KLOTHO, A NOVEL REGULATOR OF SOMATOTROPE PROLIFERATION AND HORMONE SECRETION

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Klotho is a transmembranal protein which can be also act as a circulating hormone. Klotho is highly expressed in the pituitary, and studies of klotho-deficient mice revealed smaller growth hormone (GH) secreting cells (somatotropes) and reduced number of secretory granules compared to control mice. The basic fibroblast growth factor (bFGF) pathway regulates secretion of pituitary hormones and klotho is a major regulator of this pathway. We, therefore, aimed to study the role of klotho in the regulation of pituitary cell proliferation and hormone production.

We first studied klotho mRNA expression in the pituitary, and found lower klotho expression in human acromegaly samples (n=22) compared to normal human pituitary (n=3) and non functioning adenomas (n=22). Next we studied the effects of klotho on somatotropes cell proliferation using MTT assay. Klotho inhibited somatotropes cell proliferation by up to 45% ($P < 0.01$) in a dose dependent manner. The effects of klotho on GH secretion were studied in GH3-somatotropes cell line, in primary cultures of human acromegaly (n=4) and rat normal pituitary (n=2). Klotho treatment (24hrs) elevated GH secretion by 20-50% ($P < 0.01$) in the different cultures tested. Klotho regulates bFGF activity in breast and pancreatic cancer, and bFGF increases hormone secretion in GH3 cells. Therefore, we studied the effects of bFGF, klotho and their combination on GH secretion. bFGF elevated GH secretion in normal rat pituitary by 44%, and by 50% in acromegaly. Co-treatment with klotho further elevated GH secretion by 80%. Finally, we discovered that klotho enhanced bFGF-mediated activation of the ERK pathway in GH3 cells.

Our findings indicate, for the first time, a role for klotho and bFGF in somatotroph proliferation and GH secretion.

IGF-1 INDUCES TUMORIGENESIS IN HUMAN PITUITARY TUMORS - FUNCTIONAL BLOCKADE OF IGF-1 RECEPTOR AS A NOVEL THERAPEUTIC APPROACH IN NON-FUNCTIONING TUMORS

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Non-functioning pituitary tumors are usually large and invasive with considerable growth potential, and unlike hormone secreting adenomas, no effective medical therapy is available for these common adenomas. The growth hormone/insulin-like growth factor (IGF-1) axis plays a fundamental role in growth and development. IGF-1 and its receptor display potent proliferative and antiapoptotic activities and are considered key players in malignancy. The objective of the study was to explore the role of IGF-1 and its downstream pathways in the tumorigenesis of non-functioning pituitary tumors and to develop a targeted therapeutic approach for the treatment of these tumors. Our results show that IGF-1 elevated the number of viable cells and cell proliferation of human non-functioning pituitary tumor cells and non-secreting immortalized pituitary tumor cell line, MtT/E, respectively. IGF-1 induced the phosphorylation of ERK, Akt and p70S6K and the expression of cyclins D1 and 3. Our results also indicate the abrogation of IGF-1- induced cell viability as well as IGF-1 receptor phosphorylation and downstream signaling in human non-functioning pituitary tumor cells and MtT/E cells by the selective IGF-1R inhibitor, NVP-AEW541. Our results suggest that IGF-1 controls cell proliferation and may contribute to pituitary tumor development and progression. Our results also indicate the blockade of IGF-1- induced cell proliferation and signaling by the selective IGF-1R inhibitor, NVP-AEW541, which may constitute an attractive novel strategy for the treatment of these invasive tumors that, as yet, do not respond to any known medical treatment.

INHIBITION OF LUNG CANCER CELLGROWTH BY COMBINED TREATMENT OF 1,25-DIHYDROXYVITAMIN D3 WITH KETOCONAZOLE AND SODIUM VALPROATE

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Background: The unrestrained growth of lung cancer cells is impeded by 1,25-dihydroxyvitamin D3($1,25(OH)_2D_3$). However, effectiveness of $1,25(OH)_2D_3$is limited because of its catabolism by 24-hydroxylase (CYP24). This enzyme, a member of the cytochrome P450 super-family, is inhibited by the anti-fungal drug ketoconazole. In addition, the $1,25(OH)_2D_3$activityis restricted by transcriptional co-repressor complexes which include histone deacetylases (HDAC). Sodium valproate (VPA), a well-known HDAC inhibitor, decreases the activity of these co-repressors.

