THE MOLECULAR BASIS FOR APOPTOSIS AND CELL-CYCLE DELAY FOLLOWING IONIZING RADIATION OF PROSTATE CANCER CELLS PRETREATED WITH 1,25(OH)2D3 AND SODIUM VALPROATE.

Dr. Vladimir Gavrilov [1] Mr. Yaron Leibovich [2] Prof. Samuel Ariad[2] Dr. Konstantin Lavrenkov [2] **Prof. Shraga Shany [1]**

1. Department of Clinical Biochemistry, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel
2. Department of Oncology, Soroka Medical Center and Faculty of Health Sciences, Ben-Gurion University

Introduction: Our previous study has shown that pretreatment of prostate cancer (PCa) cells with a combination of the anticancerogenic vitamin D active metabolite, 1,25(OH)2D3, and sodium valproate (an inhibitor of histone deacetylase enzymes with antitumor activity) effectively potentiates the antitumor effect of ionizing radiation. We have demonstrated that this antitumor effect is mainly due to increased apoptosis and cell-cycle arrest in S phase. The present study aims to investigate the molecular mechanisms underlying these phenomena. For this purpose, generation of DNA double-strand breaks (DSBs), the most serious DNA lesion caused by ionizing radiation, and activation of cell-cycle-checkpoint kinases Chk1 and Chk2 were assessed.

Methods: Androgen-refractory PCa DU145 cells were grown in RPMI-1640 medium containing 10% FCS. Cancer cells were seeded into 96-well plates and pretreated for 3 days with 100 nM 1,25(OH)2D3 or 1 mM sodium valproate, or their combination. Treated and control cells were irradiated with a dose of 4 Gy and incubated for an additional 3 hours. Expression of DSBs (phosphorylated H2A.X) and activated (phosphorylated) checkpoint kinases was assessed by the cell-based ELISA method, using relevant antibodies.

Results: Irradiation caused a significant generation of DSBs in both control and pretreated DU145 cells. However, the damage of DNA in pretreated cells was greater and reached a maximum in cells pretreated with a combination of sodium valproate and 1,25(OH)2D3. DNA damage was evident by the increased phosphorylation of H2A.X. Levels of phosphorylated H2A.X 3 hours after irradiation increased by 11.7% in control cells, by 18.7% in cells pretreated with sodium valproate, by 20.6% in cells pretreated with 1,25(OH)2D3 and by 34.7% in cells pretreated with a combination of both drugs ($p<0.001$, $p<0.006$, $p<0.03$ and $p<0.001$, respectively). Following irradiation, Chk1 kinase showed no activation. However, the activity of Chk2 kinase was significantly elevated in PCa cells subjected to irradiation. Phosphorylated Chk2 kinase levels were increased by 32.0 - 43.4% 3 hours after irradiation ($p<0.02$). Maximal activation of Chk2 kinase was observed with the combined pretreatment.

Conclusions: The results show that pretreatment of PCa cells with a combination of 1,25(OH)2D3 and sodium valproate is highly efficient in potentiating the DNA damaging effect of ionizing radiation. Using such a pretreatment may increase the therapeutic effect of irradiation, decrease doses of radiation and consequently limit side effects of the treatment.

The beta-cell KATP channel is required for replicative decisions

Dr. Rachel Schyr [1,2] Mrs. Sharona Tornovsky [1] Dr. Shay Porat[2] Mrs. Mozhgan Heyman [1] Prof. Benjamin Glaser [1] Dr. Yuval Dor [2] Dr. Frances M. Ashcroft [3]

1. Endocrinology and Metabolism Services, Department of Internal Medicine Hadassah-Hebrew University Medical Center, Jerusalem, Israel
2. Department of Cellular Biochemistry and Human Genetics, The Hebrew University Medical School,
3. Wellcome Centre for Gene Function, Department of Physiology, Anatomy and Genetics, Oxford University

Introduction: The mechanisms by which the endocrine pancreas controls its mass are poorly understood, but it is generally thought that glucose, the trigger for Insulin secretion, is also a key determinant of beta cell proliferation and mass. However the molecular mechanisms governing this fundamental circuit, although crucial for the understanding and treatment of diabetes, remain obscure. We hypothesize that the molecular cascade controlling Insulin secretion, involving increased glucose metabolism, membrane depolarization via ATP-dependent potassium channels, calcium entry and Insulin exocytosis, also regulates beta cell proliferation. Aims: To test the involvement of glucose metabolism and membrane depolarization in beta cell proliferation.

