effects of inhibition of its production by direct inhibition of ChoK in normal and tumoral proliferation. Methods: Cell culture assays were performed in 4 non solid tumoral cell lines, K562 (Human erythroleukemia), IM-9 (Human multiple myeloma), Jurkat (Human lymphoma), U-937 (Human histocytic lymphoma), in comparison with a non solid primary cell line (Human lymphocytes). On the other hand, we have compared 2 solid tumoral cell lines, HT-29 (Human adenocarcinoma of colon grade II) and Hela (Human Epitheloid cervix carcinoma) in contrast to 2 solid primary cell lines, CCD986 sk (Human skin fibroblast) and IMR-90 (Human lung fibroblast). We have also compared NIH with LP8-3 (NIH transformed by H-ras). Flow-cytometry (apoptosis and cycle analysis), radiolabeling (synthesis of DNA, RNA and lipids metabolites), crystal violet (sensibility and recovery from treatment) and westernblotting (expression and phosphorylation analysis) were used. ChoK inhibitors developed by our group were used to inhibit PCho production. Results: A differential effect by ChoK inhibitors among non tumoral and tumoral cell lines is reported. All tumoral cell lines tested were very sensitive to the antiproliferative effect of these drugs, and were promoted to apoptosis. By contrast, under similar conditions, non tumoral cell lines were arrested but recovered normal proliferation rates after withdrawal of the drug. These results imply the absence of an unespecific toxic effect derived from ChoK inhibition that is corroborated by the observed bypass of arrest when the cultured medium is saturated with growth factors. Furthermore, no alteration in mitogenic signalling pathways like MAPK, PI-3K or lipid stress was observed. Conclusions: Phosphocholine production is required for normal cellular progression in normal human cells. Inhibition of PCho production may be a new element for the development of a strategy against abnormal cell proliferation of human tumors.

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Significant experimental decrease of the hepatocarcinoma (HPC) incidence in C3H/SY mice after administration of EB1089, a vitamin D analogue

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EB1089, a Vitamin D analogue, without the acute side effects of the original Vitamin, exerts strong antiproliferative activities in malignant cells, including hepatocytes, in vitro and in experimental HPCs in animals, as well. It also induces cell cycle arrest and apoptosis, a fact suggesting its application in chemopreventive trials.

We examined the possible chemopreventive effect of EB1089 on the incidence of HPCs in C3H/Sy virgin female mice, a strain developing 58% incidence of spontaneous HPCs. A total of 95 mice, 4 months old, were used. EB1089 injections of 0.5 g/ml/kg of BW were given i.p. every other day for 2, 4 and 6 months to 18, 19 and 14 mice respectively. The rest 44 mice were divided into three control groups accordingly and injected with the vehicle solution. The mice which developed disease were sacrificed just before they died. The rest of the mice were sacrificed at the age of 18th months. A full autopsy was performed and liver tissue was processed for histological examination. The results obtained are shown

Treatment Period (months)	Experimental groups		Control groups	
	HPC mice/Total mice	%	HPC mice/Total mice	%
2	2/18	11.1	7/18	38.9
4	0/19	0	7/18	38.9
6	0/14	0	2/8	12.5
Total	2/51	3.9*	16/44	36.4*

*P<0.0001 vs. control for all groups

Our results show that the chemopreventive administration of EB1089 causes a very statistically significant inhibitory effect on the incidence of hepatocellular carcinomas on C3H/Sy. These data suggest a potential application of EB1089 in the chemopreventive control of hepatocarcinomas.

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Inhibition of growth of human breast cancer cell lines with the combination of zoledronic acid and a COX-2 inhibitor

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Purpose: Cyclo-oxygenase (COX) is prostaglandin H synthase which is the principal enzyme mediating the formation of prostanoids (a collective term for prostacyclins, prostaglandins and thromboxanes). COX-2 is up-regulated in a high percentage of common human cancers and is associated with invasive and metastatic tumor behavior. COX-2 inhibitors suppress colon cancer growth in vitro by inducing apoptosis. Zoledronic acid, a new generation bisphosphonate used in the treatment of breast cancer-induced bone disease, significantly reduces cell number and induces apoptosis in human breast cancer cells. The purpose of this study was to assess the effect of combining a COX-2 inhibitor with zoledronic acid on breast cancer cell growth.

Methods: The effect of combining the COX-2 inhibitor (SC236) and zoledronic acid compared to either agent alone was tested in a HER-2/neu transfected human breast cancer cell line (MCF/18) and the control vector transfected line (MCF/neo). Cell number was determined after a 3 day incubation using the MTT tetrazolium dye assay.

