hypermethylation of tumour suppressor genes, is a hallmark of cancer. Information about which methylation events are disease specific has a great potential in diagnostics and drug development. The aim of this study was to investigate the methylation status of the tumour suppressor genes SHP1, SOCS1 and STAT1, their counterparts SOCS3 and SHP2, as well as the drug resistance gene MGMT, and their effect on protein expression and cytotoxic drug sensitivity in glioblastoma cell lines. A further aim was to investigate the possibility to increase cytotoxic drug sensitivity in the glioblastoma lines by demethylation treatment.
Methods: To study methylation patterns, bisulfite treatment of total DNA followed by PCR amplification and Pyrosequencing® analysis was employed. Protein expression of total lysates was evaluated by Western blot analysis. Cytotoxic drug sensitivity was analysed by the fluorometric microculture cytotoxicity assay. Demethylation was obtained by treatment with the drug decitabine. Six glioblastoma cell lines were used in the studies.
Results: MGMT, SHP1 and SOCS1 were methylated at varying levels in the analyzed gene regions, whereas SHP2, SOCS3 and STAT1 were not methylated. The observed methylation levels in MGMT and SHP1 were associated with a reduction of protein expression. In addition, a low degree of methylation and a high protein level of MGMT were related to a decreased sensitivity to the cytotoxic drugs 5-fluorouracil, 17-AAG, bortezomib, and picropodophyllin. Finally, it was possible to increase the sensitivity in the glioblastoma cells lines to several cytotoxic drugs by demethylation treatment with the drug decitabine.
Conclusions: Epigenetic regulation of MGMT and SHP1 appear to affect tumor phenotype in glioblastoma. The correlation between a low degree of methylation of MGMT and SHP1, a high protein level and low sensitivity to several cytotoxic drugs constitutes a potential predictive marker for chemotherapy of glioblastoma. Finally, the possibility to increase cytotoxic drug sensitivity by demethylation treatment points to novel therapeutic strategies in combination drug therapy of glioblastoma.

Natural products and marine compounds

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Role of ERK activation in triptolide-induced apoptosis in MDA-MB-231 human breast cancer cells

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Background: Triptolide (PG490), a compound isolated from *Trypterygium wilfordii*, has been shown to have potent activity in a variety of xenograft tumor models. However, very little is known about the molecular mechanism by which triptolide acts in cancer cells. Therefore, the aim of this study was to investigate the role of extracellular signal-regulated protein kinase (ERK), a member of the mitogen-activated protein kinase family, in triptolide-induced cell death using the human breast cancer cell line MDA-MB-231.
Materials and Methods: MTT assay was used to determine cell viability upon treatment with 0−40 ng/mL triptolide. Apoptosis was assessed by annexin-V/7AAD staining, and caspase 3/7 activity was measured by a fluorescence-based assay kit. To assess the involvement of ERK and caspases, phosphorylated ERK and cleaved PARP were probed by western blot, respectively, as well as by the use of a MEK inhibitor, U0126, and the pan-caspase inhibitor, Z-VAD-FMK. Expression of phosphorylated eIF2α was determined by western blot.
Results: Dose-dependent reduction in MDA-MB-231 cell viability was observed upon a 72-hour exposure to triptolide, with an IC50 value of 1.9 ng/mL. A 3.2-fold increase in annexin-V+/7AAD− cells was observed when cells were treated with 4 ng/mL triptolide for 48 hours, indicating induction of apoptosis. Triptolide-induced apoptosis was caspase-dependent, as supported by significant increases in caspase 3/7 activity, PARP cleavage and cell viability in the presence of caspase inhibitor Z-VAD-FMK. ERK was activated as early as 2 hour post triptolide treatment, and remained activated for 48 hours. eIF2α was also activated in a time-dependent manner in triptolide-treated cells. The concomitant use of MEK inhibitor, U0126, attenuated triptolide-induced caspase 3/7 activation, PARP cleavage, and significantly increased cell viability from 49% to 98%, indicating that ERK activation acts upstream of caspase activation.
Conclusion: Our data demonstrated for the first time that ERK activation played an important role in triptolide-induced apoptosis, in contrast to the general view that ERK activation contributes to cancer cell survival and proliferation. Furthermore, the sustained activation of ERK, together with eIF2α activation, suggested a possible link of triptolide-induced apoptosis to endoplasmic reticulum stress which warrants further characterization.

