Poster Abstracts Group A

RAPAMYCIN ENHANCES AUTOPHAGY AND AMELIORATES DIABETES IN THE AKITA MODEL OF ER STRESS-INDUCED DIABETES

Etty Bachar-Wikstrom[1], Jakob D Wikstrom[1], Yafa Ariav[1], Nurit Kaiser[1], Erol Cerasi[1], Gil Leibowitz[1]

1 Endocrinology, Hadassah-Hebrew University medical school, Jerusalem, Israel

Stimulation of autophagy may protect cells through clearance of misfolded proteins and damaged organelles. mTORC1 is an important nutrient sensor which inhibits autophagy. We have recently shown that hyperglycemia stimulates mTORC1 in beta-cells, resulting in exacerbation of ER stress and apoptosis, while using mTORC1 inhibitor rapamycin results in the alleviation of beta-cell stress and survival.However, the mechanisms of rapamycin protection against ER stress-induced diabetesin vivoare currently not known. We studied the effects of rapamycin on autophagy, ER stress and beta-cell function inAkitamice, an animal model of ER stress induced diabetes. DiabeticAkitamutant and healthy wild-type mice were treated by daily injections of rapamycin for 2 weeks. The glucose tolerance of rapamycin-treated animals was improved together with partial preservation of pancreatic insulin content and enhancement of glucose-stimulatedinsulin secretion. Morphometric analysis revealed distorted islet morphology ,increased apoptosis and proliferation in the diabetic animals vs. controls, with no change in beta cell mass while rapamycin treatment partially corrected this phenotype. Treatment ofclonal Akitabeta–cells with rapamycin for 16 h reduced cellular stress, as evident by decreased JNK and cJUN phosphorylation CHOP and cleaved caspase 3 expression, without affecting protein synthesis., LC3-IIlevels with and without the lysosome inhibitor Bafilomycin A1 were higher in Akita than in wild-type cells. Rapamycin stimulated autophagy as shown by increased expression of LC3-IIlevels and decreased expression of P62. Live imaging analysis in clonalAkitacells showed increased accumulation of P62 and LC3 in the akita compare to WT, while treatment of rapamycin enhanced P62 accumulation and co-localization with the lysosomal marker LAMP1. Inhibition of autophagy by chloroquine or Bafilomycin A1 induced cellular stress and apoptosis and abolished the beneficial effect of rapamycin. Collectively, we suggest that rapamycin alleviates ER stress-induced diabetes by stimulating autophagy leading to enhanced disposal of misfolded proteins, thereby improving beta-cell function and survival.

ER MORPHOLOGY IN PANCREATIC BETA CELLS: REGULATION BY FATTY ACIDS AND AMPK

Jakob Wikstrom[1], Avital Swisa[2], Erol Cerasi[1], Gil Leibowitz[1]

1 Endocrinology and Metabolism Service, Hadassah-Hebrew University Medical Center, Jerusalem, Israel

2 Department of Developmental Biology and Cancer Research and Molecular Biology, The Institute for Medical Research Israel-Canada, The Hebrew University-Hadassah Medical School,, Jerusalem, Israel

The morphology of organelles is critical to their function. Previous studies showed that the ER is dilated in diabetic beta-cells and under conditions of fatty acid-induced ER stress; however the mechanisms involved are not known. Here we present a detailed description of ER morphology in healthy and stressed beta cells and report on its regulation. INS-1E beta cells and mouse islets were treated with palmitate, oleate and different pharmacological inducers of ER stress. Live cell confocal microscopy and electron microscopy showed that treatment with the saturated free fatty acid (FFA) palmitate or the SERCA inhibitor thapsigargin, but not with the unsaturated FFA oleate resulted in a rapid transition of ER morphology from a predominantly tubular to a cisternal appearance. Fluorescence Recovery After Photo-bleaching did not show any change in ER connectivity. ER transformation in beta-cells treated with palmitate preceded the stimulation of the unfolded protein response (UPR) and the induction of apoptosis. Notably, the observed changes in ER morphology were reversible following removal of palmitate from the incubation medium. Inhibition of mitochondrial beta-oxidation by etomoxir did not reverse the morphology changes. By contrast, the AMP-activated protein kinase (AMPK) stimulators AICAR and metformin nearly completely reversed the ER morphology changes induced by palmitate. In summary, we found that in beta-cells, induction of ER stress by palmitate or thapsigargin results in a unique transition from tubular to cisternal ER and identify AMPK as a novel regulator of ER morphology.

