tumor cells. 2. Stability of chelating agents chosen complex with different metals was proven.

3. Stability of chelating agents complex with mini-antibody created was also proven (in-vitro). Hybrid nanoparticles designed are being evaluated by in-vivo biodistribution studies in animal (rats) models.

Conclusions: Tumor-targeted nanoparticles with conjugated specific antitumor antibodies are promising tools for the reduction of malignant tumors. Our results form basics for creation of new targeted radiopharmaceuticals.

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Dolichyl phosphate dependent P-glycoprotein expression in Doxorubicin resistant MCF-7 breast cancer cells

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Introduction: Multidrug resistance (MDR) in breast cancer cells during chemotherapeutic course restricts the possibilities of Doxorubicin (Dox) application. The investigations reveals that MDR correlates accumulation of P-glycoprotein (Pgp) in plasma membrane. The resent results are in favour of the idea that glycoprotein synthesis in malignant tissues is limited by Dolichyl Phosphate (DolP). The aim of the present study is to investigate the effect of polyprenol (PP) which provides a DolP substitute in regulation of N-glycosylation on MCF-7 breast cancer MDR.

Experimental procedures: Breast cancer cell lines, MCF-7 and MCF-7 cells with induced resistance to Doxorubicin (MCF-7/ADR) were used. Pol concentration in the culture medium made up 10-2 -10-6 Pgp expression was detected with monoclonal antibodies using flow cytometry and immunohistochemistry. Intermediates of DPC fractions were analysed by HPLC method. Dolichyl phosphate N-acetylglucosamine-1-phosphate transferase (GPT) due to DPAGT1 polymorphism was assessed in T-cells.

Results: Pol in concentration 10-2 -10-3 M induced apoptosis in MCF-7/ADR cells within 3-4 hours. It is confirmed that plasmatic membrans of MCF-7 cells contain 5,6 - 6,4% of Pgp (the total protein amount) as a resistance marker. Resistant MCF-7/ADR cells differ from sensitive ones MCF-7 in Pgp content by 10-12 times. The study showed 8,5-fold DPC intermediates decrease in MCF-7/ADR cells. The investigations demonstrate that the situation can be changed by treatment with Pol. The DPC concentration in MCF-7/ADR cells was returned to the normal level. It is established that Pol in the concentration 10-4 M aid 7-9-fold reducing Pgp in membranes of MCF-7/ADR cells. The MCF-7/ADR cells cultivation in medium with Pol proceeded to give lowered Pgp content in membranes no over 0,4-0,6 %, which amount was consistent with the level of Pgp in MCF-7 cells.

Conclusions: These results indicate that noncontrollable accumulation of Pgp, which cause MDR can be overcomed using stimulation of DPC with Pol, which provides a DPC substitute in regulation of Pgp. Pol is a promising new agent which clinical usage can open up possibilities to tackling the problem of Dox resistance in breast cancer chemotherapy. It is, also, a hypothesis, which has suggested that there is a genetic polymorphism of DPAGT1 in MCF-7 breast cancer that could mediate Pgp expression and blunt the response to Dox.

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MAPK/ERK signaling mediates melatonin-induced neuroendocrine differentiation in prostate cancer cells

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Prostate cancer has become one of the most frequently observed tumours among men and a major cause of death in Western countries. Although a minor cellular component in the normal tissue, neuroendocrine (NE) cells increase in number and importance as long as prostate tumors develop. It is though that NE cells may secrete some factors that help cancer cells to grow, which explains why the presence of NE cells in prostate tumors is sometimes associated with a bad prognosis. However the exact role of NE cells in prostate cancer is still a matter of debate. Previously we have shown that the endogenous antioxidant pineal indole melatonin is able to reduce cell growth and induce NE differentiation in a human prostate cancer cell line, LNCaP, without modifying intracellular cAMP levels or protein kinase A activity. Thus, the aim of this study was to find out the intracellular pathways involved in NE transdifferentiation induced by melatonin and compare it with other NE-inducing stimuli. For this purpose we cultured androgen-dependent LNCaP cells with melatonin, androgen-withdrawn serum or cAMP analogues in order to induce NE differentiation. To discard the involvement of other mechanisms reported so far, we studied by using ELISA assay the production of IL-6 in NE-like LNCaP cells. Although melatonin is well known by its properties as a stimulator of immune system, we did not find any detectable changes in IL-6 levels when cells were treated with the indole. We found that all, melatonin treatment, androgen-withdrawal or cAMP rise, induced a transient activation of MAPK/ERK phosphorylation. Also, melatonin showed the fastest and higher effect in p-ERK activation. On the contrary, no increment in p38 or SAPK/JNK phosphorylation was observed after treatment. Melatonin and androgen withdrawal but not cAMP analogues also induced AKT activation after 24h. In conclusion, melatonin induces NE differentiation in androgen dependent prostate cancer cells by increasing p-ERK levels. This work was supported by “Instituto de Salud Carlos III (FISS-07-PI061715)”

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Mechanisms of tumour-selective apoptosis induced by the histone deacetylase inhibitor vorinostat

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Histone deacetylase inhibitors (HDACi) are new anti-cancer agents demonstrating promise in clinical trials for the treatment of haematological malignancies. Vorinostat, the first HDACi to be approved as a cancer therapeutic agent, inhibits the enzymatic activities of histone deacetylases, resulting in hyperacetylation of histone and non-histone proteins and the induction of various biological processes including cell cycle arrest and apoptosis. We have used a novel system of human cell transformation, in which tumorigenic cells were created from normal counterparts through the introduction of hTERT, SV40 large T and small t antigens and an oncogenic allele of H-RAS (Hahn and Counter et al. 1999), to investigate the molecular basis of vorinostat-mediated tumour-cell-selective apoptosis.