Results: Pharmacologic activation of glucokinase resulted in increased proliferation (193%±31% vs. 100±14% in control) despite hypoglycemia (85±13 mg/dL vs. 146±51 mg/dL in control), suggesting that glucose flux and not hyperglycemia per se regulates proliferation. Diazoxide, a drug that prevents depolarization, resulted in hyperglycemia, but totally blocked the effect of glucokinase activation on proliferation. To confirm this finding using a genetic approach, we used mice expressing in beta cells a mutant KATP channel (Kir6.2 V59M), which prevents membrane depolarization. Patients with this mutation have permanent neonatal diabetes. Eight days after induction of the transgene, beta cell proliferation rate dropped by 33% despite marked hyperglycemia (>600mg/dL).

Conclusions: We conclude that glucose controls beta cell replication via glucose flux and membrane depolarization. The results provide a link between the physiologic function of beta cells and the homeostatic mechanism that controls their number.

Identification of insulin-like growth factor-I receptor (IGF-IR) gene ‫׃‬ promoter-binding proteins in estrogen receptor (ER)-positive and ER-depleted breast cancer cells.

Dr. Rive Sarfstein, Prof. Haim Werner

1. Department of Human Molecular Genetics and Biochemistry, Sackler School of Medicine, Tel Aviv University

Introduction: The insulin-like growth factor I receptor (IGF-IR) has been implicated in the etiology of breast cancer. Identifying the factors that regulate IGF-IR gene expression may help us better understand the molecular mechanisms underlying the involvement of the IGF axis in breast cancer. Overexpression of the IGF-IR gene is a typical feature of most primary breast cancers, whereas low IGF-IR levels are seen at advanced stages. Hence, evaluation of IGF-IR levels might be important for assessing prognosis. Aim of study: In the present study we employed a novel and reliable DNA affinity chromatography protocol linked to proteomic analyses in order to identify IGF-IR promoter-binding proteins in breast cancer cells. This method allowed us to detect known and new potential biomarkers in ER-positive (MCF7) and ER-depleted (C4.12.5) cells, which may reflect early and advanced stages of the disease.

Methods: A biotinylated IGF-IR promoter fragment was bound to streptavidin magnetic beads and incubated with nuclear extracts of breast cancer cells. IGF-IR promoter-binding proteins were eluted with high salt and analyzed by Western blotting and mass spectroscopy (MS). The biological relevance of selected transcription factors in regulation of the IGF-IR gene was confirmed by evaluation of IGF-IR promoter activity in cotransfection assays and by chromatin immunoprecipitation (ChIP) analyses.

Results: Among the proteins that were found to bind (either directly or indirectly) to the IGF-IR promoter, MS and Western analyses allowed us to , p53, c-jun, and polyαidentify zinc finger transcription factor Sp1, KLF6, ER- (ADP-ribosylation) polymerase (PARP). ChIP analysis confirmed the direct binding of some of these transcription factors to DNA. In addition, we identified a number of non-DNA sequence-specific nuclear proteins that are involved in IGF-IR gene regulation. Furthermore, cotransfection experiments using c-jun expression vector revealed that this nuclear protein stimulated IGF-IR promoter activity.

Conclusions: We identified a collection of known and novel IGF-IR promoter-binding transcription factors in breast cancer cell lines. Identification of differentially expressed IGF-IR promoter regulatory transcription factors may help elucidates the mechanisms responsible for the differential expression of the IGF-IR gene in both cell types. Furthermore, these proteins may constitute potential biomarkers characteristic of ER-positive (early) or ER-negative (advanced) stages. These results need to be confirmed by future studies including biopsies from patients at early and advanced stages, and correlated with conventional prognostic factors such as tumor size, lymph nodes status, histological grade, and ER status.

Plant polyphenols enhance all-trans retinoic acid-induced maturation and growth inhibition in acute promyelocytic leukemia (APL) cells.