GROWTH AND METABOLIC CONTROL IN PATIENTS WITH TYPE 1 DIABETES AND CELIAC DISEASE- A LONGITUDINAL OBSERVATIONAL

Irit Taler[1], Moshe Phillip[2,4], Yael Lebenthal[2], Liat De Vries[2,4], Ranan Shamir[3,4], **Shlomit Shalitin[2,4]**

1 Department of Pediatrics B, Schneider Children's Medical Center of Israel, Petach Tikva, Israel

2 Jesse Z and Lea Shafer Institute of Endocrinology and Diabetes, National Center for Childhood Diabetes, Schneider Children's Medical Center of Israel, Petach Tikva,, Israel

3 Institute of Gastroenterology, Nutrition and Liver Diseases, Schneider Children's Medical Center of Israel, Petach Tikva, Israel

4 Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

Background: The occurrence of celiac disease (CD) in patients with type 1 diabetes (T1D) is increasing.

Objective: To determine the effect of CD on growth and glycemic control in patients with T1D, and the effects of adherence to gluten-free diet (GFD) on these parameters.

Patients and Methods: A longitudinal retrospective case-control design was used. The medical files of 68 patients with T1D and jejunal-biopsy-confirmed CD were reviewed for data on weight, height, HbA1c, frequency of diabetic ketoacidosis (DKA) and severe hypoglycemic events before and after diagnosis and treatment of CD. Findings were compared with 131 patients with T1D only matched for age, gender, and duration of diabetes.

Results: CD was diagnosed in 5.5% of all patients with T1D attending our center during the study period; 26% of the patients with CD were symptomatic. There were no significant differences in glycemic control or frequency of severe hypoglycemia or DKA events between the study and control groups**.** Body mass index-standard deviation score (SDS), height-SDS, and HbA1c values were marginally but not significantly higher in the control than the study group, and similar in subjects with CD with good or fair/poor adherence to a GFD throughout follow-up.

Conclusions: There is limited evidence that adherence to GFD in patients with T1D and asymptomatic CD improves growth or metabolic control. At present, the decision to impose more dietary restrictions of GFD on patients with T1D and CD should be weighed against possible short- and long-term consequences of no intervention.

DIFFERENTIAL REGULATION OF NOX1 AND NOX4 IN ESTRADIOL-17β MEDIATED INHIBITION OF HUMAN VASCULAR SMOOTH MUSCLE CELL PROLIFERATION

Sigal Shaklai[1], Meital Grafi-Cohen[1], Gary Weisinger[1], Esther Knoll[1], Ariel Many[1], Rona Limor[1], Naftali Stern[1,2], Dalia Somjen[1]

1 Endocrinology, Sourasky Medical Center, Israel
2 Medical School, Tel- Aviv University, Israel

Background: The lower incidence of cardiovascular disease (CVD) in premenopausal women than men has led to the speculation that estradiol-17β (E_2) plays a protective role in vascular pathologies. Clinical studies however, have shown equivocal results. The "timing hypothesis" attempts to settle the discordance, suggesting that E_2 treatment is protective only in early menopause before accumulation of vascular pathologies. E_2 is a redox active substance. Reactive oxygen species (ROS) function as second messengers but upon accumulation lead to age related vascular pathologies. Thus, if ROS mediate E_2 effects on the vasculature, when overplayed or poorly timed, could prove harmful.

Aims: We hypothesized that E_2 inhibition of human vascular smooth muscle cell (hVSMC) proliferation, a presumably protective vascular effect, is mediated at least in part by NADPH oxidase (Nox) dependent ROS formation.

Methods: hVSMCs treated with E_2, PPT and DPN (ERα and ERβ agonists respectively) were assessed for ROS, Nox1 and Nox4 expression and proliferation using the 2',7'-DCF fluorescent and NBT colorimetric methods, western blot analysis, semi quantative RT-PCR and ^{3}[H]-thymidine incorporation.