Results: Treatment of the HER-2/neu transfected MCF/18 and control MCF/neo cell lines with the SC236 COX-2 inhibitor (1-10 uM) resulted in dose-dependent growth inhibition (15-41% inhibition and 18-53% inhibition, respectively). Treatment with zoledronic acid (1-10 uM) also gave dose-dependent growth inhibition. The HER-2/neu overexpressing MCF/18 cells, however, were less sensitive to zoledronic acid (11-56% inhibition) than the MCF/neo cells (16-70% inhibition). The combination of zoledronic acid (5 uM) and SC236 (5 uM) appeared to have an enhanced inhibitory effect on the MCF/neo cells and a synergistic effect on the MCF/18 cells.

Conclusion: The bisphosphonate, zoledronic acid, gave dose-dependent growth inhibition in both a HER-2/neu transfected human breast cancer cell line (MCF/18) and a control vector transfected line (MCF/neo). The MCF/18 line, however, was less sensitive to zoledronic acid. The combination of zoledronic acid with the SC236 COX-2 inhibitor gave an enhanced inhibitory effect on the control MCF/neo breast cancer cells and a synergistic effect on the HER-2/neu transfected MCF/18 cells compared to either agent alone.

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Basic HGF-like peptides have anti-angiogenic and anti-metastatic effects

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Purpose: The majority of cytokines, responsible for auto- or paracrine regulation of normal and transformed cells are characterized by heparin-binding properties. We have postulated, that the peptide domain(s) of the heparin-binding cytokine(s) might have biological activity which theoretically could be exploited for modulation of the biological behavior of cancer cells. Furthermore, the major angiogenic factors are also heparin-binding proteins.

Methods: We have used HGF as model heparin-binding cytokine and synthesized two HGF b-chain domains, HHRGK (HGP1) and RYRNKH (HGP2) as well as four scrambled variants. As target cells, we have used three cancer cell lines (HT25 human colonic carcinoma, M1/9 human melanoma and 3LL-HH murine lung carcinoma), all characterized by high liver metastatic potentials, as well as normal (HBE) and transformed (KS-IMM) human endothelial cells. For liver metastasis assay, we used SCID mice and intraspenic injection of tumor cells while chicken CAM assay served as angiogenesis model.

Results: All the basic penta- or hexapeptides exhibited similar antiproliferative effects in vitro on cancer cells in a dose range of 0.1-1 mg/ml. None of the HGP peptide exhibited significant antitumoral effect on the primary tumors in form of systemic treatment but HGP1, but not HGP2, had inhibitory effect on liver metastatisation of all the tumor lines studied. Furthermore, one out of the four scrambled hexapeptides, BP4 (KRKRKR), had similar activity. Interestingly, HGP1,2 and BP4 all inhibited the growth of normal human endothelial cells in vitro and angiogenesis in vivo in the chicken CAM assay. Local treatment of HT25 human colon carcinoma in SCID mice with HGP1 resulted in significant inhibition of tumor growth and maturation of intratumoral vessels.

Conclusion: We suggest the small basic penta-hexapeptides as a new class of biological response modifiers which can modulate both the metastatic properties of cancer cells and angiogenesis.

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Pattern of combine activity of 8-CL-cAMP and paclitaxel on the growth of murine melanoma in vitro and in vivo

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Purpose: 8-Cl-cAMP (ICN Pharmaceuticals) is site-selective cyclic AMP analogue that specifically down-regulates type I protein kinase A, involved in cell proliferation and neoplastic transformation. Paclitaxel (P) promotes microtubule assembly and stabilizes the tubulin polymers. Modern chemotherapeutic strategies are based on the combination of substances having different targets within malignant cells. Safety profile of 8-Cl-cAMP is not anticipated to have myeloablative effects, what makes it an ideal candidate for combination therapy with traditional cytotoxic treatment.

Material and methods: B16 cells were seeded in 96 well plates using standard RPMI 1640 medium supplemented with 10% fresh FBS. Cells were left for 24 hours to settle down, when were treated with 8-Cl-cAMP (1, 3 and 10 microM) or P (3, 10 and 30 nM) alone, and both in coincubation for 48, 72 and 96 hours of incubation period. Results were evaluated by SRB assay and expressed as a percent of growth inhibition. In vivo growth inhibition of B16 tumors in C57Black mice was evaluated using 65 and 100 mg/kg of 8-Cl-cAMP i.p., d 1- 14 and P 20 mg/kg, d 1-5, alone and in combination.The antitumour activity was examened by measuring tumor volume on days 5, 8, 11 and 14 and determining life span for each group. The analysis of combination treatments was made using the isobole method (combination index D for in vitro and interaction index I.I. for in vivo).

Results: We demonstrated that 8-Cl-cAMP, in a time- and dose dependent manner, inhibited growth of murine melanoma B16 in vitro, with IC50 value (7.6 microM) obtained after 72 hours incubation period. We further determined the IC50 values of P on B16 cell line (26, 7.83, 6.87 nM for 48, 72 and 96 hours respectively). The D values for low dose P (3 nM) showed no synergistic activity while at higher P concentrations (10 and 30 nM) showed mostly synergistic relations. Analysis of combination treatment on tumor growth in vivo, confirmed synergy between these two compounds.