Rina Wassermann [1] Victoria Rossova [1] Prof. Michael Danilenko[1]

1. Department of Clinical Biochemistry, Ben Gurion University, Beer Sheva

Introduction: All-trans retinoic acid (ATRA) induces granulocytic differentiation of myeloid cells via interaction with the retinoic acid receptor (RAR) that forms a heterodimer with the retinoic X receptor (RXR). ATRA has been successfully used in differentiation therapy of APL. However, its therapeutic (high) doses may cause a severe retinoic acid syndrome. We have previously shown that plant polyphenols, such as carnosic acid (CA) from rosemary and silibinin (SIL) from milk thistle, synergistically enhance the in vitro antileukemic effects of 1,25-dihydroxyvitamin D3 in human leukemic cells. The Nrf2/antioxidant response element (Nrf2/ARE) transcription system was found to be important for such enhancement. Here we determined whether CA and SIL could also enhance the antileukemic activity of physiologic doses of ATRA, and if this effect correlates with Nrf2/ARE activation.

Methods: Cell proliferation and viability were determined by the trypan blue exclusion assay. Cell differentiation was assessed by the expression of CD11b and CD11c surface markers and superoxide production. The levels of RAR-alpha and RXR-alpha as well as Nrf2/ARE responsive genes NADP(H)-quinone oxireductase (NQO1), gamma-glutamylcysteine synthetase (gamma-GCS) and thioredoxin reductase (TrxR) were examined using real-time RT-PCR and Western blotting. The intracellular levels of reactive oxygen species (ROS) and total glutathione were measured by dichlorofluorescein fluorescence, and the glutathione reductase recycling assay, respectively.

Results: Both CA (10 microM) and SIL (10-30 microM) enhanced the differentiation of the APL cell line (NB4) induced by 0.5-1 nM ATRA, whereas only additive effects on cell proliferation were observed. Differentiation enhancement by CA was associated with the prevention of RAR-alpha and RXR-alpha loss induced by ATRA. As expected, CA reduced ROS levels and elevated total glutathione content, i.e. acted as an antioxidant. In contrast, ATRA had a prooxidant effect, which was prevented by CA. Consistent with its antioxidant activity, CA increased the expression of Nrf2/ARE-responsive gene products (gamma-GCS, NQO1 and TrxR). Surprisingly, ATRA suppressed CA-induced expression of both NQO1 and TrxR but significantly enhanced gamma-GCS induction, which correlated with elevation of glutathione content. However, glutathione depletion did not affect cell differentiation.

Conclusions: We suggest that the antioxidant effect of plant polyphenols per se does not play a role in their differentiation-enhancing activity. However, Nrf2/ARE activation by these agents may contribute to differentiation induction, possibly via regulation of RAR-alpha and RXR-alpha gene expression and/or protein stabilization.

Human Medullary Carcinoma cell line TT Growth is Estrogen-Sensitive and t-Boc Derivatives of Carboxy- Alkyl Isoflavones Can Serve As Novel Anti-Cancer Agents for TT cells in vitro.

Dr. Yona Greenman [1] Mrs. Meital Grafi-Cohen [1] Dr. Sara Katzburg[1] Mrs. Orly Sharon [1] Mrs. Etty Knoll [1] Dr. Zaki Kraiem [2] Prof. Fortuna Kohen [3] Prof. Naftali Stern [1] Prof. Dalia Somjen [1]

1. Institute of Endocrinology, Metabolism and Hypertension, Tel-Aviv Sourasky Medical Center and the Sackler Faculty of Medicine, Tel-Aviv University
2. Department of Medicine, The Rappoport Institute, Haifa Med School Technion
3. Department of Biological Regulation, The Weizmann Inst, Rehovot.

Introduction: Currently available treatments for patients with medullary thyroid carcinoma (MTC) with residual or recurrent disease after primary surgery have low efficacy rates. Here we explore whether or not human TT cell growth, can be curbed by novel phytoestrogen derivatives generated in our laboratory, which we previously found to possess potent anti-cancer effects in human ovarian cancer cells through interaction with ER-β.

Methods: mRNA expression of estrogen receptors (ER) α and β was quantified by real time PCR. Cell proliferation was determined by 3[H] thymidine incorporation, cell viability and apoptosis assays. Creatine kinase specific activity (CK activity), which is linked to the growth modulation effects of estrogen, was also used as a marker for cell proliferation.

