

Profile, target genes and regulation of microRNAs in ovarian carcinoma tumor progression

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Introduction: Ovarian cancer is the leading cause of death from gynecological cancers in western countries. The disease is asymptomatic in the early stages, and is usually diagnosed at an advanced stage. Primary solid tumors, solid metastases, and effusions to the peritoneal and (ascites) pleural cavities characterize the tumor as it progresses. MicroRNAs (miRNAs) are small non-coding RNAs that exert a regulatory effect post-transcriptionally by binding target mRNAs and inhibiting gene translation. miRNA expression is deregulated in cancer. The aim of this study was to characterize the differences in miRNA expression pattern and the miRNA-regulating machinery between ovarian carcinoma cells in primary tumors vs. effusions.

Patients/ Methods: We analyzed snap-frozen primary ovarian carcinoma tumors and cells derived from peritoneal and pleural effusions. microRNA-array platforms were used to profile the expression of miRNAs at the two sites. The results of the array were validated on an independent set of samples by real-time PCR. Putative targets for miRNAs of interest were predicted using web available algorithms. Expression of target genes was assessed by Western blot, expression of the machinery molecules was analyzed by real-time PCR and Western blot.

Results: Using miRNA-array platforms, we identified three sets of miRNAs, one that is highly expressed in both primary carcinomas and effusions, one overexpressed in primary carcinomas, and one overexpressed in effusions. The most significant miRNAs were validated by real-time PCR on a validation set of samples. Our results show concordance between the training and the independent test cohorts for the reduced miR-145 and miR-214 and for the elevated let-7f, miR-182, miR-210, miR-200c, miR-222 and miR-23a in effusions. Using in-silico target prediction programs we identified potential target genes for the above miRNAs. We analyzed the expression of ZEB1 and c-Myc, targets of miR-200c. In addition, we analyzed PAK1 and PTEN, both predicted targets of miR-222. We found inverse correlations between the expression levels of the indicated miRNAs and of the predicted target genes. We further observed higher expression of the miRNA processing molecules Ago1, Ago2 and Dicer in effusions compared to primary carcinomas.

Conclusions: our data are the first to document different miRNA expression and regulation profiles in primary and metastatic ovarian carcinoma, suggesting a role in tumor progression.

Analysis of the interplay between the insulin receptor and IGF-I receptor signaling pathways in prostate cancer

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Introduction: Hyperinsulinemia is a consequence of insulin resistance. Beside its normal spectrum of metabolic effects, insulin also acts as a growth factor and has the ability to promote mitogenic activity. Thus, hyperinsulinemia is regarded a potential risk factor for the development of cancer in patients with diabetes. However, the mechanism of action of insulin in prostatic cancer has not yet been completely elucidated. The aim of this study was to investigate whether insulin induces a mitogenic activity in prostate cancer-derived cell lines via its own receptor or the IGF1 receptor.

Patients/ Methods: we employed a number of prostate cancer cell lines (P69, C4-2 and PC3) representing early and advanced stages of the disease. Insulin doses ranged between 0-500 ng/ml. Insulin-stimulated proliferation rates were measured by hemocytometer cell counting or with an MTT assay. Cell-cycle dynamics were evaluated by propidium iodide staining. Activation of the insulin receptor (IR) was assessed by immunoprecipitation assays. Expression levels of the receptor were measured by western immunoblotting.

Results: Insulin induced cell proliferation in a dose-dependent fashion in the PC3, C4-2 and P69 cell lines. Cell cycle analyses showed that insulin can positively influence C4-2 and P69 cells to progress towards the G2/M phase. With the insulin doses used immunoprecipitation assays showed significant activation of IR, but not IGF-IR.

Conclusions: In the model studied, insulin exhibited direct mitogenic activities mediated exclusively through the IR. Further research is needed to fully dissect the molecular mechanism underlying the biological actions of insulin in prostate cancer.

The t-Boc derivative of 7-(O)-carboxymethyl daidzein is cytotoxic to the human follicular carcinoma cell line WRO and a negative growth modulator in non-malignant human goiter cells in vitro: Potential role of reactive oxygen species (ROS)

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Introduction: The incidence of thyroid cancer is up to 3 folds higher in women than in men, suggesting that estrogenic effects may be involved in the pathogenesis of this malignancy. We previously reported that a novel phytoestrogen derivative generated in our laboratory, the N-t-boc-hexylenediamine derivative of 7-(O)-carboxymethyl daidzein [cDtboC], possess potent anti-cancer effects several human cancer cell types expressing preferentially mRNA for estrogen receptor (ER) β relative to ER α . These earlier studies indicated that cDtboC exerted cytotoxic effects in thyroid cancer by the induction of apoptosis and not through cell necrosis. In the present study we compared the effect of cDtboC in the follicular thyroid carcinoma cell line WRO and cultured primary goiter cells originally harvested from 3 different patients.

