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Autocrine human growth hormone expression leads to resistance of MCF-7 cells to tamoxifen

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Background: Tamoxifen is the most common antiestrogen used in Treatment of estrogen positive breast cancer but its adverse effect and also resistance to this drug are serious challenges in treatment of breast caner. Characterization of mechanisms responsible for these adverse effects can lead to design more efficient therapeutic strategies to treatmentof breast cancer.
Materials and Methods: Here we prepared a cellular model of autocrine expression of human growth hormone (hGH), by production of stable MCF-7 cell line expressing hGH, to evaluation of autocrine expression of hGH effects on response of cells to tamoxifen. By using of microculture tetrazolium test (MTT) we compared tamoxifen antiproliferative effects between MCF-HGH, MCF-7 cells expressing active hGH, and MCF-MUT, MCF-7 having translation deficient hGH coding region. To finding that which of esterogen receptors are responcible to difference between MCF-HGH and MCF-MUT cells response to tamoxifen, we performed real-time RT PCR reaction using primers specific for either of esterogen receptor (ER)-a, Er-β or G-coupled estrogen receptor (GPR30) and beta-actin as housekeeping gene.
Result: MTT results show that tamoxifen treatment of MCF-MUT cells leads to inhibiotion of cell proliferation. But autocrine expression of growth hormone in human breast adenocarcinoma cell line, MCF- 7, in MCF-HGH cells results that treatment of cells by tamoxifen not only does not decrease cell proliferation, but also, partially, increase it. real-time RT PCR results show that expression levels of ER-a and Er-β does not change under effect of autocrine hGH, while expression level of GPR30 in MCF-HGH cells is 4.5 fold higher than its expression level in MCF-MUT.
Conclusion: Our results suggest, autocrine hGH results to non-responsive phenotype of MCF-HGH cell to antiproliferative effects of tamoxifen. This effect may be as a result of upregulation of G-coupled estrogen receptor, GPR30, which does not inactivates by tamoxifen. These conclusion suggests, determination of autocrine hGH expression status of breast tumor cells can help to design appropriate therapeutic strategies in treatment of breast cancer patients.

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Correlation of BRCA1, DAXX, TXN, TXR1 and TSP1 tumoral expression with resistance to docetaxel-based chemotherapy in patients with advanced/metastatic Non Small Cell Lung Cancer

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Background: Taxanes are among the most active antitumor agents in the treatment of Non-Small Cell Lung Cancer (NSCLC). However, an increasing number of patients being treated with taxanes develop resistance that finally limits chemotherapeutic efficacy. The prognostic and predictive value of tumoral expression of 5 genes *(BRCA1*, *DAXX*, *TXN*, *TXR1* and *TSP1)* related with the mechanism of actions of taxanes was evaluated in patients with NSCLC treated with first-line docetaxel-based chemotherapy.
Material and Methods: Tumor samples from 184 patients, with stage IIIB (with pleural effusion) or IV NSCLC were analyzed for *BRCA1*, *DAXX*, *TXN*, *TXR1* and *TSP1* mRNA levels by quantitative real-time PCR, from microdissected cells derived from patients' primary tumors.
Results: The mRNA levels of the *BRCA1*, *DAXX* and *TXN* were significantly correlated with each other. Also, the mRNA levels of *TXR1-TSP1* were inversely correlated (Spearman's test: −0.38; $p = 0.002$). Low *TXR1* mRNA levels were associated with higher response rate (RR $p = 0.0014$), longer median time to tumor progression (TTP $p < 0.003$) and median overall survival (mOS $p = 0.004$), while high *TSP1* expression was also, correlated with higher RR ($p = 0.021$), longer TTP ($p < 0.0001$) and mOS ($p < 0.0001$). In addition, patients whose primary tumors presented higher *BRCA1* mRNA expression experienced higher RR ($p = 0.002$) in comparison with those with low *BRCA1* tumoral expression. Higher *DAXX* mRNA levels were significantly correlated with prolonged survival ($p = 0.028$) and a trend for higher RR ($p = 0.07$) and improved TTP ($p = 0.1$). No significant association was found for *TXN* expression. Multivariate analysis demonstrated that high *TXR1*/low *TSP1* expression was an independent prognostic factor for decreased TTP (HR 2.6; 95% CI: 1.7−4.1; $p < 0.0001$) and mOS (HR 3.4; 95% CI: 2.1−5.7; $p < 0.0001$).
Conclusions: These data indicate the *TXR1-TSP1* mRNA expression could be used for the prediction of taxanes' resistance in the treatment of NSCLC and merits further evaluation.

