nude mice. These xenografts grew multifocally in both lungs and all lobes of the lung and were resistant to intravenous Cisplatin treatment, closely mimicking the NSCLC patients who have unresectable and chemo-resistant tumors. Our therapeutic approach included an efficient nonviral gene delivery system (ENGD) that is composed of multiple cationic polymers at an optimal combination ratio, and a therapeutic gene (*badp*) that is a modified proapoptotic gene, *bad*, that carries mutatant Ser/Thr phosphorylation sites.
Results: *In vitro*, the ENGD-carried mutant *bad* (ENGD-*badp*) significantly induced apoptosis in human NSCLC cell lines H322, H358, H460, and A549. The apoptotic index of cells treated with *badp* was 2- to 6-fold higher than that of the cells treated with wild-type *bad* under the same experimental conditions. *In vivo*, intratracheal injections of ENGD-*badp* effectively inhibited the growth of H358 (%TGI = 61%) and A549 (%TGI = 78%) xenografts in nude mice. In contrast, iv Cisplatin at the maximum tolerated dose was not effective. Moreover, the combination of the ENGD-badp and Cisplatin further increased the average lifespan of the tumor-bearing mice by 60% to 210% compared with the single-agent therapeutics alone (68% vs 7% and 219% vs 7%).
Conclusions: Our studies support the hypothesis that locoregional administration of a proapoptotic gene could effectively inhibit the local chemo-resistant NSCLC tumors and sensitize them for further chemotherapy.

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Introduction of specificity into cytotoxic drugs and improvement of therapeutic index by kinase-mediated trapping

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Non-specific cytotoxic agents continue to play a major role in cancer therapy. In addition to their traditional role, they are essential partners for optimal activity of signal transduction inhibitors in most solid tumor settings and may also be useful in metronomic anti-angiogenic regimens. Despite the continued and potentially expanded use of these agents, their activity is constrained by dose-limiting side effects. Some cytotoxic drugs have been improved via the use of extracellular targeting and pro-drug approaches. However, improvements have been highly drug and disease specific, and suffer from drawbacks with respect to the efficiency of cellular uptake and drug release. We have developed broadly applicable methods for engineering selectivity into non-specific cytotoxic drugs. Our approach takes advantage of well-validated drug discovery targets, i.e. kinases that are aberrantly activated or overexpressed in tumor cells and tumor associated endothelium. Instead of making inhibitors of these cancer-causing enzymes, we have developed methods to covalently conjugate protein and small molecule kinase substrates to cytotoxic drugs. The resulting peptide and small molecule conjugates retain both drug and kinase substrate activities, are stable in serum, and are able to diffuse across cell membranes. We have proposed that selective phosphorylation of the conjugate by an elevated or aberrantly activated kinase can trap the conjugate in the disease or disease-associated cell, preventing exit by passive diffusion and increasing therapeutic index. We have produced bifunctionally active conjugates of paclitaxel and vinblastine with peptide substrates of Src tyrosine kinase and Akt serine/threonine kinase. We have also produced paclitaxel-thymidine conjugates. The conjugates retain 50 to >100% of the parent drug activity and 35 to >100% of the substrate phosphorylation potential. Furthermore, peptide and small molecule conjugates were produced that are stable in serum, exhibit cytotoxic EC50s within 5 to 10-fold of the values obtained for the parent drugs, and in the case of paclitaxel-peptide conjugates, are water soluble. The therapeutic index of each conjugate was determined by comparing cytotoxic EC50s against normal fibroblasts to those obtained with breast, lung and colon carcinoma cells, as well as normal endothelial cells. Conjugates from 4 different drug-substrate classes were obtained that exhibit a 4 to 31-fold increase in therapeutic index, relative to parent drug. Our results demonstrate that it is possible to significantly increase the cell-based therapeutic index of a non-specific cytotoxic agent by linking it to the substrate of a disease-causing kinase. This approach appears to be broadly applicable to non-specific drugs used for the treatment of cancer and many other diseases caused by chronic or undesirable activation of kinases.