Patients/ Methods: Cells were cultured and treated with the different compounds and DNA synthesis, CK specific activity, ROS formation and microscopical appearance were analysed.

Results: Both WRO and primary thyroid cells expressed ER α and ER β with only slightly higher abundance of ER β over ER α . In both WRO and goiter cells DNA synthesis and creatine kinase (CK, a marker of genomic response to estrogen agonists) increased in response to E₂, the ER α agonist PPT and the ER β agonist DPN. Very significantly, cDtboC abolished these effects completely in WRO but only partially in non-malignant goiter cells. Acting in the absence of E₂, cDtboC alone was cytotoxic to WRO, as determined by DNA synthesis indices, the XTT assay and direct microscopic visualization, and much less so to human non-malignant goiter cells. A functionally critical effect of cDtboC was its ability to markedly increase reactive oxygen species (ROS) formation, which was particularly prominent in WRO, but was also detectable in goiter cells. The NADPH-oxidase inhibitor DPI not only abolished ROS formation but also partially inhibited the cytotoxic effects of cDtboC on WRO.

Conclusions: Hence, cDtboC possesses some antiestrogenic properties in thyroid cancer cells and is cytotoxic to thyroid cancer cells and, to a lesser extent, to non-malignant goiterous cells, acting in part via induction of ROS formation. Since WRO cells are clearly estrogen sensitive, thus resembling the estrogen-sensitivity of other thyroid cancer cell lines reported by us earlier (NPA, MRO and ARO), this property can be utilized to design highly effective estrogen-related, anti-thyroid cancer drugs. Further, the present study in goiter cells opens novel pathways through which the growth of non-malignant goiters can possibly be modulated.

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

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Introduction: Endometrial cancer is the most frequent gynecologic cancer in Western countries. The majority of the cases can be divided into two categories: Type I cancers, which represent more than 80% of the cases, exhibit an endometrioid histology, are generally estrogen dependent, and have a good prognosis. Type II 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 evaluate the biological and molecular effects of treatment with a monoclonal antibody against IGF-IR in endometrial cancer cell lines.

Patients/ Methods: Type 1 (ECC-1 and Ishikawa) and Type II (USPC-1 and USPC-2) endometrial cancer cell lines were treated with the A12 monoclonal antibody (ImClone Systems Inc, New York) and assayed for proliferation, apoptosis, cell cycle progression, internalization, and IGF-IR and downstream mediators activation.

Results: A12 effectively inhibited IGF-IR activity in ECC-1, Ishikawa, USPC-1 and USPC-2 cells, whereas it abolished AKT and ERK activity only in ECC-1 and USPC-1 cells. In addition, treatment with A12 on top of IGF-I exhibited a pro-apoptotic activity, as demonstrated by PARP and Caspase-3 cleavage. Furthermore, proliferation assays showed that the inhibitor caused a significant decrease in proliferation rate in ECC-1 and USPC-1 cells. Cell cycle analyses revealed that the antibody caused a progressive accumulation of ECC-1 cells in G0/G1 phases compared to IGF-I-treated cells, with a marked decrease in the percentage of cells in S and G2/M phases. Results of internalization assays revealed that A12 treatment shifted the distribution of IGF-IR from the cell membrane periphery to the cytoplasm in ECC-1 and USPC-2 cells. Furthermore, treatment with the antibody caused a reduction in IGF-IR expression after 24 h and 48 h in all cell lines. Finally, immunoprecipitation analysis revealed that A12 blocked IGF-I-signaling without detectable effects on insulin receptor activation.

Conclusions: Taken together, our results demonstrate that A12 may be an effective therapeutic tool for the treatment of endometrial cancer in which deregulated expression of the IGF-IR plays a critical role. Receptor internalization and degradation seem to be important aspects of the mechanism of action of A12 in these tumors.

Carotenoid derivatives prevent cancer and improve bone health by Inhibition of NFkB and induction of Nrf2 transcription systems

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Introduction: Nrf2 mediates induction of phase II detoxifying and antioxidant enzymes which are responsible for reducing the mutagenic effects of carcinogens and reactive oxygen species. In contrast, activation of the NFkB transcription system increases cancer cell proliferation and tumor metastasis and decreases apoptosis, all of which lead to cancer progression. Recently, a harmful effect of NFkB on bone health has also been shown. Interestingly, under un-stimulated conditions, both Nrf2 and NFkB transcription factors are retained in the cytoplasm by their inhibitory proteins, Keap1 and Ikb, respectively. Phosphorylation of Ikb by the Ikb kinase (IKK) complex is an obligatory step prior to Ikb degradation and activation of NFkB. In addition, both Keap1 (Nrf2 system) and IKK (NFkB system) have been shown to harbor cysteine thiols that are critical for their activity. Various electrophyles have been shown to interact with these cysteines, resulting in activation of Nrf2 and inhibition of the NFkB system. Intact carotenoids such as lycopene and beta-carotene lack such electrophylic groups and we have recently demonstrated that carotenoid derivatives, but not the intact carotenoids, activate the Nrf2 transcription system. The aim of the current study was to examine whether carotenoid derivatives and not the intact carotenoid molecule prevent cancer and improve bone health by stimulating Nrf2 and inhibiting NFkB transcription systems in cancer as well as in bone cells.