Compared to normal cells, transformed cells were hypersensitive to the apoptotic activities of vorinostat and displayed biochemical characteristics consistent with induction of the intrinsic apoptotic cascade. Vorinostat-mediated apoptosis required de novo protein synthesis and we subsequently employed microarray profiling and quantitative real-time polymerase chain reaction techniques to identify vorinostat-regulated genes that might mediate the tumour-selective effects of the compound. Gene ontology and pathway analysis revealed a dominant vorinostat-induced pro-apoptotic gene expression signature in the tumour cells. Transcripts encoding pro-apoptotic Bad, Bak, Bmf and Bik were selectively induced in transformed cells, while the pro-survival regulator BclA1 was selectively repressed. These transcripts remained largely unaltered in normal cells, consistent with tumour cell-selective pro-death signalling. Ectopic expression of BclA1 protected tumour cells from vorinostat-induced apoptosis. Furthermore, the transcriptional signature was specific for HDACi such that it could be induced by the structurally different HDACi depsipeptide but not the topoisomerase II inhibitor etoposide.

These data suggest that altered expression of transcripts encoding apoptotic regulators following HDACi treatment may underpin the tumour cell-selective apoptotic effect of these agents. We have subsequently extended our functional studies to determine which of the differentially regulated pro-apoptotic genes are necessary and/or sufficient for the tumour-selective activities of vorinostat.

Hahn W.C and Counter C.M et al. (1999) Nature 400: 464-468

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N-cadherin as a predictor of brain metastases in NSCLC

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Introduction: We have been screening genes encoding transmembrane/secretory proteins that are up-regulated in lung cancers and their brain metastasis, with cDNA microarrays and tumor cells purified by laser-capture microdissection. To verify the predictive value of these gene products from the point of wiew of brain metastases, we have been

performing tumor tissue microarray analysis of clinical lung cancer materials.

Preliminary results: Gene expression microarray study: RNA from 28 primary NSCLC, 8 samples of normal lung that were taken from the same patients, seven independent brain metastases and one specimen of normal brain (commercial RNA that is derived from a pool of normal brains) were hybridized to Affymetrix U95 Chips (containing 12625 probe sets). Of the 28 primary NSCLC cases 6 developed brain metastases and 7 extra-cranial metastases during a minimal follow-up of three years. Limited space precludes a detailed description of the analysis). The microarray results were confirmed by RQ-PCR of selected genes. ADAM8 and N-cadherin are according to these analyse genes associated with brain metastasi in NSCLC patiens screned above. After werrification on symplex from independent NSCLC patient files, collected both in Israel and Czech republic we found a significant association between n-Cadherin expression and brain metastasis (p= 0,008).

Conclusion: n-Cadherin is a wery strong predictor of brain metastasis in NSCLC patiens.

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Pilot study of neo-adjuvant intra-arterial (i/a) chemotherapy in patients with sarcomas of a head and neck

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Purpose. Reduction the volume of operation in patients with sarcomas of head and neck.

Methods. 19 pts (6 female, 13 male) with various histological types of head and neck sarcomas, age 19-59 years (middle age 39 years). Primary sarcomas were diagnosed at 15 people, recurrent at 4. Sarcomas of soft-tissue (SST) G1-G2 were diagnosed for 6 pts, the primary bone sarcomas G1-G2 at 13. Treatment regimen were: doxorubicin 45 mg/m2 i/a for 1 hour 1-2 days, cisplatin 75 mg/m2 i/a for hour, 1-2 days with one-stage intravenous hydratation, cyclophosphamide 800 mg/m2 intravenously 3 day. Interval between cycles was 14 days; number of cycles were 2-4. For i/a chemotherapy was used a. carotis externe.

Results. Clinical efficacy was registered at 15 pts (SST 4, bone sarcomas 11). According to beam methods of diagnostic, partial response was noted at 10 pts, stabilization at 5. All of them were underwent operation. Follow-up period in this group was from 5 till 22 months. At 4 pts treatment was inefficient. After 4 month the progress of disease was noted at 3 people and local recurrence of tumor in the same time at 1. Medical pathomorphosis III was noted in 4 cases (including bone sarcomas G1-G2), accordingly, changes of II and I wers noted in 8 and 7 cases.

Conclusion. Preoperative intra-arterial chemotherapy allows to reduce the volume of surgery, in the patients with widespreaded sarcomas of head and neck.