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Inactivation of Notch signaling by Withaferin-A in human colon cancer

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Background: Colorectal cancer is the third most frequently diagnosed cancer and the third leading cause of cancer-related deaths in men and women in the United States. The medicinal plant, *Withania somnifera*, is extensively used in Asian herbal medicines to treat a variety of ailments, including cancer. We identified Withaferin-A (WA), a major bioactive compound in *Withania somnifera* exhibits potent anti-cancer effects on colon cancer cells that underscores the necessity of studying the molecular mode of action of WA to clarify its potential clinical merit.
Materials and Methods: To determine the effect of WA on Notch signaling we used three colon cancer cell lines (SW-620, SW-480 and HCT-116). Cell viability and apoptosis was determined using Trypan Blue exclusion assay and Annexin V-FITC staining respectively. Western Blot analysis was performed to determine WA-mediated modulation in the expression of Notch signaling proteins. To study whether WA transcriptionally regulates Notch and its downstream genes, we isolated total RNA and subjected it to RT-PCR to determine WA-mediated modulation of mRNA expression of Notch and its downstream genes.
Results: Our results suggest that WA inhibits cell proliferation and induces apoptosis in colon cancer cells (SW-480, SW-620 and HCT-116). While dissecting the mechanism of action of WA on colon cancer cells, we found that WA inhibits Notch-1 signaling, which resulted in the downregulation of pAkt and Bcl-2 expression. In addition, inhibition of mTOR signaling by WA resulted in the down regulation of pS6K and p4E-BP1 expression in SW-480, SW-620 and HCT-116 colon cancer cells. We also observed, WA activates caspase-3 and PARP cleavage suggesting that it triggers the pro-apoptotic machinery in colon cancer cells. Interestingly, WA causes a strong mitotic catastrophe by arresting the cells G2/M phase of cell cycle in colon cancer cells. Finally, oral administration of WA resulted in significant tumor regression of xenografts comprised of highly metastatic colon cancer cells in nude mice.
Conclusions: Our results suggest that WA inhibits cell viability and induces apoptosis in colon cancer cells. Additionally, we found that the biological effects of WA were due to inhibition of Notch and its downstream signaling molecules. The results from our study suggest that WA can be explored for its potential as a targeted therapy for colon cancer.

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Influence of chemotherapy on breast cancer cells in the conditions of 24-hour incubation in vitro

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Background: Nowadays the spectrum of medicaments for treatment of oncologic patients constantly extends, but there is no common opinion about therapeutic advantages of these agents and practically there are no standardized schemes of their use in a combination with traditional therapy. In our opinion more perspective method is restoration of function of lymphocytes by using of ultrasonic influence. The technique of allocation of lymphocyte stimulation factor by ultrasound influence on serum, worked out in our centre, showed the high efficiency of restoration of the lost or weakened function of immune cells.
Materials and Methods: the **s**tudy of morphological tumor structure of 28 breast cancer patients showed that selected influences significantly differ by the character and expression of changes. In our work 5 series of the experiment are presented. In the 1 series of the experiment – tumor was processed by Cycloferon. In the 2 series of the experiment – tumor was processed by Doxorubicin. In the 3 series of the experiment – tumor was processed by the combination of Cycloferon and Doxorubicin. In the 4 series of the experiment – processing of tumor by combination of Doxorubicin and autoserum after ultrasound influences on it. In the 5 series of the experiment – tumor was processed only by autoserum after ultrasound processing.
Results: In the 1 series the rise of a number of mitosis till 1.3% and insignificant induction of apoptosis till 0.8% was marked. In the 2 series the quantity of mitosis considerably decreased to 0.6% and expression of apoptosis increased to 1.5%. Simultaneously a toxic damage of cells

