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Inhibition of polo-like kinase 1 by antisense oligonucleotides and RNA interference

B. Spaenkuch-Schmitt[1], J. Bereiter-Hahn[2], M. Kaufmann[1], K. Strebhardt[1]. [1]*Medical School, University of Frankfurt, Department of Obstetrics and Gynecology;* [2]*J.W.Goethe-University, Department of Biology, Frankfurt/Main, Germany*

Background: A central role for polo-like kinases (PLK) in regulating several stages of mitotic progression has been born out in several species. Overexpression of PLK1 is observed in numerous human tumors and it is a negative prognostic factor in patients suffering from various carcinomas. In order to define the role of PLK1 for mitotic progression of human cells and for neoplastic proliferation, phosphorothioate antisense oligonucleotides (ASOs) and 21-nt-long small interfering (si) RNAs were tested to selectively downregulate PLK1 expression in cancer cells (MDA-MB-435 breast, MCF-7 breast, HeLa S3 cervix, A549 lung, SW-480 colon) and in primary human mammary epithelial cells (HMECs). In eukaryotes, double-stranded (ds) RNA induces sequence-specific inhibition of gene expression referred to as RNA interference (RNAi).
Methods: After transfection Northern and Western blot analyses were used to examine the potential of ASOs (250nM) and siRNAs (56nM) targeted against human PLK1 for specific inhibition of PLK1 gene expression. Furthermore, the influence of ASOs and siRNAs on kinase activity, cell cycle, apoptosis, phenotype and proliferation of cancer cells and HMECs was monitored.
Results: ASO and siRNA treatment of cancer cell lines and HMECs resulted in a sequence-specific decrease in the level of mRNA and protein expressed from the human PLK1 gene. The analysis of ASO- or siRNA-treated cancer cells by fluorescence microscopy revealed centrosomes that lost their ability for microtubule nucleation and an elevated percentage of apoptosis in contrast to HMECs. Moreover, ASO and siRNA treatment against PLK1 resulted in G2/M arrest and a potent antiproliferative effect in tumor cells of different origin. In contrast, HMECs exhibited inefficient uptake of PLK1-specific siRNA and no proliferative inhibition even at high concentrations (2μM). Considerable antitumor activity of ASOs was observed *in vivo* against A549 cells.
Conclusions: These data indicate that PLK1 function is essential for centrosome-mediated microtubule events and consequently for spindle assembly. Our observations implicate that ASOs and siRNAs targeted against human PLK1 may be valuable tools as antiproliferative agents that display activity against a broad spectrum of neoplastic cells. The limitation of ASOs as pharmacological agent due to the potential toxicity seems to be less likely in the case of siRNA, because efficient 'knock-down' of target genes can be achieved at very low doses.

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Antisense to a novel, hepatocyte growth factor-induced and cancer cell-specific mRNA inhibits carcinoma cell migration

J.S. Lindsey, T. Tavarez, S. Crouch. *Texas Tech University Health Science Center, Pharmaceutical Sciences, Amarillo, USA*

Hepatocyte growth factor, also known as Scatter factor (HGF/SF), is involved in tumorigenesis and metastasis and initiates cell migration by binding to and activating the tyrosine kinase, protooncogene c-Met receptor. In epithelial carcinoma cells, c-Met activation causes the breakdown of E-cadherin cell-cell contacts leading to cell spreading and cell migration. While the breakdown of E-cadherin contacts is immediate, HGF/SF-induced migration requires transcription. To test the hypothesis that this de novo mRNA synthesis includes cancer cell-specific transcripts, we performed a PCR-based subtraction hybridization using an endometrial carcinoma cell line that responds to HGF/SF with migration and invasion. After hybridization, we isolated HGF-induced transcripts, and characterized expression in cell lines as well as in normal and metastatic tissues. One HGF-induced transcript we call Mig-7 was detected in human endometrial, prostate, breast, and ovarian epithelial carcinoma cell lines as well as in metastatic tumor tissues. In contrast, we did not find Mig-7 mRNA even by RT-PCR in HGF-responsive human umbilical vascular endothelial cells (HUVECS) or in normal tissues known to express HGF/SF and c-Met such as small intestine, bone marrow, uterus, term placenta, and spleen. In addition, we found that Mig-7 specific antisense oligonucleotides inhibited HGF/SF-induced migration of RL-95 endometrial epithelial carcinoma cells by 83% *in vitro* as compared to control oligonucleotides or no oligonucleotides. These results suggest that Mig-7 expression may be used as a cancer cell-specific target to inhibit metastasis.