Results: ER α mRNA was more abundant than ER β with a ratio of 48:1. Estradiol-17β (E2) (0.03-300 nM) increased DNA synthesis in a dose dependent manner. E2 (30 nM) and the ERβ agonist DPN (42 nM) similarly stimulated 3[H]thymidine incorporation by 68%, whereas the ERα agonist PPT caused a 117 % stimulation only at a higher concentrations (390 nM). The ER α antagonist raloxifene abolished E2 and PPT, but not DPN stimulation of CK specific activity. cD-tBoc inhibited TT cell growth as assessed by thymidine incorporation (31 to 49%), XTT assay (39-70%), CK activity (38%) and microscopic analysis of culture wells. Furthermore, cD-tBoc potently increased apoptosis (1350-1750% stimulation of histone-DNA fragments) and cell necrosis (67% increase in LDH activity). Co-incubation with the antiapoptotic agent ZV(Z-VAD-FMK) reversed the growth inhibitory effect elicited by cD-tBoc as measured by thymidine incorporation, induction of apoptosis and CK activity.

Conclusions: These results suggest that estrogens are involved in proliferation of the human MTC cell line TT, and that this property can be utilized to design highly effective anti-cancer drugs both in vitro and in vivo.

Role of the antioxidant effect and VDR transcription system in the enhancement of vitamin D-induced differentiation of leukemic cells by plant polyphenols

Mrs. Victoria Rossova [1] Dr. Marina Khanin [1] Mrs. Stella Pesakhov[1] Prof. Ofer Shpilberg [3] Dr. Itai Levi [2] Dr. Ory Rouvio [2] Prof. Michael Danilenko [1]

1. Department of Clinical Biochemistry, Ben-Gurion University, Beer-Sheva,
2. Institute of Hematology, Soroka Medical Center, Beer-Sheva
3. Department of Hematology, Rabin Medical Center, Petah-Tikva

Introduction: Acute myeloid leukemia (AML) is an aggressive cancer without effective treatment. Differentiation therapy is a promising approach to treat AML. 1α,25-dihydroxyvitamin D3 [1,25D] is a powerful differentiation agent, but it is highly toxic at pharmacologic doses. Here we determined the ability of plant-derived polyphenolic antioxidants (PAOs), such as carnosic acid (CA), carnosol (CN), rosmarinic acid (RMA), curcumin (CUR), and silibinin (SIL) at non-cytotoxic concentrations (5-60 uM) to potentiate the differentiation of AML cells induced by a low dose of 1,25D (1 nM).

Methods: The expression of surface differentiation markers (CD11b and CD14) was determined by flow cytometry. The functional differentiation was measured by the levels of superoxide production using cytochrome c reduction assay. Total glutathione level was determined by the glutathione reductase recycling assay. Intracellular levels of reactive oxygen species (ROS) were measured by DCFH-DA oxidation method. Protein levels were estimated by Western blotting.

Results: All the PAO tested, except RMA, enhanced 1,25D-induced expression of CD11b and CD14 and superoxide generation in HL60 myeloblastic leukemia cell line. The order of potencies was CUR > SIL > CA > CN. The differentiation-enhancing ability of PAOs correlated well with their antioxidant activity. Interestingly, this antioxidant effect markedly increased (2-4-fold) in the presence of 1 nM 1,25D, which also slightly decreased the ROS levels when added alone. Surprisingly, only CA and CUR were capable of markedly elevating cellular glutathione content (2.0-2.5-fold). No further elevation was observed in the presence of 1,25D, which alone was ineffective. Thus, the antioxidant effects of different PAOs and their combinations with 1,25D are likely mediated by different cellular mechanisms. However, irrespective of the mechanisms involved, only those PAOs that were capable of decreasing ROS levels were also capable of synergizing with 1,25D in the elevation of vitamin D receptor (VDR) and retinoid X receptor alpha (RXRalpha) protein levels, which may contribute to their differentiation-enhancing activity. Most importantly, similar to their prodifferentiating effects in the HL60 cells, CA, CUR and SIL markedly potentiated 1,25D-induced differentiation in leukemic blasts isolated from AML patients, which was associated with an increase in VDR and RXRalpha protein levels in these cells.

Conclusions: Together with our recent data showing synergistic antileukemic activity of combinations of low-calcemic 1,25D analogs and rosemary polyphenols in mouse AML models, the results of the current translational study suggest novel therapeutic and preventive strategies against AML.