Methods: We analyzed the structure-activity relationship of a series of dialdehyde carotenoid derivatives in NFkB inhibition.

Results: diapocarotene-dials inhibited NFkB-driven reporter gene expression in both, cancer and bone cells. Moreover, similar to our previous findings regarding the Nrf2 system, we found that the activity of the carotenoid derivatives depends on the relative position of the methyl group to the terminal aldehyde, which determines the reactivity of the conjugated double bond in reactions such as Michael addition to SH groups in proteins (e.g. Keap1, IKK). Importantly, these derivatives also attenuated IKK activity as seen in western blot analysis of its product: phosphorylated-Ikb. In addition, the carotenoid derivatives inhibited NFkB nuclear translocation and reduced mRNA level of the target gene TNF alpha. These results suggest a novel mechanism for carotenoid beneficial effect on bone health through inhibition of NFkB activity in osteoblast cells.

Conclusions: we suggest that electrophylic carotenoid derivatives contribute to cancer prevention as well as bone health maintenance by two mechanisms: Nrf2 activation and NFkB inhibition. Both could be mediated by modification of SH groups of upstream proteins.

The histone deacetylase inhibitor vorinostat exhibits a potent pro-apoptotic activity in endometrial cancer cell lines

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Introduction: Endometrial cancer is the most common gynecologic cancer in the Western world. Several studies have been shown a correlation between components of the insulin-like growth factor (IGF) system and endometrial cancer risk. Vorinostat (Merck Oncology) is a novel histone deacetylase inhibitor which induces growth arrest, differentiation and/or apoptosis in a variety of transformed cells, including prostate, leukaemia, breast, and colon cancers. The mechanism underlying the antitumour action of vorinostat is not entirely clear but may involve changes in the expression of specific genes via acetylation of histones and transcription factors as well as nontranscriptional effects such as inhibition of mitosis. The aim of this study was to establish whether vorinostat can modify the expression of specific genes related to the IGF-IR signaling pathway and revert the transformed phenotype.

Patients/ Methods: To investigate the effect of vorinostat on the IGF-I signaling pathway, human endometrioid Ishikawa (Type I) and serous papillary (USPC-2, Type II) endometrial cancer cell lines were treated with vorinostat (5uM) for 24 h, in the presence or absence of IGF-I during the last 10 min of the incubation period. The expression and activation (phosphorylation) of specific genes involved in IGF signaling was evaluated by Western blots. Apoptosis was evaluated by cleavage of PARP and caspase-3 measurements.

Results: Vorinostat increased IGF-IR phosphorylation, BRCA1, pTEN, and p21 expression, and reduced Sp1 and p53 protein levels in Ishikawa cells. In addition, vorinostat up-regulated the expression of total IGF-IR, p21 and down-regulated the expression of total AKT, BRCA1, Sp1, p53, and pTEN in USPC-2 cells. Vorinostat did not alter the expression of ERK1/2 in neither cell line. Of interest, IGF-IR activation was associated with a major elevation in IGF-IR promoter activity. In addition, vorinostat treatment induced apoptosis in both cells lines and abolished the anti-apoptotic activity of IGF-I. Next, we investigated whether the pro-apoptotic effect of vorinostat is connected with IGF-IR levels. For this purpose, cells were treated with vorinostat for 24 h, separately and in combination with MK-0646, a humanized monoclonal anti-IGF-IR antibody (Merck Oncology), in the presence or absence of IGF-I. Western blot analysis revealed that vorinostat abolished the anti-apoptotic activity of IGF-I both in the absence or presence of MK-0646, thus suggesting that the pro-apoptotic action of vorinostat is not correlated with IGF-IR levels.

Conclusions: In summary our studies demonstrate that vorinostat exhibits a potent pro-apoptotic effect in both Type I and Type II endometrial cancer cell lines. The mechanism of action of vorinostat, at least in the specific context of endometrial cancer, is most probably an IGF-IR independent mechanism. Future studies will address the molecular nature of these biological effects.