observed at conservation of tumor stroma. In the 3 series the number of mitosis was 1.4% and apoptosis was 3.6%. It was marked a plural toxic damage of cells and signs of destruction of stroma. In the 4 series mitosis decreased to 0.2% and apoptosis was 2.8%. It was marked the necrosis of cells and destruction of tumor stroma. Results of the 5 series were most interesting. Mitosis was 0, i.e. process of cell fissions practically stopped. Apoptosis was 8.1% against the expressed destructive changes of stroma and almost total cell destruction.
Conclusion: The results received in the 5 series of the experiment open quite new prospects in breast cancer treatment.

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Functional characterization of TRAP1 pathway in multidrug resistance in human colorectal carcinoma

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Background: TRAP1 has been recently characterized by our group as a mitochondrial chaperone up-regulated in human colorectal carcinoma (CRC) and involved in favoring a phenotype resistant to apoptosis and chemotherapeutic agents in tumor cells. Interestingly, these findings correlate with the observation that TRAP1 is a component of a mitochondrial pathway, which antagonizes the proapoptotic activity of cyclophilin D and is responsible for maintenance of mitochondria integrity, favoring cell survival.
Materials and Methods: To further characterize TRAP1 function in multidrug resistance of human CRC and to identify novel targets involved in TRAP1 antipoptotic pathway, GST-pulldown experiments and mass spectrometry analysis were performed that allowed us to identifify several TRAP1 ligands. Among others we selected sorcin, a Ca^{2+}-binding protein involved in the development of MDR phenotype in leukemia cells.
Results: Co-immunoprecipitation analyses confirmed TRAP1/sorcin interaction in CRC cells and preliminary experiments suggest a concomitant enrichment of TRAP1 and sorcin, which was known to be a cytosolic protein, in the mitochondrial fraction of CRC cells resistant to 5-fluoruracil, irinotecan and oxaliplatin and in human CRC specimens. Indeed, TRAP1 and sorcin are up-regulated in 60−70% of human CRCs where a significant correlation between the two proteins has been observed (Pearson Correlation test $r = 0.60$; $p = 0.001$). All these findings are in agreement with the observation that HT-29 CRC cells transfected with TRAP1 exhibit a phenotype resistant to 5-fluorouracil-, oxaliplatin- and irinotecan-induced apoptosis and that the inhibition of TRAP1 activity by the TRAP1 ATPase antagonist, shepherdin, or the transfection of a dominant negative TRAP1 mutant increase the sensitivity to apoptosis induced by chemotherapeutic agents in wild type HT-29 CRC cells or in CRC cells resistant to single antiblastic drugs.
Conclusions: It is likely that sorcin and TRAP1 cooperate in a pro-survival pathway responsible for resistance to chemotherapy and that such a pathway may represent a novel molecular target to overcome drug resistance in human CRCs.

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The effect of sorafenib on Indium-111 labeled bevacizumab uptake in patients with clear cell renal cell carcinoma (ccRCC)