Targeting of the IGF-IR as a potential therapeutic strategy in endometrial cancer

Mrs. Zohar Attias [1] Ilan Bruchim, Haim Werner

1. Department of Human Molecular Genetics and Biochemistry, Sackler School of Medicine, Tel Aviv University

Introduction: Endometrial cancer is the most frequent gynecologic cancer in Western countries. The majority of the cases can be divided into two broad categories, based on clinic-pathological and molecular characteristics. Type I cancers, which represent more than 80% of the cases, exhibit an endometroid histology, are generally estrogen dependent, and have a good prognosis. Type II endometrial cancers, also termed uterine serous papillary endometrial cancer (USPC), have serous papillary or clear cell histology and have a poor prognosis. The insulin-like growth factors (IGFs), have been implicated in the etiology of a number of malignancies, including endometrial cancer. However, no study has so far evaluated the expression of the IGF system in USPC. Moreover, no study has addressed the potential impact of IGF-IR targeting in endometrial cancer. The aim of our research was to investigate the anti-proliferative potential of a targeted therapy approach against the IGF-IR in endometrial cancer.

Methods: To assess the impact of IGF-IR inhibition on IGF-I-mediated signaling, ECC-1 and Ishikawa endometroid carcinoma cells and USPC-1 and USPC-2 serous papillary carcinoma cells were treated with IGF-I in the absence or presence of increasing amount of the selective IGF-IR inhibitor NVP-AEW-541 (Novartis Pharma, Basel, Switzerland).

Results: Results obtained showed that NVP-AEW-541 abolished the IGF-I-stimulated IGF-IR phosphorylation in all of the cell lines, whereas it abolished AKT and ERK phosphorylation only in ECC-1 and USPC-1 cells. Furthermore, in order to evaluate the effect of IGF-IR inhibition on apoptosis, the cells were treated with IGF-I in the absence or presence of the inhibitor. Results showed that addition of the IGF-IR inhibitor on top of IGF-I prevented from IGF-I from exerting its antiapoptotic effect in ECC-1, USPC-1 and USPC-2 cells. Furthermore, proliferation assays showed that the inhibitor NVP-AEW-541 cause a significant decrease in proliferation rate compared to control cells in all of the cell lines.

Conclusions: our results suggest that inhibition of IGF-IR signaling by AEW-541 abolished the antiapoptotic activity of IGF-IR and abrogated IGF-I-mediated signaling events. Taken together, these results indicate that specific IGF-IR inhibition is a potential proapoptotic tool in endometrial cancer cells.

Can auxology, IGF-1, IGFBP-3, MRI and genetic tests replace GH stimulation tests for the diagnosis of GH deficiency?

Dr. Ora Hess [1,3] Dr. Yasir Hujeirat [3] Dr. Osnat Admoni[1] Dr. Stavit Allon-Shalev [2,3] **Dr. Yardena Tenenbaum-Rakover** [1,2]

1. Pediatric Endocrine Unit, Ha'Emek Medical Center, Afula, Israel,
2. Technion Faculty of Medicine, Haifa, Israel
3. Genetic Institute, Ha'Emek Medical Center, Afula, Israel

Introduction: Two different growth hormone stimulation tests (GHSTs) are traditionally used to identify children with growth hormone deficiency (GHD). Since GHSTs are imprecise, time-consuming, costly and unsafe, the use of auxology, IGF-1 and IGFBP-3 levels and then performing brain MRI and genetic tests has been recently proposed for the diagnosis of GHD. The aim of the current study is to analyze whether this new approach is reliable and can replace the traditional approach to diagnosing GHD in children.

Patients/ Methods: Fifty-three children (18F/35M), diagnosed as having GHD by two different GHSTs and under GH treatment, were enrolled (aged 1.2 to 11.2 years, mean 5.4 ± 2.6 SDS). They all underwent auxology, IGF-1 and IGFBP-3 measurements and brain MRI prior to GH treatment. Sequence analysis of GH-1, ghrelin (GHRL) and ghrelin-receptor (GHSR) genes was carried out.