Differential effects of plant polyphenols on leukemia cell differentiation are associated with distinctive changes in Nrf2/ARE and VDR-RXR/VDRE transcription systems

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Introduction: Acute myeloid leukemia (AML) is a hematological malignancy with poor prognosis. Differentiation therapy is an alternative to cytotoxic therapy of AML. 1 α ,25-dihydroxyvitamin D₃ (1,25D) is a strong differentiation inducer in AML cells, but it causes severe hypercalcemia at pharmacologic doses in vivo. We have shown that plant polyphenols, e.g. carnosic acid (CA), curcumin (CUR) and silibinin (SIL), at non-toxic concentrations synergistically enhance the differentiation effects of physiologic concentrations of 1,25D in myeloblastic leukemia cells (HL60). Since CA, CUR and SIL are known antioxidants, here we determined whether this enhancement is associated with activation of the Nrf2/antioxidant response element (Nrf2/ARE) transcription system and/or changes in the levels and activity of the nuclear receptor for 1,25D (VDR/RXR).

Patients/ Methods: Cell proliferation and viability were determined by the trypan blue exclusion assay. Cell differentiation was measured by the expression of CD11b and CD14 markers using flow cytometry and real-time RT-PCR. The levels of VDR and RXR α as well as Nrf2/ARE responsive genes NAD(P)H-quinone oxidoreductase and gamma-glutamylcysteine synthetase were examined using real-time RT-PCR and Western blotting. Activity of vitamin D responsive element (VDRE) and ARE was assessed by the luciferase reporter gene assay.

Results: Plant polyphenols CA (10 μ M), CUR (10 μ M) and SIL (60 μ M) strongly enhanced HL60 cell differentiation induced by 1 nM 1,25D. This was associated with a strong increase in both VDR and RXR α protein levels and VDRE activity. However, in U937 promonocytic leukemia cells, CA and CUR showed only a moderate enhancement of differentiation, and SIL even had an inhibitory effect. Accordingly, CA hardly affected and SIL strongly reduced both 1,25D-stimulated VDRE activity and RXR α levels, though VDR levels tended to increase. Consistent with their antioxidant features, CA and CUR induced ARE transactivation in both HL60 and U937 cells. On the other hand, the antioxidant SIL only moderately activated ARE in HL60 cells and was without effect in U937 cells.

Conclusions: Our results demonstrate that various polyphenols differentially affect cell maturation, depending on AML subtype. Furthermore, polyphenol-induced changes in VDR and RXR α levels are associated with the extent of Nrf2/ARE activation. These findings suggest that Nrf2/ARE activation is required for the upregulation of VDR and RXR α and, thus, for the differentiation-enhancing effect of plant polyphenols. On the other hand, the lack of Nrf2/ARE activation may result in a decline in cellular RXR α levels, leading to inhibition of 1,25D-induced differentiation.

Pathways involved in 1,25(OH)₂D₃ and valproic acid – induced enhanced response of prostate cancer cells to ionizing radiation

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Introduction: We have previously shown that pre-treatment of prostate cancer (PCa) cells with a combination of the active metabolite of vitamin D (1,25(OH)₂D₃) and the sodium salt of valproic acid (VPA) markedly enhanced the damaging effect of ionizing radiation (IR) resulting in increased apoptosis and cell-cycle progression delay. The aim of the present investigation was to study in PCa cells the effect of this combinatorial pre-treatment on intracellular pathways involved in the enhanced response to IR. For this purpose, generation of DNA double-strand breaks (DSBs), activation of DNA-damage checkpoint kinases Chk1 and Chk2, and levels of cell-cycle inhibitory proteins p21Cip1/Waf1 and p27Kip1 were assessed.

Patients/ Methods: Androgen-refractory PCa DU145 cells were grown in RPMI-1640 medium containing 10% FCS. Cancer cells were seeded into two 96-well tissue culture plates. Twenty four hours later, cells were treated with 100 nM 1,25(OH)₂D₃ or 1 mM VPA, or their combination. Control cells were treated with medium containing vehicle 0.1% ethanol. Seventy-two hours later, a test plate was irradiated with a dose of 4 Gy and both irradiated and non-irradiated PCa cells were incubated for additional 3 hours. Induction of DSBs, expressed as levels of phosphorylated histone H2AX, activation (phosphorylation) of Chk1 and Chk2, and expression of p21Cip1/Waf1 and p27Kip1 were assessed by cell-based ELISA.