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PET imaging of HSV-1 amplicon vector-mediated gene expression

A.H. Jacobs[1-3], A. Winkeler[1], C. Dittmar[1], M. Hartung[1], M. Slack[1], C. Kummer[1], K. Wienhard[1], W.D. Heiss[1-3]. [1]*MPI for Neurological Research, Laboratory for Gene Therapy and Molecular Imaging, Köln, Germany;* [2]*Department of Neurology, University of Cologne, Köln, Germany;* [3]*Center for Molecular Medicine, University of Cologne, Köln, Germany*

Objective: To non-invasively assess HSV-1 amplicon vector-mediated gene expression *in vivo* by positron emission tomography (PET). Background: PET of herpes simplex virus type 1 thymidine kinase (HSV-1-tk) gene expression mediated by replication-conditional HSV-1 vectors reveals TK-expression only in viable target tissue not yet been destroyed by vector replication.
Methods: To non-invasively assess the 'total'dose of HSV-1 vector-mediated TK-expression, non-toxic helper virus-free HSV-1 amplicon vectors were constructed carrying multi-functional imaging genes. 24 hours after HSV-1 amplicon vectors (2×10^7 t.u.) had been injected into subcutaneous growing human Gli36dEGFR gliomas in nude rats (n=2) and nude mice (n=4), the levels and locations of HSV-1-TK expression were assessed after intravenous administration of 9-(4-[18F]fluoro-3-hydroxymethyl-butyl)guanine ([18F]FHBG; 300 μCi/rat; 100 μCi/mouse) using new generation ECAT HRRT (Siemens, CTI) and microPET (Concord) sanners.
Results: Various levels of HSV-1-tk gene expression ([18F]FHBG % ID/g) could be assessed by FHBG-PET mostly located around injection sites. The highest levels of TK-expression were observed for tkgfp fusion (TG17; gfp green fluorescent protein gene) and tkIRESgfp (TIG; IRES internal ribosome entry site) constructs. In comparison to stabily expressing TG17-gliomas (positive control), the levels of FHBG-accumulation were comparatively low. No significant FHBG-accumulation was observed in non-transduced gliomas.
Conclusions: These results demonstrate for the first time, that HSV-1 amplicon vector-mediated gene expression can be monitored non-invasively by PET and that subtle differences of HSV-1-TK expression can be differentiated by high-resolution PET imaging. With a therapeutic gene proportionally coexpressed with tk as PET marker gene, TK-imaging indirectly reveals the level and location of the 'total tissue dose' of any therapeutic gene expression mediated by helper virus-free HSV-1 amplicon vectors.

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CRE-enhancer DNA decoy as a tumor target-based genetic tool to treat cancer

Y. Cho-Chung[1], M. Nesterova[1], Y.S. Cho[1], C. Neary[1], R.E. Klem[2]. [1]*National Insititutes of Health, USA, National Cancer Institute;* [2]*Genta, Inc., Berkeley Heights, USA*

Enhancer DNA decoys bind transcription factors away from the enhancer, thereby inhibiting transcription *in vivo*. A 19mer decoy oligodeoxynucleotide (ODN) (G4224) containing a consensus sequence of the cAMP response element (CRE) inhibits CRE-directed transcription *in vivo* and tumor growth. Cancer cell lines of a variety of cell types, including breast, ovarian, prostate, colon, epidermoid and non-small cell lung carcinoma cells, are sensitive to growth inhibition by G4224 (IC_{50}: 100-270 nM), whereas immortalized non-cancerous cell lines are totally resistant to G4224 treatment. Control decoy ODN (G4233) has no effect on CRE-directed transcription or cancer cell growth. The functional genomics of the CRE-decoy (G4224) in cancer cell lines and in human tumors grown in nude mice are analyzed using cDNA microarrays. In tumors, but not in the livers of host animals, treatment with G4224 (5mg/kg/day for 4 days, i.p.) results in upregulation of the expression of a cluster of genes involved in development and cell differentiation. Concomitantly, another cluster of genes involved in cell proliferation and transformation are downregulated. Induction of these expression signatures by the decoy is permissive in cells with wild type CREB (CRE-binding protein), but not in cells overexpressing the CREB mutant KCREB, which no longer binds CRE. These findings indicate that CRE-directed transcription favors tumor cell growth but not normal cell growth. Thus, the CRE-decoy ODN (G4224) may serve as a tumor target-based genetic tool to treat cancer.