Results: Prior to GH therapy, mean height (SDS) was -2.5 ± 1.5 (range, -5.1 to -1.3), IGF-1 (SDS) -1.3 ± 0.6 (-2.9 to 0.1), IGFBP-3 (SDS) -1.2 ± 1.6 (-4.0 to 1.8). Anatomical pituitary abnormalities were demonstrated in 28 out of 48 children (58%): in 26 of them the adenohypophysis was hypoplastic and 13 had an ectopic posterior pituitary. Fourteen children (26%), belonging to two different core families, carried the G6664A mutation in GH-1 on one allele and the others had no mutations in GH-1, GHRL or GHSR. Using cut-off levels of -1.5 SDS for each of the following parameters: height, IGF-1 and IGFBP-3, excluded GHD diagnoses of 13%, 64% and 60% of the children (respectively), while a cut-off of -2 SDS excluded 25%, 85% and 72% of the children (respectively). MRI and genetic tests disregarded 42% and 74% of the children with GHD (respectively).

Conclusions: Using the approach of auxology, IGF-1, IGFBP-3 levels following by brain MRI and genetic tests for the diagnosis of GHD will not identify 13 to 85% of the children with GHD who are currently being treated with GH. We recommend that until future helpful tools become available, the diagnosis of GHD in children be judged on combined clinical, auxological, GHSTs, MRI and molecular parameters.

Effect of chronic stress and antidepressant therapy on IGF-1 and BDNF systems in the brain, and on cognitive performance of rats

Dr. Michal Taler [1] Dr. Irit Gil-Ad [1] Ms. Maya First[1] Mrs. Nurit Novak [1] Prof. Abraham Weizman [1]

1. Lab of Biological Psychiatry, FMRC and Sackler Faculty of Medicine, Tel Aviv University, Israel

Introduction: Introduction: Depression is associated with hippocampus (Hip) volume loss. Chronic mild stress (CMS) in rats is an established model of depression. Antidepressants appear to protect against hip volume loss and to reverse the symptoms of stressed animals. Our goals were to evaluate the effect of CMS and antidepressants (fluoxetine, reboxetine) therapy on hip and prefrontal cortex (PFC) IGF1 and BDNF systems levels in rat and on behavioral and cognitive parameters in the Morris Water Maze (MWM).

Methods: Male rats (SD) were exposed sequentially, over a period of 3 weeks, to a variety of mild stressors (Wilner 1997). Animals received antidepressant therapy (5mg/kg/day ip). Control rats received the same treatment without stress. After 3 weeks of CMS animals were exposed to MWM. After autopsy brains were dissected and the PFC and the hippocampus were separated.

Results: Body weight gain and food intake was decreased in the antidepressant treated rats. In the MWM we found a facilitating effect of fluoxetine in the acquisition phase of non-stressed animals, and in the reboxetine animals we found an improvement on the 1st day of acquisition in the stressed rats. Fluoxetine increased IGF-1 receptor levels in the Hip and normalized the mRNA levels of IGF1 in PFC of stressed animals. Reboxetine increased BDNF and its receptor TrkB in the PFC and Hip of stressed animals, while fluoxetine increased BDNF in the PFC of control rats and normalized the BDNF levels in Hip of stressed animals.

Conclusions: We suggest that decreased neurotrophins in the brain following CMS may contribute to depression and anxiety, which are reversed by antidepressant therapy. The spatial cognitive performance in the MWM was also slightly improved by the antidepressants.

The role of Urocortin peptides in regulating the central stress response: Evidence from a novel Urocortin triple knockout mouse model

Dr. Michael Tsoory [1] Miss Adi Neufeld [1] Mr. Dimitry Getselter[1] Dr. Alon Chen [1]

1. Department of Neurobiology, Weizmann Institute of Science, Rehovot, Israel.

Introduction: Introduction: The CRF-peptide family includes corticotropin releasing factor (CRF) and Urocortins (Ucn) 1, 2 and 3. These peptides integrate the neuroendocrine, autonomic and behavioral responses to stressors through selective activation of two receptors, CRFR1 and CRFR2, both widely expressed in stress-related nuclei in the brain. CRF activates CRFR1, Ucn1 activates both CRFR1 and CRFR2, whereas Ucns 2 and 3 activate CRFR2. Accumulating evidence suggest opposing roles in regulating the central stress response for these two CRF receptors systems, the CRF-R1 system was found critical for initiating stress responses while the CRF-R2 system appears principal for reestablishing homeostasis.

Methods: This study evaluated the role of Ucn 1, 2 and 3 in regulating the central stress response by utilizing a novel knockout mice model we generated, lacking all three known Ucns (tripleUcnKO). We compared anxiety indices of tripleUcnKO mice with those of wild-type mice (WT), obtained from the same colony, under basal conditions and both immediately and 24 hours following exposure to an acute stressor.