Results: IR caused a significant generation of DSBs. However, DNA damage in pre-treated cells was greater and reached a maximum in cells pretreated with both VPA and 1,25(OH)₂D₃. DSBs level after IR treatment was increased by 11.7% (p<0.001), and by 18.7%, 20.6% and 34.7% in cells pretreated with VPA (p<0.006), or 1,25(OH)₂D₃ (p<0.03) or both drugs (p<0.001) respectively. Although no effect on Chk1 activity was found following IR treatment, a significant increase in Chk2 activity was observed, Chk2 activity was increased in non-pretreated cells by 23.8% and by 33%-39% in pretreated cells (p<0.05, compared to cells exposed to IR only). IR and VPA alone raised p21Cip1/Waf1 levels by 19.0% (p=0.01) and 32.7% (p<0.005) respectively, while VPA treatment followed by IR increased p21Cip1/Waf1 level by 54.9% (p<0.001). The dual pre-treatment with VPA and 1,25(OH)₂D₃ followed by IR was most effective and increased p21Cip1/Waf1 level by 94.0% (p<0.02). The p27Kip1 expression level was increased by VPA alone by 34.7% (p<0.003), and by VPA followed with IR by 52.6% (p<0.0001). Here again, the most prominent increase (86.5%, p<0.0001) was observed in cells irradiated after combinatorial pretreatment with VPA and 1,25(OH)₂D₃.

Conclusions: The results show that a combination of 1,25(OH)₂D₃ and VPA efficiently sensitizes PCa cells to radiation-induced damage through enhanced activation of intracellular pathways comprising DNA-damage checkpoint kinase Chk2, and cyclin-dependent kinase inhibitors p21Cip1/Waf1 and p27Kip1. The present results suggest the possible use of such pretreatments to enhance IR effect in the treatment of prostate cancer.

Endothelin converting enzyme (ECE)-1: a plausible target gene for hypoxia inducible factor (HIF)

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Introduction: Experimental diabetes is characterized by diminished renal parenchymal oxygenation, particularly at the medulla, with enhanced expression of HIF. This condition, as well as ambient hypoxia per se, triggers ET-1 synthesis. We have recently reported that diabetes augments the expression of ECE-1. The aim of the study is to explore the potential role of HIF α as the link between evolving renal tissue hypoxia and the regulation of ECE-1 expression, by the inhibition of HIF-degradation.

Patients/ Methods: Rats were subjected to the prolyl-hydroxylase inhibitor L-mimosine (600 mg/kg), or to vehicle, and sacrificed 6h later. The right kidney was perfusion-fixed for immunostaining, while the left kidney was dissected and samples of cortex, outer medulla and inner medulla were analyzed for prepro ET-1 and ECE-1 mRNA, and for ET-1 and ECE-1 protein.

Results: Mimosine led to HIF-1 α accumulation mainly in S3 segments of the outer stripe of the outer medulla. This was associated by enhanced pSTAT-3 expression, principally in distal nephron segments both in the cortex and in the outer medulla, and with a 51% and 66% increase in pSTAT-3 in the outer and inner medulla respectively, without a significant effect in the cortex. Both induction of HIF-1 α and pSTAT-3 were associated by a three folds increase in ECE-1 protein expression in the inner and outer medulla.

Conclusions: Enhanced ECE-1 expression in the hypoxic kidney might be triggered by HIF through pSTAT-3.

Identification and characterization of novel pate-like genes that code for secreted, cysteine-rich proteins expressed in reproductive and nervous systems

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Introduction: We previously reported on gene clusters in both humans and mice that code for secreted proteins each comprising ten cysteine residues (Levitin JBC 2008). These conform to TFP/Ly-6/uPAR domains that shape three-fingered proteins. The founding gene is PATE, expressed primarily in prostate and less in testis. We identified 3 additional similar human PATE-like genes that co-localize with the PATE locus. Our study elucidates tissue expression of the human PATE-like genes, and determines their function and expression level in some pathological states. Finally, as PATE-M protein interacts with nicotinic acetylcholine receptors (nAChRs), PATE gene expression in Alzheimer's disease (AD), was investigated.

Patients/ Methods: Anti sera and mAbs were generated in Tel Aviv University. Effect of PATE proteins at the nAChRs were evaluated using the *Xenopus* oocyte cell expression system. In mice, hormonal effect of murine Pate expression was evaluated in the prostate following castration and in the mammary gland during pregnancy. Expression of Pate genes in the mammary glands of pregnant and/or lactating mice was examined by immunohistochemical staining of the relevant tissues. Similar staining was employed for evaluation of human tissues and sperm cells. Expression level of PATE-M gene in Alzheimer's disease patients was determined by using real-time PCR of cDNA from brain samples obtained from normal and AD patients. The results were confirmed at the protein level by immunohistochemical staining in relation to amyloid plaques stainable by the anti-amyloid mAbs.

Results: PATE-like proteins show selective expression in prostatic neuroendocrine cells (Fig. 1 A, black arrows) and human sperm (Fig. 1 B, white arrows). In male mice, the expression of syntenic Pate-like genes was modulated by the androgenic status. However, in ventral lobes the expression was reversibly suppressed in an androgen-deprived milieu. In female mice, expression of certain Pate-like genes was observed only in mammary glands from pregnant or lactating mice and not from virgin mice. Various forms of nAChRs expressed in the *Xenopus* expression system revealed modulation of the nAChRs by the PATE-like proteins. Low expression of PATE-M gene was found in temporal lobes of AD patients (Fig 2, I), as compared to normal controls. It co-localized with the characteristic AD A β -containing plaques (Fig 2, II).

