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Tumour specific delivery of cytostatics encapsulated in novel phospholipase A2 degradable liposomes

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Cancer treatment using traditional chemotherapeutics is often problematical due to severe side effects. These side effects could be diminished using specific tumour targeting of the drugs, thereby increasing the drug concentration in the tumour area and lowering the systemic exposure. Liposome based drug delivery has been thought to alleviate these problems, but so far no tumour specific release mechanism has been demonstrated.

We have designed a new generation of liposomes that are specifically degraded by secretory phospholipase A2 type IIA (sPLA2), which is secreted into the tumour microenvironment in a broad range of human tumours. Accumulation of the prodrug liposomes in the tumour is facilitated by the leaky tumour vasculature, known as the enhanced permeability and retention effect.

As a result of the high levels of sPLA2 in the tumour, liposomal phospholipids are cleaved at the *sn-2* position resulting in the release of encapsulated drug and production of free fatty acids and lysolipids, which can act as locally generated permeability enhancers.

We have analysed liposomes loaded with various cytostatics e.g. doxorubicin and cisplatin. The liposomes were evaluated *in vitro* for sPLA2 mediated degradation and concomitant release of the encapsulated compounds. In presence of sPLA2, cisplatin liposomes were degraded with release of cisplatin and lysolipids, acting jointly to cause a strong cytotoxic activity on the cultured cells. In contrast, cisplatin liposomes added to the cells in absence of sPLA2 showed very little cytotoxic activity towards the cells. In contrast, liposomal stealth formulations of cisplatin (known as SPI-077) did not cause cell lysis, in accordance with earlier results.

Xenograft studies with encapsulated doxorubicin and cisplatin in the sPLA2 secreting MT-3 breast cancer model, showed increased therapeutic activity at equimolar doses for both compounds, whereas parameters of toxicity of these formulations indicated similar or weaker toxicity compared to free drug.

These data strongly suggest that sPLA2 triggered tumour specific release of the encapsulated cytostatics is a promising approach for increasing the therapeutic index of current and new cytostatics.

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A new multifunctional drug delivery system based on polymalic acid to inhibit angiogenesis and invasion of human gliomas in vitro and in vivo

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Introduction. Specific drug delivery is crucial for treating tumors and reducing side effects for normal cells. Simultaneous inhibition of several molecular targets at the level of protein synthesis may be highly effective in preventing tumor growth and progression. Laminin-8 chains overexpression is associated with glioma progression, and laminin-8 blocking inhibits glioma invasion *in vitro*.

Material and Methods. A multifunctional drug delivery construct consists of modules attached to polymalic acid (PMLA) from *Physarum polycephalum*. The modules are (1) Morpholino antisense oligonucleotides (MOA), which are cleaved in the cytoplasm to release the free drug, (2) antibody to transferrin receptor (ATR) for cancer cell targeting and receptor-mediated endocytosis, (3) short chain PEG-conjugated L-leucine and directly coupled L-valine, to provide pH-dependent lipophilicity to disrupt endosomal membranes, (4) long chain PEG for protection, (5) fluorescent reporter (fluorescein or Cy5) to detect the construct in tissue/cell.

Drug 1: MOA to laminin α 4 and β 1 chain conjugated to PMLA; **Drug 2:** MOA to laminin α 4 and β 1 chain plus ATR conjugated to PMLA. **Controls** were the carrier conjugates with corresponding sense oligonucleotides. Human U-87MG glioblastoma was used for *in vitro* experiments and injected intracranially into NIHRNU-M nude homozygous rats.

Results: The functional effect of module 1 (Morpholinos) was detected as reduced immunostaining for laminin α 4 and β 1 chains; their syntheses were blocked by the antisense oligonucleotides. 2. The functional effect of module 2 (ATR) was detected by fluorescence in cell cultures via fluorescein (PMLA-vehicle) and rhodamine-labeled ATR. They were visible in endosomes and in the cytoplasm equally at different time points. The drug was not toxic in three different concentrations *in vitro* and *in vivo*.