Results: ROS production peaked at 1h exposure to E_2, PPT and DPN. E_2 led to an early transient reduction in Nox1 protein with a concomitant increase in Nox1 mRNA. hVSMC proliferation was inhibited after 24h exposure to 30nM E_2, DPN and the phytoestrogen Daidzein. Pre-treatment with Nox inhibitors DPI and STK prevented E_2 mediated ROS production and inhibition of cell proliferation.

Conclusions: Our results suggest that Nox derived ROS participate in E_2 mediated inhibition of hVSMC proliferation. It appears that in hVSMCs Nox1 expression is induced by E_2 while Nox4 expression is unaffected. Our results may further suggest that Nox1 activity is regulated at the protein level through a feedback mechanism in which increased activity leads to early protein degradation with rapid return to baseline via induction of transcription.

THE PRO-ATHEROGENIC EFFECT OF CHRONIC NITRIC OXIDE (NO) SYNTHESIS INHIBITION IN APOE-NULL MICE IS DEPENDENT ON THE PRESENCE OF PPARα

Michal Vechoropoulos[1], Maya Ish Shalom[1], Jessica Sack[1], Naftali Stern[1], **Karen Tordjman[1]**

1 Institute of Endocrinology, Metabolism, and Hypertension, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel

Inhibition of NO synthesis accelerates plaque formation in apoE-null mice. We have previously shown that PPARα-deficient ApoE-null mice are protected from atherosclerosis. We hypothesized that the absence of PPARαcould protect apoE-null mice from the worsening of atherosclerosis induced by NO synthesis inhibition with L-NAME.

Methods: At 4 weeks, apoE-null or apoE/PPARα-null mice were given a sub-pressor dose of L-NAME in the drinking water or regular water (control). 4 weeks later, the mice were switched to a Western diet for 12 weeks, while L-NAME treatment continued throughout the experiment. Each group consisted of approximately 20 mice. At the end of the experiment, serum chemistry was analyzed, aortas were harvested for NADPH oxidase activity determination, an indirect assessment of angiotensin II action, and atherosclerosis was assessed from oil-red-O-stained lesions at the aortic sinus.

Results: As expected, lipid levels were higher in control apoE/PPARα-null mice: cholesterol 1451±147 vs 737±93 mg/dl for apoE-null, P=0.0001; triglycerides 289±48 vs 86±6 mg/dl, P<0.0001. However, L-NAME treatment increased cholesterol in the apoE-null mice to 1021±63 mg/dl, P=0.01, but was without effect in the apoE/PPARα-null animals. A similar effect of L-NAME was seen for triglycerides. Aortic NADPH oxidase activity was identical in control animals 1243±259 RLU/mg/min in apoE-null vs 1280±159 inapoE/PPARα-null. However, in the apoE-null mice it rose to 2587±476 RLU/mg/min with L-NAME treatment, while it remained essentially unchanged in the apoE/PPARα-null animals, P<0.05.

Finally, while atherosclerosis was about 18% less pronounced in the apoE/PPARα-null control animals, it was also completely unaffected by L-NAME treatment, whereas it went up by about 32% in the apoE-null mice. Hence, by the end of treatment it encompassed 53.8+1.6% of the sinus in the apoE-null mice but only 31+2.7% in the apoE/PPARα-null animals, P<0.05.

Conclusions: The data suggest that in apoE-null mice, in the absence of NO the pro-oxidative and pro-atherogenic effects of angiotensin II are driven by PPARα, independently of the prevailing lipid levels.