Conclusions: 1.PATE –like genes are expressed in the reproductive system and are modified by sex hormones. 2.PATE –like genes are also expressed in the nervous system and modulate the activity of nAChRs. 3. PATE-M may have a role in the pathogenesis of AD.

Construction of gene therapy viral vectors targeting thyroid cells: infection of thyroid cancer cells with Adeno-associated viral (AAV) vector serotypes

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Introduction: The successful use of tissue specific promoters in targeted gene therapy for cancer depends on a high level of cell type specificity. Thyroglobulin (Tg) is a thyroid-specific protein that is expressed in the normal thyroid and a majority of thyroid tumors. As a step towards the development of gene therapy vectors directed toward human thyroid carcinoma, we previously characterized a minimal Tg enhancer fragment which provided maximal, thyroid-specific, transcriptional activity from the Tg promoter in human thyroid carcinoma cells. Furthermore, we clarified the mechanism of thyroid specificity for the Tg minimal enhancer at the level of thyroid-specific transcription factors and with agents modulating the cAMP pathway. In the present study, we screened different serotypes of recombinant adeno-associated viral (rAAV) gutless vectors to define a relevant AAV viral envelope for potential thyroid cancer therapy. We compared these rAAV gutless serotypes expressing green fluorescent protein (GFP) under the control of the CMV promoter/enhancer. Additionally, we compared two forms of the GFP protein, eGFP and the nuclear localization signal (nls) GFP. Gutless rAAV vectors are considered safe for in vivo use for both animals and humans. In fact, numerous human clinical trials are currently in progress using different rAAV serotypes. Our aim was to screen four serotypes of rAAV vectors in order to determine the serotype which is the most efficient and effective for infecting human thyroid carcinoma cells

Patients/ Methods: Four gutless rAAV serotype (2,4,5 and 12) vectors expressing eGFP or nlsGFP protein were prepared. Their infection of human thyroid carcinoma cells, papillary (NPA) and follicular (WRO), were characterized in a time- and concentration-dependent manner. Infection efficiency was measured by calculating the percentage of green fluorescent cells following different time periods.

Results: Thyroid carcinoma cells were infected at a virus/cell ratio of 2000 (modality of infection, MOI): AAV12nlsGFP, follicular carcinoma cells 92+/-5.9%, papillary carcinoma cells 82.7+/-3.6%. This peak response was seen 3 days post-infection. AAV12CMVGFP, follicular carcinoma cells 47.8+/-10.5%, papillary carcinoma cells 1.4%. This peak response was also seen 3 days post-infection. AAV4nlsGFP, follicular carcinoma cells 4.8+/-1.14%, papillary carcinoma cells 27.9+/-9.1%. This peak response was seen 19 days post-infection. AAV5CMVGFP, follicular carcinoma cells 26+/-6.6%, papillary carcinoma cells 18.4+/-4.9%. This peak response was seen 3 days post-infection. For the virus, AAV2CMVGFP, which was infected a virus/cell ratio of 4000: follicular carcinoma cells 32.3+/-6%. This peak response was seen 6 days post-infection. Papillary carcinoma cells 27.9+/-9.6%. This response was seen 9 days post-infection.

Conclusions: The rAAV12 data obtained will permit the completion of the AAV-Tg enhancer/promoter driven vector with and without GFP and/or an appropriate killing gene construct(s). Furthermore, these constructs will allow us to confirm thyroid tissue specificity and hopefully be the basis for future gene therapy trials for the treatment of thyroid cancer.

Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia

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Introduction: Normal adult lungs contain scattered pulmonary neuroendocrine cells (PNEC). Reactive PNEC hyperplasia is commonly observed in persons who live at high altitude, in cigarette smokers, and in numerous lung diseases. DIPNECH is a rare entity in which PNEC hyperplasia appears without predisposing conditions. According to the 1999 WHO lung tumor classification, DIPNECH is thought to be primarily a neuroendocrine proliferative process, which can be associated with carcinoid tumors and with a clinical picture of constrictive obliterative bronchiolitis. The available data regarding the treatment and the prognosis of this rare condition is very limited.

Aims: To describe the clinical, radiological and pathological characteristics of patients with DIPNECH, and the effect of different therapeutic modalities on disease progression and patient well-being.

Methods: We have retrospectively studied 9 consecutive patients with DIPNECH followed at two referral centres in Israel between 2001 and 2009. Clinical, biochemical, pathological and imaging data were collected from the medical files.