Aim: To enhance the inhibitory action of $1,25(OH)_2D_3$ on lung cancer cells growth by combination with ketoconazole and VPA.

Methods: Human non-small cell lung cancer (NSCLC) cell line A549 was grown in RPMI-1640 medium containing 10% FCS at 37^0C with 5% CO_2in air. Cell proliferation and cell-cycle distribution were assessed using crystal violet and flow cytometry assays, respectively. Dose response incubations of cells with each agent were carried out for 72 hours. Different drug combinations were also tested. Statistical analysis was performed using Student's t-test and $p \leq 0.05$ was considered significant.

Results: $1,25(OH)_2D_3$, ketoconazole and VPA tested singly exerted a dose dependent inhibitory effect on NSCLC cell proliferation. Combination of a sub-effective concentration of $1,25(OH)_2D_3$(10 nM) with 3.125μM ketoconazole and with 0.5 mM VPA synergistically suppressed cancer cell growth (28.1%, $p<0.001$). Cell-cycle studies also indicated that the triple drug combination was the most effective, leading to the arrest of cell-cycle in G1 phase.

Conclusions: The prevention of $1,25(OH)_2D_3$catabolism by ketoconazole, combined with the inhibition of histone deacetylase activity by VPA, enhanced the anti growth action of $1,25(OH)_2D_3$on a human NSCLC cell line The present results emphasize the importance of combined drug administration, in the treatment ofNSCLC using low concentrations of agents,a therapeutic approach which may also afford diminished side effects.

A PREFORMED SIGNALOSOME IN GNRH-STIMULATED GONADOTROPES MAY DIRECT ERK PHOSPHORYLATION OF FAK AND PAXILLIN AT FOCAL ADHESIONS: POSSIBLE ROLE IN GONADOTROPES MIGRATION

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We have recently described a preformed multi protein complex (signalosome) associated with the GnRH receptor (GnRHR) in pituitary gonadotrope cells. This signalosome included c-Src, focal adhesion kinase (FAK), paxillin, vinculin, tubulin, caveolin-1, protein kinase C (PKC) δ, PKCε, PKCα, Ras, kinase suppressor of Ras-1 (KSR), MAPK kinase (MEK) 1/2, ERK1/2 and the GnRHR. Incubation of LβT2 gonadotrope cells with GnRH resulted in a rapid phosphorylation of caveolin-1, FAK, vinculin, and paxillin on Tyr residues by the GnRH-activated c-Src. Then, GnRH activated ERK1/2 in the complex in a c-Src-dependent manner, and the activated ERK1/2 subsequently phosphorylated FAK and paxillin. Addition of GnRH to LβT2 cells transfected with GnRHR-mCherry and ERK-GFP resulted in bleb formation, ERK accumulation in the blebs and apparent cell migration. Also, addition of GnRH to LβT2 gonadotrope cells transfected with GnRHR-mCherry and paxillin-GFP resulted in enrichment of paxillin in focal adhesions in the newly formed blebs. We therefore propose that the role of the signalosome is to sequester a cytosolic pool of activated ERK1/2 to phosphorylate FAK and paxillin at focal adhesions apparently to mediate gonadotropes migration.

WHAT IS SAUCE FOR THE GANDER IS NOT SAUCE FOR THE GOOSE: SEXUAL DIMORPHISM OF SKELETAL MUSCLE STEM CELLS IN AGE AND EXERCIS

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Background: Aging is associated with a progressive decline in muscle mass and strength termed sarcopenia.The functional units of skeletal muscles are myofibers, their repair depends on satellite cells (SCs), muscle stem cells. Human and rodent studies show an age-associated decline in SC numbers.This decline may play a prominent role in sarcopenia.**Aim:**to investigate gender-related differences in SCs in response to age and endurance exercise carried in an attempt to reduce sarcopenia.

Methods: Gastrocnemius or extensor-digitorum-longus muscles of rats and mice, respectively, were assessed for the effect of age, gender and exercise on SCs. Rodents ran on a treadmill for 20 minutes/day/6 days a week, for 13 weeks. Ages (in months) at the end of exercise: rats: 6.5, 18-20; mice: 8, 18-20.