Results: Under basal conditions and immediately following exposure to acute stress, tripleUcnKO mice did not differ from WT mice in most anxiety indices of the Open- Field and Dark- Light transfer tests. However, 24 hrs following the exposure to stress tripleUcnKO mice exhibited increased levels of anxiety compared to WT mice. Furthermore, tripleUcnKO mice continued to appear anxious even 10 days following the stress exposure as indicated by increased freezing in the fear conditioning context test. Furthermore, tripleUcnKO mice exhibited an altered corticosterone profile in response to restraint stress.

Conclusions: Collectively, the results suggest an important role for endogenous CRFR2 ligands in mediating the coping mechanisms following stressful experience and support the tripleUcnKO mouse line as a useful stress sensitive mouse model. Further elucidating the role of central Ucns in mediating the central stress response may provide new insights for developing therapeutic tools for stress- related psychopathologies.

Perifornical Urocortin 3 mediates the link between stress-induced mood and energy homeostasis.

Mrs. Yael Kuperman [1] Miss Orna Issler [1] Mrs. Limor Regev[1] Miss Ifat Musseri [1] Dr. Alon Chen [1]

1. Department of Neurobiology, Weizmann Institute of Science, Rehovot, Israel .

Introduction: In modern western societies, the high stress load correlates with increasing incidence of both obesity and metabolic syndrome which have reached epidemic proportions over the past decades. Understanding the mechanisms that mediate the reciprocal relationships between stress-related behaviors and metabolic homeostasis may provide novel insights into the pathophysiology of both mental and metabolic conditions. Studies conducted using corticotropin-releasing factor receptor type-2 (CRFR2) -null mice provide evidence that in addition to its role in mediating stress-related behavior central CRFR2 is important in modulating metabolic rate, appetite and feeding behaviors. Urocortin 3 (Ucn3), a selective CRFR2 ligand, expressed by the perifornical area (PFA) projects to both the lateral septum and the ventromedial hypothalamus. These brain nuclei express high levels of CRFR2 and are considered as behavioral and metabolic related nuclei, respectively. Therefore, PFA-Ucn3 is a potential modulator of the stress response and its metabolic consequences

Methods: In order to study the involvement of this PFA-Ucn3-CRFR2 pathway in mediating stress-induced behavioral and metabolic changes we generated transgenic mice model that over-express Ucn3 in an inducible manner using the Tet-On system. Transgenic mice, carrying the tetracycline responsive element sequence upstream to the Ucn3 coding sequence, were injected with lentiviruses expressing the reverse tetracycline transactivator protein, specifically into the PFA. These mice were tested, for variety of stress-related behavioral performances and their metabolic parameters were evaluated with or without the presence of the inducer doxycycline.

Results: Ucn3 over-expressing mice showed an increase in anxiety-like behavior, as measured by the light/dark transfer and the open field tests. Indirect calorimetry revealed augmentation in oxygen consumption, carbon dioxide production and heat production, indices of whole body energy expenditure. In addition, though PFA Ucn3 over-expressing mice have normal glucose tolerance, they demonstrate a reduction in insulin sensitivity.

Conclusions: Our results support a regulatory role for PFA Ucn3 in anxiety-like behaviors, energy expenditure and glucose metabolism. We suggest that PFA Ucn3 may mediate the link between stress-induced mood and energy homeostasis

Amygdalar CRF knockdown attenuates stress-induced anxiety

Ms. Limor Regev [1] Dr. Alon Chen [1]

1. Department of Neurobiology, Weizmann Institute of Science, Rehovot, Israel .

Introduction: The corticotropin-releasing factor (CRF) neuropeptide is essential regulator of the neuroendocrine stress response and is implicated in the control and maintenance of homeostasis. Flaws in the regulation of the stress-response can have severe psychological and physiological consequences. Dysregulation of the CRF system has been proposed to be involved in the development of anxiety disorders and depression. Central administration of CRF was demonstrated to increase anxiogenic-like behavior in rodents, affect that could be blocked by specific CRF receptor antagonist, thus strengthening the importance of the CRF as central players in modulating anxiety-like behavior.

