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