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

as high-grade gliomas. Thus, AP 12009 is now applied in comparison to standard chemotherapy in an international phase II/III study with currently 26 study centers.

440 POSTER

Effects of bispecific antisense oligonucleotide targeting both insulin-like growth factor binding proteins 2 and 5 on cell survival and apoptosis in prostate cancer model

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Novel treatment modalities designed to prevent androgen-independent prostate cancer progression and metastasis are the subject of strong interest given the lack of success with currently available therapies to prevent or treat this lethal stage of disease. Antisense oligonucleotides (ASO), designed to a chosen cancer-relevant target gene, show enhanced specificity for malignant cells. Insulin-like growth factor binding proteins 2 and 5 (IGFBP2 and IGFBP5) are the members of IGF-I axis that is known to be critical in the regulation of neoplastic tumor progression and differentiation. IGFBP2 is a major binding protein in the prostate that is up regulated in prostate cancer during progression. IGFBP5 has been suggested to play a role in the metastasis of prostate cancer through its role in the bone microenvironment. Since both binding proteins are involved in prostate cancer development and progression, they provide potential targets for antisense strategies.
Methods: A prostate cancer tissue microarray spotted with 382 untreated and post hormonetherapy treated cancers was used to evaluate changes in IGFBP-2 and -5 after androgen ablation and in osseous metastases. Sequence similarity between the genes coding for IGFBP2 and IGFBP5 permits the design of bi-specific antisense oligonucleotide (bs-ASO) to target both IGFBP2 and IGBP5 mRNA. Dose-dependent sequence-specific effects of bs-ASO on mRNA level of IGFBP2 in LNCaP and C42 prostate cancer cell lines and IGFBP-5 in the SaOS-2 osteosarcoma cells were evaluated using Northern Blotting, while flow cytometry and MTT assay were performed to evaluate effects of bs-ASO treatment on cell cycle, cell growth, and apoptosis.
Results: Prostate cancer tissue microarray confirmed that IGFBP2 increased during prostate cancer progression to the androgen independent (AI) state. High level of IGFBP5 was found in prostate cancer metastasis. Northern blot showed dose-dependent sequence-specific down-regulation (up to 90%) of mRNA in cells expressing BP2 and BP5 respectively after bs-ASO treatment. bs-ASO treatment showed dose-dependent sequence-specific cell growth inhibition (from 50% to 90% depending on cell type), and 2-fold increase in subG0 apoptotic fraction and 3 fold G2/M arrest in prostate cancer cells. In order to identify the way by which bs-ASO may affect cell biological behavior, LNCaP and C42 cells were treated with the PI3K inhibitor LY294002. IGF-I is known to overcome LY toxicity, which was measured by AKT phosphorylation. bs-ASO completely inhibited the ability of IGF-I to overcome LY toxicity compare to control.
Conclusion: Bispecific antisense oligonucleotide targeting IGFBP-2 and IGF-BP5 could be seen as a potential therapeutic approach in prostate cancer patient, targeting both local disease and metastatic progression.

441 POSTER

Sensitizing NSCLC to chemotherapy by Bcl-2 siRNA – what is the optimal chemo combination?