Intracranial tumor treatment. Drug 2 concentrations of 0.5 and 2.5 mg/kg were equal for the treatment in the survival study. After intracranial administration of four doses of Drug 2, the animal survival time was increased by 30%, $p<0.0074$, compared to control groups. Drug 1 (without ATR) did not affect survival. Therefore, the mechanism of drug cell delivery is transferrin receptor-mediated endocytosis.

Conclusions. Antisense oligonucleotides to laminin-8 chains combined with a novel drug delivery vehicle, PMLA, efficiently inhibited laminin-8 expression in a xenografted intracranial human glioma in rats and increased animal survival. The ability of the drug vehicle to penetrate BBB and BTB is important for potential intravenous treatment of patients. These data hold promise for an efficient brain tumor inhibition using laminin-8 as a therapeutic target.

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KCa channel-mediated regulation of metastatic brain tumor permeability and proliferation

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Background: Brain metastases from breast and lung cancer are a significant source of mortality and morbidity. Since abnormality in receptor kinases (RTKs)/epidermal growth factors is implicated in most cancers, patients with advanced cancer are treated with RTK inhibitors like Trastuzumab (Herceptin), Gefitinib (Iressa), Gleevac and SU11248. A significant number of cancer patients who respond well to these anti-cancer agents, however, develop CNS metastasis. This is most likely due to the inability of anti-cancer agents to cross the blood-brain barrier (BBB) and blood-brain tumor barrier (BTB) to reach cancer cell in the brain in effective quantities. We discovered that calcium-activated potassium (K_{Ca}) channels regulate both BTB permeability and tumor cell proliferation in metastatic brain tumors. The critical role of K_{Ca} channels in tumor cell proliferation provides an opportunity to attenuate tumor cell growth.

Material and Methods: We investigated whether K_{Ca} channels are involved in metastatic brain cancer cell proliferation by FACS, immunohistochemistry and Western blot methods. We also tested whether K_{Ca} channel-mediated BTB opening (with K_{Ca} channel agonists) enhances delivery of RTK inhibitors and enhance survival of rodents with metastatic brain tumors. Animals with metastatic brain xenografts were intravenously administered with [^{14}C]α-aminoisobutyric acid (AIB) and contrast-enhancing agent to develop quantitative autoradiography (QAR) and dynamic contrast-enhanced (DCE) T1-weighted magnetic resonance images, respectively.

Results: K_{Ca} channels are over-expressed in metastatic brain tumor capillary endothelium and brain tumor cells. The prolonged activation of K_{Ca} channels in metastatic brain tumor cells by specific openers, NS-1619 and NS-004 induced K^+ flux, caused membrane hyper polarization, down-regulated K_{Ca} channel expression and activity causing cell death and cell-cycle arrest in the G1 and G2 phases. Normal cells, however, were not significantly affected. BBB/BTB permeability changes measured by DCE-MR imaging before, during and after BTB permeability modulation nicely co-registered with QAR images of rat brain sections. Therefore a novel transitional strategy for high-throughput screening of enhanced anti-cancer drug delivery selectively to metastatic brain tumor was developed.

Conclusion: It is anticipated that these translational experiments will provide a basis for targeting K_{Ca} channel over-expressing metastatic brain cancer cells with pharmacotherapeutic specific openers of K_{Ca} channel openers. Monitoring the outcome of increased RTK inhibitor delivery in patients with metastatic brain tumor should lead to beneficial clinical results.

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Mutant bad effectively inhibited human nsclc xenografts in nude mice

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Background: Non-small cell lung cancer (NSCLC) patients frequently present, or relapse, with unresectable disease that is resistant to standard chemotherapy. There is, therefore, an urgent need for new treatments for NSCLC. In order to explore a novel therapeutic method, we have developed orthotopic human NSCLC models and a new therapeutic approach.

Materials and Methods: The human NSCLC models were generated by orthotopically inoculating human NSCLC cell lines H358 or A549 into