Conclusion: Results indicate synergy between 8-Cl-cAMP and paclitaxel on murine melanoma growth in vitro and in vivo.

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Modulation of phospholipase d by hexadecylphosphorylcholine: a putative mechanism for its antitumoral activity

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Purpose: Hexadecylphosphorylcholine (HePC) belongs to the new family of alkylphosphocholines with anticancer activity. Its mode of action could be mediated by interference at the level of generation of lipid-derived second messengers or to the inhibition of the corresponding regulated enzymes. We have investigated the effect of HePC on two enzymes recently reported to play a role in cell growth proliferation such as phospholipase D (PLD) and choline kinase (ChoK).

Methods: Assays of ChoK and PLD activity were used. Analysis of protein levels were carried out by Western-blot of Hek 293T cells extracts trasiently transfested with different isoforms of PLD (PLD1 and PLD2).

Results: Treatment with HePC induces a rapid stimulation of PLD. Depending on the cell line investigated, activation of PLD by HePC may be achieved by PKC- dependent or independent mechanisms. PLD1 and PLD2 isoenzymes are sensitive to HePC activation. Furthermore, a chronic exposure of the cells to HePC abrogates the response of PLD to stimulation by either phorbol esters or HePC itself with no effect on total cellular PLD levels. By contrast, no effect was observed by HePC on choline kinase (ChoK), a new target for anticancer drug development.

Conclusion: Many evidences support a role of PLD in signal transduction pathways controlling mitogenesis. The net balance between short activation and desensitisation induced by chronic treatment with HePC on PLD can be important for the final response of the different cells to membrane-active agents. This novel interpretation may be useful for a better understanding of the mechanisms of action of this family of anticancer drugs. Thus, the observed effects on PLD regulation by HePC may be related to its antiproliferative action.

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Growth inhibition of mouse autochthonous skin cancer by oral administration of new serine protease inhibitor ONO-3403

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Purpose: The purpose of this experiment is to present experimental evidence that oral administration of new serine protease inhibitor ONO-3403 is effective inhibiting the development of 3-methylcholanthrene-induced autochthonous skin cancer in mice.

Methods: The experiment was started when the tumors reached a size of 5 mm in diameter. Animals were divided into 2 groups. ONO-3403 was dissolved in sterile distilled water and 6 tumor bearing mice were given 3 times daily at a dosage 10 mg/kg with stomach tube in a 1-ml of volume of ONO-3403 for 9 weeks. While 5 mice of control group were administered saline alone same times daily.

Results: The Oral administration of ONO-3403 inhibited significantly the growth of autochthonous mouse skin cancer ($p < 0.001$) and also prolonged survival time of tumor bearing mice ($p < 0.01$).

Conclusion: Recently we have reported that orally active serine protease inhibitor ONO-3403 obtained by injection inhibited the mouse skin cancer. In the present study, the antitumor effect of oral administration of this material in the same experimental tumor system was confirmed.

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Enzyme inhibitor derivative of targeted nonbiodegradable GnRH analogue conjugate with high anticancer selectivity

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Purpose: To improve the therapeutic efficacy and tumour selectivity target specific gonadotropin hormone releasing analogue (GnRH-III) with selective anti-cancer activity and its antimetabolite enzyme inhibitor derivative were coupled to a carrier molecule poly(N-vinyl pyrrolidone-co-maleic acid).

Methods: The target specificity of the conjugates was determined by investigating their specific binding affinity and receptor-mediated internalization using radionuclide labelled compounds. Effects of conjugates on mitotic signal and cell cycle progression were also studied. The antiproliferative and antitumour activity of the compounds were tested in vitro on GnRH receptor-positive MCF-7 and MDA-MB-231 cell lines as well as in vivo on immunosuppressed MDA-MB-231 xenograft bearing mice (i.p. treated daily).

Results: Conjugation significantly enhanced the stability and receptor-mediated internalization of hormone-receptor complexes. Conjugates exerted retarding effect on the cell division cycle at G2 phase suggesting an inhibitory action in the premitotic stage. As a result of increased stability of GnRH-III the in vivo therapeutic efficacy of the conjugates versus unbound peptide hormone was significantly enhanced. By the end of the 7th week of i.p. administration with GnRH-III conjugate as well as its enzyme inhibitor derivative MDA-MB-231 tumour mass was decreased by 45% and 75% in comparison to the age-matched control.

Conclusion: Our results confirm the role of a properly selected carrier molecule in enhancement of tumour selectivity and anti-cancer activity of covalently bound target specific peptide hormone, or antimetabolic enzyme inhibitor. GnRH analogue conjugates offer new possibilities in the complex therapy of GnRH receptor-positive breast cancer.

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