Results:

***Age effects*:** There was an age-associated decline in SC numbers of sedentary male and female rodents and a sharp increase in myofibers lacking SCs. Moreover, myostatin-null mice with a large muscle mass were not spared from age associated SC decline.

Gender Effects: (1)Average number of SCs was 2-3 folds higher in males vs. females at both ages(2) At old age, male myofibers exhibited a 64% decline in SC numbers compared to a 34% decline in females.

Effect of exercise as related to age and gender: In male rats, exercise induced a 25% and 100% increase in SC numbers at both agea, compared to 66% and 50% increase in young and old females. In mice the gender difference was more dramatic with an increase of 66% and 100% in SCs in males at both ages compared to only 20% and 40% in females.

Future directions: Our studies underscore the need to further explore the potential role of sex hormones in attempted preservation of skeletal muscle in the face of aging. Our data and accumulating knowledge about the role of testosterone and estradiol in enhancing SC numbers suggest that the observed differential interaction of aging, gender and exercise depends on differential age-and gender related patterns of the decline in sex hormones.

SIRT1+/- FEMALE MICE DISPLAY THE CHARACTERISTICS OF THE AGING SKELETON

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Background: Osteoporosis and bone loss are inevitable consequences of ageing. We have recently reported that Sirt1 haplo-insufficiency results in decreased bone mass in female mice (Endocrinology in press, PMID: 21952235). We aimed to evaluate the skeletal changes in these mice as a potential model of the ageing skeleton.

Methods: μCT analysis of trabecular and cortical femoral bone was performed in 4 -and 7-months old female mice. Osteocyte apoptosis was determined by Tunnel staining of paraffin embedded sections. Marrow adipogenesis was determined by Oil-Red-O staining of bone marrow-derived mesenchymal stem cells induced to adipogenesis.

Results: Sirt1+/- female mice exhibited a reduction in trabecular bone volume, trabecular number and cortical thickness. Cortical porosity was observed at age 7 months in Sirt1+/- but not in wild type mice. Increased osteocyte apoptosis and marrow adipogenesis were also detected in Sirt1+/- mice.

Conclusions: Sirt1+/- female mice exhibit the skeletal hallmarks of ageing and thus may serve as a model to study age-related changes in bone.

THE EFFECT OF CALORIE-RESTRICTED AND RE-FEEDING –INDUCED CATCH UP GROWTH ON TRABECULAR AND CORTICAL BONE

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Growth stunting constitutes the most common effect of malnutrition. In developing countries it is mostly attributed to food shortage and infections while in developed countries it may be due to prematurity, chronic diseases, anorexia nervosa etc. When the primary cause of malnutrition is resolved, catch-up (CU) growth usually occurs, associated with a dramatic increase in EGP height. However the effect on bone structure was not reported. To investigate the effect of food restriction and CU growth on the long bones we used micro CT and mechanical testing. In our experimental model, pre-pubertal rats were subjected to 10 days of 40% food restriction (RES group), followed by a renewal of the regular food supply for one day (CU group). Rats fed ad libitum served as controls (AL). Rats from the RES group had a significantly reduced body weight (-48%; $p<0.05$) and EGP height (-44%; $P<0.05$). Micro-CT results showed no effect on BMD or cortical BV/TV but significant reduction in trabecular BV/TV (-48%; $p<0.05$), concomitant with an increase in Tb. Sp (+90%; $P<0.05$) and reduction in Tb. Nb (-48%; $p<0.05$). Trabecular BV/TV and Tb. N were significantly increased in the CU group (40% and 34% respectively; $p<0.05$). Mechanical testing showed that food restriction led to less elastic bones; interestingly, bones of the CU group were weaker and more fragile, in spite of a significant increase in ALP levels. Serum analysis showed significant reduction in IGF-1 and leptin levels in RES (-80%; -99%; respectively; $p<0.05$) and an immediate increase in their level during CU. These results may suggest that while nutritional induced CU growth leads to an increase in EGP height and active bone modeling process, it is also associated with a transient reduction in bone quality. This should be taken into consideration when treating children undergoing CU growth.