Results: All patients were female, with a mean age of 62.5 years (range 53-74). Seven patients were lifetime nonsmokers, and two patients had quit smoking more than 10 years prior to the diagnosis. Five patients presented with respiratory symptoms, such as prolonged dyspnea, wheezing and cough, while in the other four the disease was incidentally diagnosed by thoracic imaging. The mean delay in the diagnosis of DIPNECH in symptomatic patients was 15.8 years (range 2-25 years). All patients had carcinoid tumor together with multiple small pulmonary nodules on thoracic HRCT examinations. The mean size of the dominant lesion was 18.7±9.6 mm. Seven patients underwent thoracotomy and resection of the dominant lesion, while in the other two the diagnosis was made using biopsy. Ki-67 proliferation index was less than 5% in 6 patients in which it was available. The disease was stable in the 6/9 patients. In 3/9 patients it progressed, and treatment with Sandostatin LAR (30-40 mg/month) was administered, inducing disease stabilization in 2/3 patients, in 1/3 patients the treatment was terminated due to diarrhea. Metastatic disease has been diagnosed in 2/9 patients (22.2%) (to hilar lymph nodes and to adrenal gland). All patients were alive during the follow-up period (1.9±0.76 years, ongoing).

Conclusions: The association of carcinoid tumor with multiple lung nodules in female patients, together with complains of chronic cough and wheezing, shall raise suspicion of DIPNECH. While rare albeit important entity, DIPNECH seems to present with a slow-progressive course. Whenever possible, surgical excision shall be performed to resect the dominant lesion, while somatostatin analogues may be considered for the symptomatic improvement in patients unresponsive to anti-asthmatic medications.

Chronic complications of peptide receptor radionuclide therapy (PRRT)- A single center experience

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Introduction: Peptide receptor radionuclide therapy (PRRT), is an established form of treatment for gastro-entero-pancreatic neuroendocrine tumor (GEPNET) patients. PRRT has been associated with several complications including bone marrow suppression, renal toxicity and hepatic damage.

Patients/ Methods: We retrospectively analyzed our data of 90 consecutive patients with different GEPNETs who received PRRT. CBC and renal function tests were performed prior to treatment and every 2 weeks following each treatment cycle for a minimum of 8 weeks. Possible risk factors were identified. Only patients with such complications that persisted for over 8 weeks were included. Three women who developed premature ovarian failure (POF) were assessed for pituitary gonadal axis function. The CTCAE version 3 NIH/NCI adverse event grading system was used to grade adverse events

Results: Of 90 patients, 13 were excluded due to lack of follow-up. 29 (37%) of these 77 patients developed late hematologic complications: mean age 58 years, 17 men and 12 women, 19 (66%) PNET and 10 (34%) carcinoids, 55% received Y90 DOTATOC, 28% received Lu177 DOTANOC/TATE and 17% received both isotopes, mean cumulative dose 531±265 mCi. 13 (44%) developed anemia (G1-2), 21 (72%) developed leucopenia (G1-3), and 18 (62%) developed thrombocytopenia (G1-4). During follow-up (range 1-60 months, median 18 months), of patients with anemia 30% improved and 70% remained unchanged, 67% of leucopenia improved and 33% remained unchanged, and 44% of thrombocytopenia improved while 44% remained unchanged and only 7% (one patient) worsened. 12 (41%) had monocytopenia, 13 (45%) bicytopenia and 4 (14%) pancytopenia. There were a total of 8 complications secondary to hematologic toxicity: bleeding - 2, infections- 2, blood transfusions-4. Three women (mean age 41) who had prior regular menses developed POF. A total of 6 patients (~8%) developed renal impairment: mean age 66±8 yrs, 2 (33%) had carcinoid and (66%) PNET. 5 had prior renal compromise with risk factors for renal disease. 3 (50%) received Y90DOTATOC 1 (17%) received Lu177 DOTANOC/TATE and 2 (33%) received both, with a mean cumulative dose of 747±547 mCi. Only one patient had worsening of renal function 8-12 weeks from the last treatment but during follow up ranging between 2-24 months (median 16.5 month) 5 of 6 patients had further deterioration of their renal function. Only one patient required dialysis.

Conclusions: In patients with GEPNETs PRRT is a safe treatment modality. The major side effects in our cohort were bone marrow suppression that improved in the long-term in most patients. Young women may be at risk for POF. Deterioration in renal function was uncommon and usually appeared late after treatment, therefore requiring prolonged follow up.