Methods: To further study the relative contribution of CRF, endogenously expressed by anxiety-linked brain structures, to anxiety-like behavior in mice, we designed and generated lentiviruses expressing shRNA aimed to knockdown CRF expression levels. In-vitro and In-vivo validation of these lentiviruses demonstrated their ability to knock down the expression levels of CRF. Following validation, lentiviruses expressing CRF shRNA or control lentiviruses expressing shRNA for non-related gene, were stereotaxicly injected bilaterally to the central nucleus of the amygdala (CeA) of male C57B/6 mice, thus creating transgenic mice expressing lower levels of CRF specifically at site of injection. A panel of anxiety and depression-like behavior tests were performed to elucidate the effect of CRF manipulation under both basal and stress-induced conditions. These studies were followed by locomotion, learning and memory tests and histological and morphological analysis.

Results: Results obtained from this study demonstrated that CRF knockdown at the CeA does not affect basal anxiety-like behavior, but significantly attenuates the anxiogenic effect of stress, as compared with control groups. CeA CRF knockdown has also reduced depression-like behavior, compared with control-injected mice. Interestingly, additional amygdala-related functions, such as learning & memory processes were not affected by the CRF manipulation.

Conclusions: Our results support the role of amygdalar CRF in mediating stress-induced anxiety, and depression-like behaviors. Behavioral changes observed in our current model suggests that lower levels of CRF at the CeA are sufficient for inducing normal behavioral response under low levels of stressful challenge but not during higher stressful demands. Overall, our data suggests a role for amygdalar CRF in mediating the behavioral stress response under enhanced challenged conditions and further support the manipulation of amygdalar CRF pathways as a possible target for the treatment for anxiety disorders.

Three uneventful pregnancies in one patient, with ectopic Corticotropin-Secreting, SSTR2 Positive bronchial Carcinoid, treated with Octreotide LAR throughout all trimesters.

Dr. Deeb Daoud [1] Dr. Adnan Zaina [1] Dr. Michal Armoni[1] Prof. Eddy Karnieli [1] Dr. Ali Yehia [2] Prof. George Kontogeorgos [3]

1. Endocrinology, Diabetes & Metabolism. Rambam Health Campus. Haifa, Israel
2. Medicine E. Rambam Health Campus. Haifa, Israel
3. Pathology, G. Gennimatas Athens General Hospital. Athens, Greece

Introduction: Feasibility and safety of Octreotide in human pregnancy is - for the foreseen future - dependent on cumulative case reports in the medical literature. Though infrequent, ectopic ACTH-dependent Cushing syndrome, associated with bronchial carcinoid is the leading etiology (30%) among Ectopic, non-pituitary, (ACTH) corticotropin Secretion (EAS). Bronchial carcinoid EAS had a good prognosis, even in cases where it persists or has multiple lesions. Anecdotal reports in the literature supported Octreotide use in pregnancy, in contrast to common attitude of uncertain safety. In most described cases, octreotide was withheld once pregnancy discovered. However, there are seven reports of uneventful deliveries after Octreotide therapy throughout pregnancy.

Patients/ Methods: We report a case of a 25 year old female which was initially diagnosed as suffering from bronchial Carcinoid-associated EAS Cushing syndrome. After surgical removal, residual disease was treated with permanent Octreotide LAR. Subsequently, she had three successful pregnancies while octreotide LAR treatment was continued throughout all trimesters of these pregnancies. Ketoconazole with Metyrapone failed to achieve desired control of the residual disease. A positive Octreoscan prompted adequate chronic injections of octreotide LAR. Routine follow-up tomography of the chest revealed normal mediastinal lymph nodes until recently. Immunostaining for SSTR subtypes, of material from the resected carcinoid of the lung lobe, revealed positive staining for SSTR -2A and SSTR-2B, while negative for SSTR – 1,3,4 & 5 in the pulmonary tumor.

Results: This is the first time report of three cases of the same mother. This is also the first description of Octreotide use for ectopic ACTH producing bronchial carcinoid during pregnancy.

Conclusions: 1. Octreotide in pregnancy seems to be a feasible and safe treatment, although our case report is insufficient to underpin a routine & regular use of Octreotide LAR during pregnancy. 2. Positive octreoscan confounds response to treatment. We suggest to routinely subtype SSTR in pathological specimen for the sake of rational therapeutic approach once the case is unresponsive, and for future evaluation of wide enough cumulative cases, to re-analyze and draw solid recommendations.