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

D. Sahpazidou[1], T. Toliou[1], P. Stravoravdi[1], G. Geromichalos[1], K. Natsis[2], H. Nenopoulou[2], J. Boukovinas[1], P. Papakotoulas[1], P. Gigis[2]. [1] *Theagenio Cancer Hospital, Thessaloniki, Greece;* [2] *Aristotelian University of Thessaloniki, Thessaloniki, Greece*

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

L. Witters[1], M. Javeed[1], J. Crispino[1], J. Green[2], J. Seaman[2], D. Reitsma[2], T. Kamal[2], L. Engle[3], V. Chinchilli[3], A. Lipton[1]. [1] *Penn State College of Medicine, Medicine, Hershey, PA, USA;* [2] *Novartis Pharmaceuticals, East Hanover, NJ, USA;* [3] *Penn State College of Medicine, Biostatistics and Epidemiology, Hershey, PA, USA*

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

J. Timar, K. Fazekas, E. Raso. *National Institute of Oncology, Department of Tumor Progression, Budapest, Hungary*

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.