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SELDI-TOF Proteomic patterns of human and experimental brain tumors: potential for new biomarkers and for pharmaco-proteomic endpoints in anti-angiogenic therapy

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In the postgenome era, proteomics provides a powerful approach for the analysis of neoplastic tissues, and for uncovering novel endpoints for the evaluation of drug efficacy and toxicity. As an alternative to the 2-dimensional gel electrophoresis, a new technique was employed to generate protein expression patterns from whole tissue extracts. Surface-enhanced laser desorption/ionization (SELDI) allows the retention of proteins on a solid-phase chromatographic surface (ProteinChip Array) with direct detection of retained proteins by the highly sensitive time of flight-mass spectrometry (TOF-MS). Using this system, we conducted a study on human brain tumor samples including gliomas and meningioma. We present data showing that SELDI analysis is rapid, reproducible, and capable of identifying protein signatures that appear to differentiate the different histological types with specific pattern characteristics of specific clinical outcomes. We also investigated the potential of this technique for pharmaco-proteomic study of new therapeutical approaches in preclinical model of glioblastoma. The effect of the antiangiogenic agent Neovastat on serum protein patterns was thus investigated. Neovastat is a complex mixture of naturally occurring antiangiogenic agent exhibiting multifunctional mechanisms of action. It is currently in phase III study in patients with kidney and lung carcinoma and phase II in patient with refractory multiple myeloma. Preclinical efficacy of Neovastat has been documented in glioblastoma models. We observed that Neovastat induces specific modification of tissues and serum protein patterns thus indicating that with this fast and powerful ProteinChip array technology, it becomes possible to investigate complex changes at the protein level in cancer associated with tumor progression. This pharmaco-proteomic technique provides potential endpoint that can be related to specific approaches such as the use of antiangiogenic agents in preclinical and human trials.

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Halofuginone activity in relation to collagen type I, VEGF and MMP-2 expression in human tumor cell lines and xenografts *in vitro* and *in vivo*

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Halofuginone (HF) is a low molecular weight quinazolinone alkaloid which inhibits insult induced excessive transcription of collagen type I *in vitro* and *in vivo*. It has further been shown that HF can reduce metastasis formation and angiogenesis related to its effects on homeostasis of stromal proteins such as matrix metalloprotease 2 (MMP-2). Halofuginone is currently being investigated in phase I clinical trials in collaboration with the EORTC. This study was designed to correlate drug response to expression of possible surrogate markers such as collagen type I and MMP-2 in tumor cells. Collagen type I, VEGF and MMP-2 protein levels were determined in 60 human tumor xenografts and cell lines by ELISA assay of lysates and by immunohistochemistry (IHC) on tissue microarrays. Six cell lines with high and low target expression were selected for *in vitro* evaluation. HF was potently active in cells with a mean IC_{50} of 56 nM, the most sensitive line was the MEXF 462NL melanoma (IC_{50} = 2 nM), followed by the renal cancer RXF 944L (IC_{50} = 5 nM). More responsive cells showed either high VEGF and high collagen I expression or high collagen I and MMP-2 levels. More resistant models had only one elevated target protein. *In vivo* activity of HF was evaluated in the orthotopically (o.t.) growing renal cancer xenograft RXF 944LX, the soft tissue sarcoma SXF 463 and the melanoma MEXF 276 in nude mice. HF was administered i.p. at 2.5 μg/mouse daily for 3 weeks. This dose and schedule had been determined as optimal. Treatment was initiated 3 days after transplantation. Tumor growth was followed by serial caliper measurements over 2 months. Significant tumor growth inhibition was seen with T/Cs of 36 % in SXF 463 and 33 % in MEXF 276 xenografts. Animals bearing o.t. RXF 944LX were sacrificed 14 d after initiation of treatment and tumors, kidneys, lungs and livers were examined macroscopically and microscopically. HF produced a 69% reduction of primary kidney tumor growth and prevented metastasis to liver and lung. However, HF treated RXF 944LX mice had large abdominal tumor masses. Whilst IHC of control and treated RXF 944LX tissue revealed little effects on VEGF or MMP-2 expression, collagen I was markedly down-regulated by HF ($p < 0.004$). The latter might explain the inhibited invasion and adhesion of renal tumor cells after injection in the kidney. Our data suggest that collagen type I might be a useful surrogate marker for patient selection in clinical trials.