Metastatic growth hormone secreting pituitary carcinoma treated with peptide receptor radionuclide therapy

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Introduction: Pituitary carcinoma (PC) is an extremely rare condition defined by the presence of adenohypophyseal neoplastic tissue outside the pituitary. Clinical experience regarding diagnosis, management and prognosis of PC is very limited. Growth hormone (GH) secreting PC is even rarer and represents a particular challenge to clinical practice. Therapeutic modalities utilized to treat PC include surgery, radiation, hormonal therapy, and cytotoxic drugs. Peptide Receptor Radionuclide Therapy (PRRT) is an emerging therapeutic modality that involves the targeted delivery of an ablative dose of radiolabelled somatostatin analog. PRRT has been applied to various neuroendocrine tumors and results in prolonged survival and enhanced quality of life. As yet, this therapy has not been applied to malignant pituitary tumors. We present a case of GH secreting PC and suggest PRRT as an apparently effective therapeutic option.

Patients/ Methods: A 56 year-old female with a 9 year history of GH secreting pituitary macroadenoma presented with worsening headache and marked acromegalic features. Surgical removal of the pituitary tumor was attempted twice, but GH and IGF-1 levels remained elevated and the pituitary mass re-expanded despite medical therapy with Octreotide and Cabergoline. The patient became severely debilitated with increasing fatigue, musculoskeletal pain, weight loss, and uncontrolled diabetes. Serum IGF-1 and GH levels were 135 nmol/L and 241 mcg/L, respectively. Whole body CT exam demonstrated numerous osteoblastic bone lesions. A CT guided biopsy obtained from a bone lesion confirmed the diagnosis of metastatic GH secreting PC. Octreoscan demonstrated numerous somatostatin avid lesions in the pituitary and the skeleton. Pasireotide therapy was attempted but not tolerated, Temozolamide was not available due to cost.

Results: Three courses of PRRT (117Lutetium-DOTATOC and 90Yttrium-DOTATOC, 200 mCi each) were administered. Post treatment scan after the 3rd PRRT course demonstrated a decrease in uptake intensity in several bone lesions with other metastases stable-appearing. However, the GH level continued to rise and palliative external radiation therapy to skeletal lesions was administered for local relief. The patient died 18 months after the initial PRRT and survived significantly longer than previously reported in PC patients.

Conclusions: The clinical course in this patient favors an aggressive therapeutic approach early in the management of malignant pituitary tumors. PRRT may be an effective therapeutic modality in PC. Additional studies are needed to examine the benefit of PRRT in selected cases of somatostatin receptor positive, unresectable, radiation resistant pituitary tumors.

Metformin displays antiproliferative activities in endometrial cancer cells via interaction with the IGF-IR signaling axis

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Introduction

Accumulating epidemiological evidence shows that obesity is associated with an increased risk of several types of adult cancers. Chronic hyperinsulinemia, a typical hallmark of diabetes, is one of the leading factors responsible for the obesity-cancer connection. Numerous cellular and circulating factors are involved in the biochemical chain of events leading from hyperinsulinemia and insulin resistance to increased cancer risk and, eventually, tumor development. Metformin is an oral anti-diabetic drug of the biguanide family. It is the first-line drug of choice for the treatment of type 2 diabetes, particularly in overweight and obese people. Metformin lowers glucose levels by reducing glucose production in liver cells *via* activation of AMPK and by increasing insulin sensitivity. Recently, metformin was shown to exert an anti-neoplastic effect in ovarian cancer cells, although the mechanism/s responsible for this non-classical metformin action remain unclear. The insulin-like growth factors (IGFs) play a prominent role in cancer biology and their mechanisms of action are tightly interconnected to the insulin signaling pathways. Given the cross-talk between the insulin and IGF signaling pathways, the aim of this study was to examine the hypothesis that the anti-proliferative actions of metformin are potentially mediated via suppression of the IGF-I receptor (IGF-IR) pathway.

Materials and Methods

To address the effect of metformin on IGF-IR activation, human endometroid (ECC-1, Ishikawa) and serous papillary (USPC-1, USPC-2) endometrial cancer cell lines were treated with metformin (10 mmol/L) for various periods of time in the absence or presence of IGF-I during the last 20 min of the incubation. IGF-IR expression and activation, as well as the activation of downstream mediators, was assessed by Western immunoblots. Apoptosis was evaluated by PARP cleavage and cell proliferation by MTT assays. The effect of metformin on IGF-IR promoter activity was assessed by transient transfection assays.

Results

Results of Western blots with anti-phospho antibodies revealed that metformin abrogated the IGF-I-induced IGF-IR phosphorylation. This effect was associated with a reduction in Akt phosphorylation. In addition, metformin activated AMPK and reduced mTOR phosphorylation. Of interest, metformin was able to abrogate the anti-apoptotic action of IGF-I, as measured by PARP cleavage. Furthermore, metformin had a potent inhibitory effect on cell proliferation. Finally, metformin treatment led to a significant reduction in IGF-IR promoter activity.

Conclusions

Taken together, our data indicates 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.