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Background: Patients (pts) with ccRCC are treated with the anti-angiogenic drug sorafenib (sor), a Raf kinase/VEGFR2 inhibitor. In this study we explored the effect of sor on VEGF expression in ccRCC as determined by scintigraphic imaging with In-111- radiolabeled bevacizumab, a humanized anti-VEGF antibody.
Materials and Methods: Pts radiologically suspected of ccRCC scheduled to undergo tumor nephrectomy were included. Adequate bone marrow, renal and hepatic function were required. Exclusion criteria were: prior use of bevacizumab or drugs targeting VEGF or VEGFR, other prior anticancer therapy, pregnancy and lactation. One hour and 5−7 days after iv administration of 100 MBq In-111-bevacizumab, a whole-body scan was performed. In-111-bevacizumab targeting to the tumors was scored qualitatively and semi-quantitatively. After four weeks of treatment with sor 400 mg bid, a second In-111-bevacizumab imaging procedure was performed. Pts underwent nephrectomy 2−3 days thereafter. In a 1-cm slice of the surgical specimen, the distribution of In-111-bevacizumab radioactivity was determined and correlated with tumor viability, VEGF expression and vessel density by immunohistochemical analysis. VEGF-A levels were also determined in tumor extracts by ELISA. As a control, in 5 untreated radiological suspected ccRCC pts a pre-operative In-111-bevacizumab scan was performed.
Results: In 5 control pts with ccRCC, In-111-bevacizumab scintigraphy depicted ccRCC, and antibody accumulation corresponded with intra-tumoral VEGF levels. Neo-adjuvant treatment with sor was well tolerated, although 3 out of 11 pts needed a dose reduction (200 mg bid) because of CTC grade 3 skin toxicity. In 10/11 pts, qualitatively preferential tumor accumulation was observed in the images that were acquired before initiation of sor therapy. One pt with urothelial cancer was negative and replaced. After sor treatment a reduction in In-111-bevacizumab was observed in 8/9 pts with histologically proven ccRCC (mean decrease 58%, range 6%-98%). In contrast, In-111-bevacizumab targeting was stable in 1 ccRCC, 1 urothelial cancer and 1 oncocytoma. Decreased targeting correlated with VEGF expression in vital tumor parts. Necrotic areas in the tumors were not targeted with In-111-bevacizumab despite high VEGF levels, due to low perfusion.
Conclusions: In-111-bevacizumab scintigraphy is able to depict VEGF in ccRCC in-vivo and to monitor the effect of anti-angiogenic therapy on tumor VEGF expression.

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Extraordinary responses to chemotherapy in metastatic gastric and cervical cancer: gene expression profiling and pharmacogenetics

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Background: Current palliative chemotherapy (CT) regimens achieve clinical benefits in less than 50% of patients treated for metastatic gastric and cervical cancers, and long term survivals are anecdotical.
Different susceptibility to the toxic effects of a given CT treatment has been demonstrated in clinically homogeneous groups of patients. Genetic polymorphisms and different expression of genes involved in drug metabolism, resistance and DNA repair can explain those differences.
We present a case-control comparison for genetic and expression profiling of *long survivors* towards *normal responders* to CT in metastatic gastric and cervical cancer. Four cases of long survivors (2 gastric and 2 cervical) and 9 of normal responders (4 gastric and 5 cervical) have been investigated.
Meterial and Methods: *Expression profiling* using a custom array of real time quantitative RT-PCR assays has been performed on an ad-hoc set of 95 genes chosen from database and literature for being of pharmacogenomic interest. RNA has been extracted from paraffin embedded tumour tissue slices.
Genetic polymorphisms analysis has been done using pyrosequencing for the determination of genetic markers in *MTHFR, DPYD, TYMS, GSTP1, ERCC1, XRCC1, ABCB1, CYP3A4*1B, CYP3A5*3* genes. Restriction enzyme analysis has been used for 5′UTR polymorphism in *TYMS* gene.
Results: *Expression analysis* revealed a consistent up-regulation of genes involved in drug catabolism: *CYP1A1, CYP2C8, CYP3A4* and down-regulation of *ABCC2* in long term survivors compared to controls in gastric tumours. The study of uterine cervix showed up-regulation of *GSTP1, PTEN AND TYMS* and down-regulation of *MMP1*, in long survivors towards normal responders. The pattern of differentially expressed genes showed interesting features of tissue-specificity.
Genetic Screening revealed polymorphisms combinations already described for being associated with prolonged survival to CT. The absence of the *GSTP1* allele, associated with diminished enzymatic activity, and the *ABCB1* polymorphism associated with increased toxicity have been observed in cervical cancer long survivors. Moreover in the group of long survivors, the “3R” allele of the gene *TYMS* presents a frequency superior than the one reported in the Caucasian population.
Conclusions: The present study shed light on a set of genes, which could play a role in driving response to CT and, therefore, could be suggested as markers to guide the choice of CTfor cancer treatment.