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Outcome of three Phase I trials of the marine compound ES-285 (3 hour infusion) in patients with refractory solid tumors

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Background: ES-285 is a marine compound originating from the mollusc *Spisula polynyma*, an edible clam, which is also known as the Stimpson or Atlantic surf clam. The drug has cytotoxic properties by disrupting actin fibers and interacting with the ceramide pathway. The agent has a broad antitumor spectrum in vitro, in vivo and in xenograft models. ES-285 was subject to 4 parallel Phase I studies in patients (pts) with solid tumors, three of them reported here, all of them using 3-hour infusions of the compound.
Material and Methods: Pts had advanced malignancies, good performance status (ECOG PS 0−2) and adequate organ function. The following intravenous schedules of ES-285 were tested: (A) 3 h d1 qwk, (B 3 h d1−5 q3wk, and (C) 3 h d1 q3wk.
Results: The dose of ES-285 per administration was ranging from 2–256 mg/m^2, depending on the study. 117 pts were entered (25−61 per trial), their median age was ranging from 52−59 yrs per trial, and there was a male predominance. The most common tumor types were colorectal, renal and prostate cancer and melanoma. Pts received a median of 2 cycles of treatment in all studies, ranging from 1−18 per patient. Less than 15% of treatment cycles were delayed. More than 80% of pts went off study due to disease progression (83−88%). Only 8.0–13.4% of patients discontinued due to toxicity. The most common clinical adverse events were nausea, vomiting, asthenia, pyrexia (all schedules) and injection site reactions. Anemia, lymphocytopenia and increases of serum liver enzymes were frequently seen, independent of treatment scheme. Ten dose-limiting events were observed, mainly consisting of grade 3/4 (CTC version 2.0) reversible increases in serum ALT, AST and reversible neurotoxicity. Only in schedule C the maximum tolerated dose (200 mg/m^2) and recommended dose (160 mg/m^2) could be established. Among 117 pts, one melanoma patient had a non-confirmed partial response (RECIST) and 29 pts had disease stabilization as best response.
Conclusions: After thorough review of the risk/benefit outcomes of the Phase I program the clinical studies with ES-285 were discontinued.

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Antiproliferative effects of fluoro-chalcone derivatives in human melanoma A375 cells and peripheral blood mononuclear cells

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Background: At present, no treatment options are available for patients with advanced melanoma providing either sufficient response rates or a significant prolongation of overall survival. Chalcones are included in fruits and vegetables, and are suggested to be cancer-preventive. In this study, we reported the effects of synthetic chalcone derivatives on proliferation of human melanoma cells and peripheral blood mononuclear cells (PBMCs).
Material and Methods: Twelve synthetic derivatives of methoxy-and/or fluoro-chalcones were included in this study. To measure the effect of chalcone derivatives on cells of a human melanoma A375 cell line, we used the 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay procedures. Effects of chalcones on the proliferation of PBMCs in response to a T cell mitogen concanavalin A was assessed by [^{3}H] thymidine incorporation. PBMCs were isolated from seven healthy subjects. Cell cycle and apoptosis was detected by TUNEL assay and PI staining of the cells, using flow cytometric analysis.
Results: Four out of the 12 chalcone derivatives: 4-trifluoromethyl-4′-methoxychalcone (CH-1), 4-trifluoromethyl-2′-methoxychalcone (CH-3), 3-trifluoromethyl-2′, 4′-dimethoxychalcone (CH-4) and 3-trifluoromethyl-4′-methoxychalcone (CH-7) exhibited the strongest antiproliferative effects on the melanoma cells with IC_{50} values of 9.6, 5.7, 5.8 and 7.2 μM, respectively. Then, we studied the effects of CH-1, CH-3 and CH-4 on apoptosis and cell cycle of A375 cells. 10 μM CH-3 induced apoptosis in 0.15, 15.3 and 54.05% of A375 cells at 24, 48, and 72 hr of culture, respectively. Percent of G2/M phase cells in control wells was 31.2, whereas CH-3and CH-4 caused accumulation of cells in the G2/M phase to be 70.1% and 90.55%, respectively. On the other hand, CH-1 reduced the G1 phase cells, as