb) decreased within 28 days to a critical length of 2.3 kb. Telomere attrition was accompanied by end-to-end chromosome fusions and replicative senescence. PC3 cells were highly β-galactosidase positive and ceased growth under treatment with 1 μM drug after 4 weeks. UXF 1138L were able to proliferate in presence of 5 μM KML001 for more than 7 weeks; MCF-7 cells did not senesce. KML001 caused chromosomal abnormalities, with chromosome end-to-end fusions seen in UXF 1138L and PC3 cells. The end-to-end fusions increased e.g. in metaphases of UXF1138L to an extent of 69% compared to controls.
Telomerase activity, however, was not inhibited. Even at supra-toxic drug levels of 1000 μM, KML001 did not inhibit telomerase or the polymerase activity in the PCR reaction, suggesting telomere poisoning by this drug. Thus, it is most conceivable that KML001 directly targets the telomeres by specific or unspecific DNA-damage in telomeric sequence regions. Senescence and genomic instability occurs and will lead to cancer cell death foremost in cells with short telomeres.
Our findings indicate that KML001 can target telomeres and that this effect should be considered in clinical trials design.

437 POSTER

Targeting telomere maintenance in childhood neuroblastoma and primitive neuroectodermal brain tumors

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Background: Primitive neuroectodermal brain tumors (PNET) and neuroblastoma are the most common intra- and extra-cranial malignant tumors in childhood. These embryonal tumors share important biological similarities. Unlimited replicative potential is an important acquired capacity of cancer. Mechanisms of telomere maintenance in cancer cells include upregulated expression of the enzyme telomerase (85–90%), or different mechanisms known as alternative lengthening of telomeres (ALT). Epigallocatechin gallate (EGCG), the major polyphenol in green tea, is a telomerase inhibitor with antiproliferative and anti-carcinogenic effects against different types of cancer. Telomestatin is a G-quadruplex intercalating drug specific for telomeric sequences.
Methods: mRNA expression of human telomerase reverse transcriptase (hTERT) was measured in 12 human neuroblastoma cell lines, 6 PNET cell lines, 50 primary PNET samples, and 14 normal human brain samples by real time RT-PCR. In cell lines, telomerase activity was determined by a quantitative telomeric repeat amplification protocol (TRAP). Telomere length was quantified using terminal restriction fragment analysis. Cell viability was quantified using the colorimetric MTS assay.
Results: Compared to normal human cerebellum, 38/50 (76%) primary PNET samples had >5-fold upregulated hTERT mRNA expression. While a positive correlation between hTERT mRNA expression and telomerase activity was detected in both PNET and neuroblastoma cell lines, no correlation was found between telomerase activity and telomere length in PNET cell lines. Both EGCG and telomestatin inhibited telomerase activity in TRAP-positive neuroblastoma and PNET cell lines. Although EGCG displayed strong proliferation inhibitory effects against TRAP-positive PNET cells, it had no significant effect against TRAP-negative D425 cells. In contrast, telomestatin inhibited proliferation in all neuroblastoma and PNET cells tested.
Conclusions: These results provide evidence for a possible role of telomerase in the pathogenesis of PNET and neuroblastoma and indicate the presence of ALT in subsets of PNET. Successful telomere-targeted anti-cancer therapy for PNET might therefore require a combination of telomerase and ALT inhibitors, such as telomestatin.

Gene therapy and antisense approaches

438 POSTER

Mesenchymal progenitor cells as gene delivery systems for cancer and leukemia therapy