ANALYSIS OF THE EFFECTS OF INSULIN ANALOGUES ON PROLIFERATION AND DIFFERENTIATION IN SKIN KERATINOCYTES

Ravid Solomon[1,2], Galina Weingarten[2], Zvi Laron[3], Haim Werner[1], Efrat Wertheimer[2]

1 Human Molecular Genetics and Biochemistry, Tel Aviv University, Tel Aviv, Israel
2 Pathology, Tel Aviv University, Tel Aviv, Israel
3 Endocrinology and Diabetes Research Unit, Schneider Children's Medical Center, Petah Tikva, Israel

Background: Exogenous insulin is the only treatment available for type 1 diabetes patients and for many type 2 diabetes patients. The long-acting insulin analogues (glargine, detemir) mimic the basal insulin secretion whereas the short-acting analogues (lispro, aspart) mimic the bolus secretion after a meal. Dermatological ailments are among the most serious complications associated with diabetes. The insulin and IGF-! receptors are expressed in skin keratinocytes and can be stimulated by insulin and IGF-1. We have previously shown that both insulin and IGF-1 lead to increased proliferation of keratinocytes. However, whereas insulin supported keratinocytes differentiation, IGF-1 inhibited this process.

The aim: of the present study was to examine the proliferative and differentiative effects of short- and long-acting insulin analogues in keratinocytes in comparison to regular human insulin and IGF-1.

Materials and methods: Primary cultures of keratinocytes were produced from newborn BalB/C mice skin using methods established in our lab. Glucose uptake was examined using 2-deoxyglucose uptake, proliferation rate was assessed by means of thymidine incorporation, differentiation was evaluated by western blots with specific antibodies against markers of skin differentiation, and IGF-1 receptor phosphorylation was assessed by immunoprecipitation.

Results: Treatment of keratinocytes with insulin, IGF-1, glargine, detemir, lispro or aspart led to a significant elevation in glucose uptake. In addition, all of these treatments resulted in marked elevations in proliferation rates. Treatment of keratinocytes with glargine or detemir resulted in an insulin-like effect on the differentiation process, while lispro and aspart led to an IGF-1 like effect. Finally, treatment of keratinocytes with aspart led to a unique dynamic of IGF-1 receptor phosphorylation.

Conclusions: Our studies provide evidence that Insulin analogues elicit atypical actions in the skin. In particular, the mitogenic effects of lispro and aspart should be taken into account when choosing a therapeutic strategy for diabetic patients.

EVALUATION OF THE BIOLOGICAL ACTIONS OF INSULIN ANALOGUES IN ENDOMETRIAL CANCER CELL LINES

Daniel Balas Aizen[1], Doron Weinstein[1], Rive Sarfstein[1], Ilan Bruchim[3], Zvi Laron[2], Haim Werner[1]

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

2 Endocrine and Diabetes Research Unit, Schneider Children's Medical Center, Petah Tikva, Israel

3 Gynecologic Oncology Unit, Obstetric and Gynecology Meir Medical Center, Kfar Sava, Israel

Background: Insulin analogues have been developed to achieve further improvements in the therapy of diabetes. The potential safety risk of insulin analogues stems from the fact that the modifications introduced into the native insulin molecule, in addition to altering their pharmacokinetic properties, may enhance their affinity for the IGF-I receptor (IGF-IR). The IGF-IR, which displays a large similarity to the insulin receptor (IR), has been correlated with a number of neoplastic processes and is usually overexpressed in many types of cancer. Endometrial cancer exhibits a significant association with obesity and diabetes. We hypothesized that insulin analogues may elicit atypical proliferative and signaling activities in endometrial cancer cells.

Methods: The ECC-1 and USPC-1 endometrial cancer cell lines were used in this study. Proliferative and antiapoptotic effects of insulin analogues were determined by MTT assays and PARP measurements, respectively. The signaling pathways elicited by the analogues were assessed by Western blots.

Results: MTT assays revealed that insulin glargine increased proliferation rates in both cell lines. The proliferative response in ECC-1 cells displayed a dose-dependent curve whereas, in contrast, cells exposed to IGF-I and regular insulin reached plateau values. In addition, apoptosis measurements demonstrated that insulin glargine prevented PARP cleavage in USPC-1 cells. Furthermore, both insulin glargine and detemir were able to induce Akt and ERK phoshorylation in both endometrial cancer cell lines.

Conclusion: In summary, our data indicate that long-acting insulin analogue glargine, but not detemir, unlike regular insulin, exhibits IGF-I-like proliferative and anti-apoptotic effects in both endometrial cancer cell lines. Short-acting insulin analogues lispro and aspart exhibit enhanced proliferative activities only in ECC cells. Finally, insulin glargine and detemir elicit atypical signaling activities in both cell lines. The clinical implications of our findings in endometrial cancer must be critically evaluated.