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A functional link between the MRN complex and the Gcn2p kinase uncovered by the antitumour drug beta-lapachone

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Background: Beta-lapachone (b-lap) is an anticancer agent that selectively induces cell death in several human cancer cells. We previously reported that, in budding yeast, b-lap was cytotoxic, induced DNA damage and activated a G1/S Mre11-Tel1p checkpoint pathway preceding death. Our aim was to gain further insights into the mechanism of b-lap action and identify the molecular targets of b-lap action.

Materials and methods: We compared the gene expression profile of the b-lap treated yeast cells with that obtained from untreated cells using cDNA microarrays. We used Significance Analysis of Microarrays to identify differentially expressed genes between untreated/b-lap treated cells. The data obtained after analysis of the microarray was validated by standard yeast genetics and molecular biology approaches.

Results: Interestingly, numerous amino acid biosynthesis genes were found to be regulated by the drug, suggesting that b-lap might activate the General Control of Nutrients (GCN) pathway in yeast. Accordingly, b-lap treatment incremented phosphorylation of the eIF2 alpha subunit in a GCN1, GCN2 and GCN20-dependent manner. Suriprisingly, phsophorylation of eIF2alpha was fully dependent on the MRN complex. Furthermore, Gcn2p kinase modulated i) checkpoint responses triggered by b-lap treatment, and ii) cell viability in response to b-lap exposure. finally, we found that Gcn2p regulated checkpoint function by mechanisms other than eIF2α phosphorylation.

Conclusions: These data uncover a functional link between the Gcn2p kinase and the MRN complex and suggest that Gcn2p may have additional functions besides regulating translation.

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Sensitization of breast cancer cells to anthracyclines by docosahexaenoic acid through loss of glutathione peroxidase response

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We found long-chain n-3 polyunsaturated fatty acids, such as docosahexaenoic acid (DHA), to enhance the sensitivity of human breast cancer cell lines (Germain 1998) and rat mammary tumors to chemotherapy containing agents that induce an oxidative stress (Colas 2004), such as doxorubicin. Since DHA, with its 6 double-bonds, is very prone to oxidation, its membrane incorporation would provide more abundant targets for ROS generated by doxorubicin. To examine the relation between chemo-sensitization by DHA and tumour cells oxidative and antioxidant status, we used two breast cancer cell lines: MDA-MB-231, in which DHA increases sensitivity to doxorubicin, and MCF-7, which is not chemosensitizable by DHA. Upon anthracycline treatment, reactive oxygen species (ROS) and lipid peroxidation levels were enhanced only in MDA-MB-231 under DHA supplementation (30 μM). This was concomitant with a decrease of cytosolic glutathione peroxidase (GPx1) activity by 30%, a crucial enzyme for protection against hydrogen and lipid peroxides, and an accumulation of glutathione, the GPx co-substrate. This lack of GPx response resulted from a decreased amount of GPx protein.

We used an autochthonous rat mammary tumour model to investigate in vivo the DHA effect on GPx1 activity and on anthracyclines treatment efficacy. Rats were fed a control diet and a DHA-enriched diet (3.6 % of DHA in the diet). When the tumour reached 1.5 cm², rats received 1 injection of epirubicin (2.5 mg/kg via intraperitoneal route) per week during 6 weeks. We found that dietary DHA enhanced tumour sensitivity to epirubicin and this effect was associated to a decrease of GPx1 activity by 20%. Furthermore we found an inverse correlation (r^2=0.488) between epirubicin efficacy and GPx activity. Conversely, when antioxidant vitamin E was added, tumour GPx1 activity was restored and the DHA effect on chemosensitization was abolished.

Thus, loss of GPx response to an oxidative stress in tumour cells may account for the ability of peroxidizable targets such as DHA to enhance tumour sensitivity to ROS-generating anticancer drugs.

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The G-quadruplex ligand RHPS4 interferes with telomere replication leading to ATR-dependent DNA damage response

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Functional telomeres are required for the replicability of cancer cells. The G-rich strand of telomeric DNA can fold into a 4-stranded structure known as G-quadruplex (G4), whose stabilization by specific ligands, can limit telomere function and cancer cell growth. RHPS4 is a telomere-interactive molecule possessing antitumoral activity because of its ability to rapidly induce telomere dysfunction and cell death [1].

Here, we show that RHPS4 induces a potent DNA damage response specifically in S-phase cells. In particular, we show that in S-arrested cells treated by RHPS4 ATR, but not ATM, is required for the formation of phospho-H2AX foci colocalizing with proliferating cell nuclear antigen (PCNA), BRCA1 and 53BP1. Interestingly, ATM is phosporylated at Ser1981 but in contrast to ionizing radiations, this activation of ATM is strictly ATR dependent, suggesting that the cellular response to pharmacological telomere deprotection follows a pathway that, most likely, represents ATR activation in response to replicational stress. By combining BrdU incorporation with CHIPs assay we clearly demonstrated that RHPS4 interferes with the replication of the telomeres, altering the dynamic association of the telomeric proteins TRF1, TRF2 and POT1. Interestingly, RHPS4 does not induce a specific DNA damage at an interstitial telomeric sequence, suggesting that it interferes with a terminal event of telomere