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We have previously demonstrated that bone marrow-derived non-hematopoietic stem cells (MSC) integrate into solid tumors as stromal fibroblasts following intravenous injection (Cancer Res 62:3603–3608, 2002). This finding suggests the development of novel anti-cancer therapies based on the local production of biological agents by gene-manipulated MSC. We examined whether human MSC producing human interferon-beta (IFNβ-MSC) can inhibit the growth of metastatic tumors in the lungs of SCID mice. MSC were transduced with an IFNβ expressing adenoviral vector. These IFNβ-MSC produced 40–50,000 I. U. of IFNβ /10^6cells/24 hours. IFNβ-MSC, but not vector-control transduced MSC, directly inhibited the growth of both A375 melanoma and MDA 231 breast carcinoma cells in co-culture experiments *in vitro*, and when injected intravenously (IV) (four doses of 10^6 MSC/week) into SCID mice bearing pulmonary metastases of carcinomas or melanomas, tumor growth was inhibited as compared to untreated or vector-control MSC controls (p=0.0073). Recombinant IFNβ protein (50,000 IU every other day) injected subcutaneously was ineffective (p=0.14). IV injected IFNβ-MSC prolonged the survival of mice bearing metastatic breast carcinomas or melanomas (p=0.001). MSC marked with β-gal were found only in tumors, where they proliferated and incorporated BudR, but not in normal tissues. Intraperitoneal injections of IFNβ-MSC in mice carrying ovarian carcinomas resulted in doubling of survival (SKOV-3) and cures of 70% of mice carrying OVAR-3 tumors. MSC injected into the carotid artery (IA) of mice selectively proliferated in human glioma xenografts, but not in normal brain tissues, and significantly prolonged survival of these animals. In a model of chronic myelogenous leukemia in blast crisis (KBM5), mifepristone (RU486) regulated production of interferon α (IFNα (in AAV infected MSC induced tumor regressions and doubled survival. MSC delivering tumor selective replicating adenovirus (delta24) exerted anti-tumor effects in ovarian cancer after I. P. injection also prolonged survival.
Data suggest that IV, IP or IA administered gene-modified MSC can inhibit the growth of leukemias, metastatic tumors of the lungs, ovarian and brain tumors. Importantly, the anti-tumor effects were only observed when MSC were integrated into the tumor microenvironment. Mechanisms responsible for MSC tropism in tumors are under investigation and will be discussed. Results suggest the use of gene-manipulated MSC for cancer and leukemia therapy.

439 POSTER

TGF-beta2 suppression by the antisense oligonucleotide AP 12009 as therapy for high-grade glioma: safety and efficacy results of phase I/II clinical studies

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Background: Tumor derived transforming growth factor beta (TGF-beta) is a pivotal factor for malignant progression by inducing metastasis, angiogenesis, proliferation and immunosuppression. High-grade gliomas are highly aggressive tumors showing marked overexpression of the TGF-beta2 isoform which is correlated with advanced tumor stage.
Methods: In 3 phase I/II dose escalation studies adult high-grade glioma patients (WHO grades III/IV) with recurrent tumor and evidence of tumor progression on MRI were treated with AP 12009, a TGF-beta2 specific phosphorothioate antisense oligonucleotide. AP 12009 was administered intratumorally by convection enhanced delivery (CED) in up to 12 cycles. In the 3rd study, an indwelling pump system was used allowing repeated treatment cycles with a single catheter placement on an out-patient basis. Safety and tolerability were primary endpoints. Secondary endpoint was clinical efficacy.
Results: In 5 of the total of 24 patients "possibly" related adverse events were observed, mostly of grade 1 or 2, one was classified as serious. Alternatively, this event could also be related to rapid reduction of steroids performed in this patient prior to study entry. There were no relevant changes in laboratory values, including hematology. Application system and CED were well tolerated without problems. Median overall survival after recurrence was 138.4 weeks for anaplastic astrocytoma (AA) and 44.0 weeks for glioblastoma (GBM) patients as compared to the published data from start of temozolomide therapy of 42.0 (AA) and 32.0 weeks (GBM), respectively. One AA patient had a complete response in all tumor sites after one cycle of AP 12009 experiencing an overall survival of 195 weeks after first recurrence. A further tumor remission with similar time course was documented for a second AA patient receiving 12 cycles of AP 12009. The remaining enhancing lesion was considered to be most likely scar tissue by the responsible neuroradiologist. Additionally, one GBM patient showed a strong reduction in tumor size.
Conclusions: AP 12009 application was safe and well tolerated. These results show AP 12009 mediated TGF-beta2 suppression to be a highly promising therapeutic approach for TGF-beta2 overexpressing tumors such