INSULIN RECEPTOR COMPENSATES FOR IGF-IR INHIBITION AND INDUCES MITOGENIC ACTIVITY IN PROSTATE CANCER CELL LINES

Doron Weinstein[1], Zvi Laron[2], Haim Werner[1]

1 Human Molecular Genetics and Biochemistry, Tel Aviv University, Tel Aviv, Israel
2 Endocrine and Diabetes Research Unit, Schneider Children's Hospital, Petah Tikva, Israel

Background: IGF-IR targeting emerged in recent years as a promising therapeutic approach in prostate cancer (PCa). The insulin receptor (IR) shares high structure homology with the IGF-IR and activates similar signaling cascades. In recent studies we have shown that insulin's mitogenic activities in PCa cells are mediated via the IR. Given the central role of IR in regulation of metabolism, most anti-IGF-IR therapeutic strategies were designed to specifically inhibit IGF-IR, but not the IR. Therefore, the aim of this study was to investigate IR ability to compensate and mediate IGF-I mitogenic signals after IGF-IR inhibition.

Methods: We employed P69 and C4-2 prostate cancer-derived cell lines, which express both IGF-IR and IR. To specifically inhibit IGF-IR we used monoclonal antibody IMAC-A12 (ImClone) or Thyrphostin (AG1024). Activation of receptors was assessed by immunoprecipitation assays. Expression levels of receptors and activation of signaling cascades were measured by western immunoblotting. Cells viability was measured by MTT assays.

Results: IGF-I mainly activates its own receptor but also led to significant cross-activation of the IR. Results of IP assays showed that specific inhibition of IGF-IR in C4-2 cells did not affect cross-activation of IR by IGF-I. In P69 cells, on the other hand, there was a minor inhibition of cross-activation. In neither cell line, IGF-IR inhibition affected the activity of signaling cascade proteins Akt and ERK. Finally, MTT assays revealed that, despite a significant reduction in IGF-IR abundance on cell membranes, IMAC-A12 had no effect on cells viability.

Conclusion: In our model, specific inhibition of IGF-IR had no effect on the activation of IR by IGF-I, providing evidence for a compensation mechanism by IR following IGF-IR inhibition. The evidence for a compensatory mechanism between the two receptors may be of future use for the local targeting of both IGF-IR and IR in PCa.

METFORMIN DISPLAYS ANTIPROLIFERATIVE ACTIVITIES IN ENDOMETRIAL CANCER CELLS VIA INTERACTION WITH THE IGF-IR SIGNALING AXIS

Rive Sarfstein[1], Yael Friedman[1], Zohar Attias[1], Menahem Sarig[2], Ami Fishman[2], Ilan Bruchim[2], Haim Werner[1]

1 Human Molecular Genetics and Biochemistry, Tel Aviv University, Tel Aviv, Israel
2 Gynecologic Oncology Unit, Meir Medical Center, Kfar Saba, Israel

Background: Endometrial cancer is the most common gynecologic cancer in the Western world. Studies have shown a correlation between obesity and endometrial cancer risk. Metformin is an anti-diabetic drug with potential anti-neoplastic actions. The mechanisms of action of IGF-I are interconnected to the insulin signaling pathways. Therefore, the aim of this study was to examine the hypothesis that the antiproliferative actions of metformin are potentially mediated via suppression of the IGF-I receptor pathway.

Materials and Methods: Human endometrioid Ishikawa and ECC (Type I) and serous papillary (USPC-2 and USPC-1; Type II) endometrial cancer cell lines were treated with metformin (10 mM), in the presence or absence of IGF-I. The expression and activation (phosphorylation) of specific genes involved in IGF signaling was evaluated by Western blots. Apoptosis was evaluated by cleavage of PARP, caspase-3, and Bcl2 measurements. Cells viability was measured by MTT assays.