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Mitochondrial-mediated apoptosis is induced by cationic polymers used in gene transfer

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A wide range of synthetic polycations in linear, branched, or dendrimer form have been used to condense DNA into structures amenable to cellular internalization via endocytosis. Polycations can destabilize endosomal membranes or act as proton sponges; they buffer the low pH in the endosomes and potentially induce membrane rupture, resulting in the release of polycation/DNA complex into the cytoplasm. The polycationic nature of the gene-delivery vehicles can induce cytotoxicity, but the mechanisms are poorly understood. Therefore, cytotoxic gene-delivery systems may compromise transcription and translation processes and potentially limit protein expression. In order to understand the molecular basis of polycation induced cytotoxicity, we studied the effect of a number of commonly used polycations on mitochondrial functions in isolated mitochondria from rat liver as well as directly in Jurkat cells. Mitochondria are key integrators of a cell's life and death decisions since they play a major role in subcellular partitioning of death-regulating biochemical signals. For example, the Bcl-2-sensitive release of proteins such as cytochrome *c* from the mitochodrial intermembrane space into the cytoplasm is a critical early event in apoptosis. Upon permeabilization or rupture of the outer mitochondrial membrane, cytochrome *c* binds to Apaf-1, leading to allosteric activation of pro-caspase-9. This in turn proteolytically activates caspase-3, one of the principal proteases that participates in the execution of cell death. A decrease in mitochondrial membrane potential ($\Delta\varphi$) due to permeability transition is also an early event in several types of apoptosis. We have demonstrated that at very low concentrations, polycations can affect mitochondrial respiration and $\Delta\varphi$; these events were followed by cytochrome *c* release from mitochondrial intermembrane in mitochondrial suspensions and in Jurkat cells. Changes in mitochondrial $\Delta\varphi$ in Jurkat cells was confirmed by Mitosensor test. Detection of phosphatidylserine translocation to the cell surface using Annexin V, and activated caspase-3 further confirmed the initiation of a mitochondrion-mediated apoptotic programme in Jurkat cells. These observations provide a molecular explanation for the previously reported immediate or delayed cytotoxicity following gene transfer with polycations. The results from this study may help to design novel materials with high transfection efficiencies suitable for clinical gene therapy.

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Plasma and tissue distribution of selenium after 5-methylselenocysteine (MSC) or seleno-L-methionine (SLM) in mice bearing human tumor xenografts

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Background: We previously reported that MSC and SLM, organic selenium compounds, increase the cure rates of human squamous cell carcinoma of the head and neck xenografts (HNSCC), FaDu and A253, in mice when combined with irinotecan. FaDu xenografts were more responsive to MSC/irinotecan or SLM/irinotecan combination (100% cure rate) than A253 xenografts (60% cure rate). MSC and SLM also protect the animals from irinotecan induced toxicities and lethalities (Cao et al., Clin. Cancer Res., *10*:2561–2569, 2004). To help understand the selectivity of selenium action and its protective effects, we initiated this plasma and tissue distribution study for selenium after MSC and SLM.
Material and Methods: Nude mice bearing bilaterally established (200–250 mg) FaDu and A253 tumors were treated daily with oral MSC at different doses (0.005, 0.01, 0.05, 0.1, 0.2 mg/mouse/d $\times$ 7) or SLM at (0.01, 0.1, and 0.2mg/mouse/d $\times$ 7). Plasma, tumor tissue, and normal tissues (liver, kidney, small intestine, large intestine, and bone marrow) samples were collected at 2h post last dose. Samples were analyzed for selenium concentration using Atomic Absorption Spectrophotometry.
Results: The data show that the base level of total selenium in the plasma of untreated mice is 4.5$\pm$0.5 μM. This level increased to 14.2$\pm$5.1 μM, 23.21$\pm$7.0 μM, and 47.7$\pm$2.1 μM at 2h post SLM administration of 0.01 (the minimal dose for modulation effect), 0.1, and 0.2 mg/mouse/d $\times$ 7 respectively. 94–96% of selenium in plasma is protein bound. The concentration of total plasma selenium increased post administration of MSC (same doses above) to 5.1$\pm$0.56 μM, 9.9$\pm$0.7 μM, and 12.8$\pm$1.6 μM respectively, with 12–21% of total selenium in free form.