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Antiangiogenic and antitumoral activity of novel heparin derivatives

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We have synthesized novel heparin derivatives with the aim to potentiate the antiangiogenic/antitumor properties and to abolish the anticoagulant effects of the original heparin. Among several compounds tested *in vitro*, ST1514 and its low molecular weight derivative ST2184, were selected to be studied *in vivo*. In the mouse model of B16/BL6 melanoma experimental lung metastases, treatment with ST1514 and ST2184 (25mg/kg coinjected i.v. with 1.5x104 cells/mouse) decreased of 63% ($p<0.01$) and 60% ($p<0.05$) the number of lung colonies, respectively. In the same animal model, subcutaneous treatment with ST2184 (100mg/kg) gave 61% and 46% metastasis inhibition, when administered within 30 min or 1 h before cell inoculation, respectively ($p<0.01$ at all time points). Moreover, all animals treated with ST2184 at 100mg/kg survived and did not show side effects. In contrast, animals treated with the same dose of heparin had pronounced bleeding and edema formation at the injection site and 25% died soon after treatment. In addition, the antitumor/antiangiogenic activity of ST 2184 was tested on a human tumor xenograft model. MeWo human melanoma cells stably expressing enhanced green fluorescent protein (EGFP) were injected intradermally in nude mice and treatment with ST2184 started 3 days later. In a first set of experiments, tumor-induced angiogenesis assessed at day 15 in the skin around the tumors, was significantly reduced ($p<0.01$) in animals treated with ST2184 (25mg/kg s.c twice daily for 10 days). In another set of experiments, tumor growth, assessed by imaging tumor cell fluorescence, was significantly decreased (53% of inhibition vs vehicle; $p<0.05$) starting from day 24 of treatment with ST2184 (50mg/kg kg s.c twice daily). In the same experiment, the administration of ST2184 (25mg/kg or 50mg/kg s.c twice daily) in combination with the camptothecin derivative ST1481 (0.25mg/kg per os qdx6/wx2w) caused further significant reduction of tumor volume compared to ST1481 alone starting from day 9 of treatment. We conclude that ST1514 and ST2184 have powerful antiangiogenic, antimetastatic and antitumor activity and could be used for the treatment of angiogenesis-related diseases. Their use in combination with chemotherapy is recommended.

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Phase I trial of recombinant human endostatin (rHu-endo) administered by continuous infusion (CI) intravenously (IV) in patients with solid tumors: a preliminary report

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Endostatin is a 20-Kb protein that has been shown in preclinical models to inhibit tumor growth. Phase I trials of rHu-Endo administered by daily short infusions did not show any significant toxicities while suggesting a biological effect. Preclinical data suggests that a continuous exposure to rHu-Endo enhanced its efficacy. A dose escalation phase I trial was conducted to determine the pharmacokinetics, toxicities, and assessment of tumor blood flow and glucose metabolism. To improve our ability to correlate dose with pharmacokinetic (PK) and biological endpoints, six patients were entered onto each dose level. Planned dose levels were 30, 60, 120, 180 and 300 mg/m^2/d of rHu-Endo IV administered by CI. To date, 18 (M=10/8=F) patients have been enrolled onto the first 3 dose levels. Median age=56.5 yrs; Median PS=1; Tumor types included: melanoma(4), lung cancer(5), hemangiopericytoma(2), sarcoma(2), and others(5); Median number of prior therapies=2. Nine patients also received XRT prior to study entry. To date, no significant drug-related toxic effects have been observed. No response have been observed to date, but 11 patients had stable disease >2 months. Preliminary PK parameter estimates are shown in the table below. With the first 3 dose levels completed, the estimated total clearance of rHu-Endo administered by CI in this trial was approximately 1/3 of that estimated when rHu-Endo was administered by short infusion. This decrease in rHu-Endo