Results: Metformin decreased the IGF-I stimulated phosphorylation of IGF-IR in ECC-1, USPC-1 and USPC-2 cells. Metformin up-regulated AKT and ERK1/2 phosphorylation in Ishikawa, ECC-1 and USPC-1 cells and down-regulated them in USPC-2 cells. In addition, metformin down-regulated the expression of total IGF-IR and insulin receptor in USPC-1 cells and up-regulated IGF-IR levels in ECC-1 cells. Metformin had no effect on ERK1/2 expression in neither cell line. In addition, the data showed that metformin induced a significant increase in cleaved PARP in USPC-1 and USPC-2 cells. Finally, MTT assays showed that metformin caused a decrease in proliferation rate compared with control cells.

Conclusions: In summary our studies demonstrate that metformin displays potent apoptotic and anti-mitogenic actions in endometrial cancer cells that are mediated, at least in part, via interaction with the IGF-IR axis. Taken together, our results suggest that rational use of metformin may help reduce endometrial cancer risk.

ROLE OF PKC ISOFORMS IN P38MAPK ACTIVATION BY GNRH IN GONADOTROPE CELLS

Shany Mugami[1], Liat Rahamim Ben-Navi[1], Shani Kravchook[1], Zvi Naor[1]

1 Biochemistry & Molecular biology, TAU University, Tel Aviv, Israel

MAP kinases (MAPKs) play a key role in in the transcriptional control of the gonadotropin subunits gene expression by GnRH. Since PKC is upstream to MAPK activation, we examine here the role of PKC isoforms (PKCs) in GnRH-stimulated p38MAPK in the αT3-1 and LβT2 gonadotrope cells. Incubation of the cells with GnRH or with phorbol-12-myristate-13-acetate (PMA; a PKC activator)) resulted in a protracted activation of p38. Gonadotropes express conventional PKCα and PKCβII, novel PKCδ, PKCε and PKCθ and atypical PKC-ι/λ. The use of dominant-negative PKCs has revealed that PKCδ and PKCε mediate p38 activation by GnRH. Furthermore, the use of siRNA for PKCδ produced a similar inhibition to that obtained with the dominant negative plasmid. Unlike the dogma that p38 is localized in the nucleus of various cells, we localized p38 to the plasma membrane. Since GnRH induces migration of gonadotropes, we will examine the role of the activated p38MAPK in migration of the gonadotrope cells. The physiological significance of gonadotropes migration is under investigation.

YIN YAN ROLE OF ESTROGENIC SIGNALING IN HUMAN MALIGNANT CELL GROWTH:CONCOMITANT UP REGULATION OF ERS, LIPOXYGENASES AND 1α-HYDROXYLASE 25- HYDROXY VITAMIN D MRNA EXPRESSION

Dalia Somjen[1], Fortune Kohen[2], Sara Katzburg[1], Orli Sharon[1], Esther Knoll[1], Naftali Stern[1]

1 Institute of Endocrinology, Metabolism and Hypertension, Tel-Aviv Sourasky Medical Center and Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Tel-Aviv, Israel

2 Department of Biological Regulation, The Weizmann Institute of Science, Rehovot, Israel

Estrogen receptors (ERs),1α-hydroxylase 25-hydroxyvitamin D(1OHase) and lipoxygenase (LO) are expressed in non-reproductive cancerous cells and the 12- and 15hydroxyeicosatetraenoic acid (HETEs) produced enhance cell survival. We investigated whether: estradiol-17β(E_2), biochainin A (BA), carboxy- BA (cBA) or dihydrotestosterone (DHT) affect adrenal (H295R), colon (320D), ovarian (A2870) and prostate (C4-24) carcinoma cells. All cells expressed ERα, ERβ, 1OHase and 15LO mRNA and produce 15HETEs. The ERβto ERαmRNA ratio was 1.8:1, 30:1, 292:1, 71:1 in H295R, 320D, A2870, and C4-24, respectively. DHT and cBA increased ERαin 320D and H295R, while BA decreased it in 320D. DHT increasedERβin A2780, 320D and H295R, decreased it in C4-24. E_2stimulated ERβin A2780 and C4-24. BA decreased ERβin A2780 and H295R and stimulated it in 320D and C4-24. cBA decreasedERβin A2780 and C4-24 and stimulated it in 320D and H295R. 1OHase was inhibited by all hormones in C4-24 whereas in 320D all compounds except BA stimulated it. In A2780 BA inhibited 1OHasewhile DHT and cBA stimulated it. In 320D all hormones except BA stimulated 1OHasewhile in H295R E2 and BA inhibited it and cBA stimulated. In H295R all hormones inhibited 15HETEs while in A2780 all hormones except DHT stimulated it. In C4-24 all hormones except BA stimulated LO and 15HETEs, in 320D DHT inhibited while BA and cBA stimulated them. In conclusion human cancer cells express different mRNAs which are hormonally modulated. This forms the basis for cancer type-specific estogen/androgen agonists/antagonists affinity drug targeting, whose blockade was shown by us to induce apoptosis (J. Med Chem.50:6405- 6410,2007). This suggests that estrogens affect cancer cell growth via opposing pathways: cell growth acceleration via induction of ER and LO expression and 1OHasethus facilitating 1,25 formation an inhibitor of cell proliferation.