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Background: Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related death in men and women and adjuvant chemotherapy resulted so far in no major survival improvement. Defective apoptosis regulation is suspected to be a fundamental aspect of the treatment resistance of lung cancer. In NSCLC the anti-apoptotic Bcl-2 is expressed in up to 71% of all cases and has been associated with significant shorter survival. Abrogating the tumour-protective function of Bcl-2 and restoring chemosensitivity in NSCLC has been suggested a novel rationale for NSCLC therapy. Only recently antisense oligonucleotides (ASO) targeting Bcl-2 has been entered clinical trials and the concept of sensitizing NSCLC to taxotere will be currently studied in a Phase III trial.
Using an alternative strategy we evaluated in the present study synthetic small interfering RNA (siRNA) compounds targeting Bcl-2 to downregulate Bcl-2 expression in NSCLC.
Material and Methods: In A549 NSCLC bcl-2 regulation by siRNA was determined on mRNA and protein level by real time PCR and western blotting, respectively. For cell growth assays, cell numbers for single-agent and combination therapies were measured by cell counting. The number of apoptotic cells was examined by PI staining using FACS analysis and activated caspase 3 ELISA.
Results: Two Bcl-2 siRNAs were screened for their potency to specifically silence Bcl-2 expression in NSCLC. Treatment with Bcl-2 siRNA compounds at low nanomolar concentrations led to a dose and time dependent reduction of bcl-2 mRNA levels (up to 6-fold) and decreased Bcl-2 protein expression down to 30%. As a result, silencing of Bcl-2 in NSCLC cells by siRNA alone (25nM) led to a clear inhibition of cell growth and increase in apoptotic cell death ($p<0.05$). However, combinations of Bcl-2 siRNA and taxotere at equipotent doses surprisingly did not show any synergistic anti-tumour activity in NSCLC, whereas combinations with other anti-tumour agents (e.g. cisplatin) indicate more favourable combination results (analysis ongoing).
Conclusion: These findings highlight Bcl-2 as an attractive target for molecular targeted therapies in NSCLC. Bcl-2 siRNA alone show a highly efficient anti-tumour activity while combination with taxotere did not result in synergistic results. Therefore, the optimal apoptosis inducing drug for combination with Bcl-2 targeting strategies needs to be determined.

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Depletion of DNA methyltransferase (DNMT)1, and/or DNMT3b mediates growth arrest and apoptosis in lung and esophageal cancer cells

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Background: Aberrant DNMT activity perturbs gene expression via chromatin remodeling mechanisms during malignant transformation. Recently we have observed induction of cancer testis antigen and tumor suppressor gene expression in biopsy specimens from thoracic oncology patients following prolonged Decitabine infusion. The present study was undertaken to evaluate the effects of DNMT depletion in cultured lung and esophageal cancer cells by antisense oligos (ASOs) as a possible prelude to evaluation of these agents in thoracic oncology patients.
Methods: A549 and CALU-6 lung cancer cells, SKGT5 and BIC esophageal adenocarcinoma cells, and normal human bronchial epithelial (NHBE) cells were transfected with ASOs specifically targeting DNMT1 or DNMT3b, or mismatch oligos using lipofectamine techniques. Quantitative RT-PCR, western blot, trypan blue exclusion, and ApoBrdU techniques were used to evaluate DNMT expression, proliferation, and apoptosis following ASO transfections. Gene expression profiles were assessed by long-oligo arrays.
Results: ASOs mediated specific, dose-dependent depletion of DNMT1 and DNMT3b, which coincided with a pronounced (80%) inhibition of proliferation of lung and esophageal cancer cells. These effects were not observed following ASO transfection of NHBE cells. Depletion of DNMT1 and /or DNMT3b mediated dramatic, caspase-dependent apoptosis in A549 (p53 wt) and CALU-6 (p53 −/−) lung cancer cells. In contrast, minimal apoptosis was observed in SKGT5 and BIC esophageal carcinoma cells following ASO transfections despite comparable inhibition of DNMT expression and proliferation. The antiproliferative effects of the ASOs were not attributable to induction of tumor suppressor genes such as RASSF1A or p16, and did not coincide with demethylation of genes encoding cancer testis antigens. p21 expression was induced in all of the cancer lines following DNMT1 and/or DNMT3b depletion; however p21 expression levels did not appear to directly coincide with apoptosis following ASO transfections. Micro-array analysis of ASO-transfected A549 cells revealed pronounced induction of a variety of genes mediating response to genotoxic stress. Interestingly, gene expression profiles following DNMT1, DNMT3b, or combined DNMT1/3b knockdown were remarkably similar, yet distinctly different from expression profiles mediated by low dose deoxyazacytidine.
Conclusions: ASOs targeting DNMT1 and DNMT3b mediate potent growth inhibition in lung and esophageal cancer cells. Further studies are warranted to define the mechanisms by which these ASOs induce apoptosis in lung cancer cells, and to examine potential strategies to sensitize esophageal carcinoma cells to the proapoptotic effects of DMNT depletion.

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Decreased expression of DNMT1 at the mRNA level following 7 day infusion of the antisense compound MG98 in a phase I study

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Background: DNA methylation in the promoter region of genes regulates gene expression and is involved in the silencing of tumour suppressor