INTRAPANCREATIC ACCESSORY SPLEEN (IPAS) MISDIAGNOSED AS NON-FUNCTIONING PANCREATIC NEUROENDOCRINE TUMOR (PNET): A CASE REPORT AND CHARACTERIZATION OF A SYNDROME BASED ON SYSTEMATIC REVIEW OF THE LITERATURE.

Etty Osher[1], J.M. Klausner[2], Y. Greenman[1], K. Tordjman[1], E. Scapa[3], A. Melhem[3], M. Ben-Haim[2], I. Nachmany[2], E. Shacham-Shmueli[4], R GEVA[4], N. Stern[1], E. Santo[3]

1 Endocrinology, Tel Aviv Sourasky Medical Center, Tel-Aviv, Israel
2 Surgery, Tel Aviv Sourasky Medical Center
3 Gastroenterology, Tel Aviv Sourasky Medical Center
4 Oncology, Tel Aviv Sourasky Medical Center

Introduction: Management of pancreatic incidentalomas(PI) is challenging due to high risk of malignancy and limitations in prediction of their histology and biological behavior. IPAS is one of such lesions and is often misdiagnosed as PNET.

Case: A 65- yrs -old male presented with PI of 1.6, 1.3cm suspected for IPAS on CT but finally misdiagnosed as nonfunctioning PNET.Post-surgical histopathology identified lesions as IPAS.

Literature review: 32 cases of IPAS were described: 15 were correctly diagnosed as IPAS by Tc-99m, FNA stain for CD-8 or contrast enhanced sonography;17 underwent surgery of IPAS misdiagnosed as PNETS .Pancreatic lesion were1)mostly solitary;2)solid on imaging;3) well defined; 4)located predominantly at pancreatic tail (97%) 5)not exceeding 3 cm; mean size 1.6±0.5cm (range 1-3); 6) detected in adults (mean age 54±16 yrs;range 22-81). 7) F/M ratio 16/16. In those referred for surgery imaging studies didn't differentiate between IPAS and PNET. FNA (5/32 cases), all were false positive for PNET. Distal pancreatectomy & splenectomy was carried out in 60%, distal pancreatecomy in 40%.

Conclusion: IPAS should be considered before surgery in PI,even when imaging or FNA are suggestive of PNET.Characteristic archiform splenic enhancement pattern on dynamic CT, imunohistichemical stain for CD-8, nuclear scan as Tc-99m, or contrast enhanced sonography can be used for better preoperative evaluation. This may provide the definitive diagnosis of IPAS and thus avoid unnecessary major surgery.

KLOTHO-INDUCED DISSOCIATION BETWEEN ACTIVATION AND NUCLEAR LOCALIZATION OF EXTRACELLULAR SIGNAL-REGULATED KINASE (ERK) 1/2: A NOVEL GROWTH INHIBITORY MECHANISM IN BREAST CANCER

Tami Rubinek[1], **Shiri Shahmoon[1,2]**, Hagai Ligumsky[1,2], Rony Seger[3], Ido Wolf[1,2]

1 Institute of Oncology, Sheba Medical Center, Israel
2 Sackler Faculty of Medicine, Tel Aviv University
3 Department of Biological Regulation, Weizmann Institute of science

Klotho is a transmembranal protein which can be cleaved, shed and act as a hormone. It is highly expressed in the brain and kidneys, but also in other tissues including pancreas and breast. Klotho-deficient mice manifest a syndrome resembling accelerated aging. Whereas, over expression of klotho in mice extends life span. We recently identified klotho as a potent tumor suppressor in breast and pancreas cancer. Klotho is also an important regulator of calcium homeostasis, as it regulates the activity of several calcium channels, mainly TRPV5/6, resulting in increased intra-cellular levels of calcium.

The ERK cascade plays a major role in tumorigenesis. Upon activation of the ERK cascade, ERK1/2 are phosphorylated and translocate to the nucleus, where they phosphorylate numerous substrates. Surprisingly, we found that klotho-induced growth inhibition is accompanied by phosphorylation of ERK1/2. It was shown that increased intra-cellular levels of calcium enhance the phosphorylation of ERK1/2 while inhibiting their nuclear translocation, resulting in apoptosis.

AsTRPV6 is abundantly expressed in breast cancer and can be activated by klotho, we hypothesize that klotho dissociates between phosphorylation and nuclear translocation of ERK1/2 by upregulating intra-cellular calcium levels, resulting in cell apoptosis

TRPV6 over-expression inhibits colony formation of MCF-7 breast cancer cells, and co-transfection of TRPV6 and klotho further inhibited colony formation. Localization studies revealed that klothorecruited and anchored TRPV6 to the plasma membrane. Co-immunoprecipitation analysis indicated direct interaction between TRPV6 and klotho. Klotho regulated several calcium-regulating genes, including S100A6, calcium binding protein and STIM1Stromal interaction molecule 1.As observed by fluorescence imaging, klotho inhibited EGF-induced ERK1/2 translocation to the nucleus. These results suggest a uniqueregulatory mechanism of klotho in breast cancer cells, involving activation of TRPV6, and leading to ERK1/2 cytoplasmtic retention, resulting in decreased cell growth.

THE MECHANISMS INVOLVED IN THE ENHANCED RISK FOR BREAST CANCER ASSOCIATED WITH HYPERINULINEMIA

Ran Rostoker[1], Keren Bitton-Worms[1], Eyal Scheinman[1], Derek Leroith[1]

1 Diabetes and Metabolism Clinical Research Center of Excellence, Ruth and Bruce Rappaport Faculty of Medicine, Haifa, Israel

Breast cancer is currently the most common cancer among women in industrialized countries. Recent epidemiologic studies demonstrate a positive correlation between circulating insulin levels and breast cancer risk and mortality.

MKR mouse serve as a model for type 2 diabetes (T2D). The female MKR mice develop a mild diabetic phenotype, which recapitulates early stages of T2D (pre-diabetes) in humans, this phenotype makes them ideal for exploring the link between high circulating insulin levels and breast cancer initiation and progression. Recent studies, with MKR female mice demonstrated accelerated mammary gland development and breast cancer progression. The signaling axis which mediates this effect includes insulin, insulin receptor (IR)/IGF-IR, and the PI3K/Akt pathways. In order to find whether insulin mitogenic signals occurs mainly throw the IR we are using the recently developed high-affinity biosynthetic insulin receptor antagonist S961. In the present study we show that the highly metastatic mouse mammary tumor cell line MVT-1 are sensitive to insulin activation, as demonstrated by significant AKT phosporylation following a 10 nM insulin stimulation, this effect was completely abolished by pre-treatment with the S961 inhibitor. The strong IR inhibition by S961 was also found in the NIH-3T3IR cells (a cell line over-expressing the insulin receptor). We also demonstrate that S961 specifically inhibits the IR and has almost no effect on the IGF-1R in both cell lines. In addition, we show that insulin stimulated proliferation of both cell lines in a similar fashion, whereas IR specific inhibition by S961 abrogated this effect. Our results suggest that S961 pre-treatment of MKR female inoculated with MVT-1 cells into the inguinal mammary fat pad to create mammary tumors, could help to identify the mitogenic signaling pathway of insulin in breast cancer.