Other Topics: Basic Research

P1-04

Glucagon-like peptide-1 stimulates lactate production by human Sertoli cells with possible implication to male reproductive potential

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Background: Glucagon-like peptide-1 (GLP-1) is a key player in energy balance, mostly attributed to its glucose-dependent insulin release stimulation ability and food intake regulation. Glucose homeostasis plays a pivotal role in male reproduction, particularly due to the metabolic cooperation established between Sertoli cells (SCs) and germ cells. We hypothesize that GLP-1 participates in spermatogenesis regulation by altering SCs metabolism.

Materials and methods: GLP-1 receptor was identified in human SCs by qPCR. Primary human SCs cultures were unexposed or exposed to increasing concentrations of GLP-1 (10, 1000 and 100,000 pM) for 6 h. Extracellular media was analysed by ^{1}H-NMR to determinate metabolites concentration. Protein levels of glucose transporters, phosphofructokinase, lactate dehydrogenase (LDH) and monocarboxylate transporter 4 were determined by Western Blot. To evaluate oxidative damages, lipid peroxidation, protein carbonylation and nitration were determined. LDH activity was evaluated by a commercial kit assay, and mitochondria membrane potential was assessed using JC-1 dye.

Results: Human SCs were shown to express the GLP-1 receptor. Cells treated with 1000 pM of GLP-1 presented a decrease in glucose consumption and an increased production of acetate. Moreover, human SCs treated with all GLP-1 concentrations dose-dependently increased lactate production. Protein carbonylation was decreased in human SCs treated with 1000 pM. Finally, mitochondria potential membrane was decreased only in cells exposed to 100,000 pM.

Conclusions: This is the first report showing that GLP-1 can alter the metabolic support of spermatogenesis by human SCs, by dose-dependently increasing lactate production which has been highlighted as crucial to improve the male reproductive potential. Further studies will be needed to unveil the possible physiological role of GLP-1 in male reproductive function.

P1-05

Fine mapping of blood pressure-related variants on epigenetic human data

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Background: High blood pressure is a major risk factor for cardiovascular disease and premature death. To better understand the epigenetics of blood pressure, we intend to assess to which extent regulatory elements influence 69 blood pressure-associated variants in different cell types.

Materials and methods: We tested the phenotypic cell-type specificity of these 69 single nucleotide polymorphisms (SNPs) and vicinity ($r^2 > 0{\cdot}8$) with H3K27ac peaks across 24 datasets of different tissues. Statistical significance was evaluated by permuting 10,000 matched sets of SNPs not associated with the phenotype to calculate a 95th-percentile threshold as cell type specificity score.

Results: The most significant enrichment was in left ventricle, adrenal gland and right atrium tissues. We found 6 SNPs mapped to tissue-specific H3K27ac peaks in left ventricle, with specificity of 0·63 or greater. These variants (related to genes NPPA, RP11-103J8.1, PLEKHA7, NPR3 and PLCE1) are in linkage disequilibrium (LD) with a SNP close to cell-type specific H3K27ac summit peak (median 167·5 bp away). Seven variants (that relate to genes ADK, PLEKHA7, HECTD4, NPR3, ULK4 and AGT) were above the specificity threshold for adrenal gland (0·24), in LD with a marker with median distance of 473 bp from a summit. Finally, 7 SNPs (genes AC092684.1, RP11-89B16.2, AKAP13, MECOM, ATXN2, FBN1 and PDE1A) showed significant phenotypic cell-type specificity (0·60) for right atrium, with median distance of 84 bp from a chromatin peak summit.

Conclusions: These preliminary results point to an enrichment of blood pressure-related SNPs with regulatory regions highlighted by H3K27ac peaks. Further analyses may help clarify the mechanisms through which these SNPs affect genes, identify the causal variants and their influence in regulatory regions.

proteins was determined by Western blot analysis and RT-PCR. For inhibition of Erk-1/2 MAP kinase was used selective inhibitor PD98059.

Results: The results showed that the redox-sensitive transcription factors Egr-1 and Sp-1 enter into competitive interactions in the early stages of stress and aspirin-induced gastric lesions in rats. Experimental ulcers caused multidirectional changes in the level of protein VEGF and bFGF. Development of erosive and ulcerative lesions in the stomach during stress action was accompanied by a decrease in the partial pressure of oxygen in the cells of the gastric mucous membrane, and caused increasing in the level of HIF-1α and reducing protein SH-groups. In the early stages of stress-induced gastric lesions increased concentrations of the stress hormone cortisol in serum is associated with activation of Erk1/2 MAP-kinase pathway and does not affect p38. Inhibition of Erk1/2 by the selective inhibitor PD98059 lead to more aggressive lesions of the stomach accompanied by a reduction in Egr-1, and accordingly, pro-angiogenic factors VEGF and bFGF.

Conclusions: So redox sensitive transcription factor Egr-1 is a leading transcription factors in the pathogenesis of stress- and aspirin-induced gastric lesions, unlike ethanol-induced ulcers. This factor is activated by hypoxia and launches gastroprotective mechanisms through Erk1/2-dependent mechanism.

P1-18
Zebrafish as a disease model for studying human Rett Syndrome

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Background: Rett syndrome (RTT) is a severe neurological disorder that affects almost exclusively females. This disease is characterized by normal growth and intellectual development until around the first year, then progression in development starts to slow down, with loss of purposeful use of the hands, distinctive hand movements, slowed brain and head growth, problems with walking, seizures, and intellectual disability. Mutations in the X-linked genes methyl-CpG-binding protein 2 (*MECP2*) and cyclin-dependent kinase-like 5 (*CDKL5*) were described in RTT and recently several bone diseases related with decreased bone mass were also described in those patients, starting early in life. Because zebrafish was largely validated as a model for human diseases, the main objective of this work was to investigate if zebrafish can be a good model to study RTT.

Materials and methods: For this purpose, we initiated studies comparing zebrafish and human MECP2 and CDKL5 regarding their chromosomal environment, gene structure, promoter regulation and protein conservation.

Results: In silico analysis showed that both human genes are localized in chromosome X In zebrafish, mecp2 and cdkl5 are located in chromosome 8 and 11, respectively and their flanking genes are conserved between species. Both genes share the same genetic structure as their human orthologs and both species present alternatively spliced isoforms for both genes. Zebrafish and human MECP2 and CDKL5 promoter regions present similar putative binding sites for known transcriptional factors. Bioinformatic analysis of corresponding protein showed a high degree of conservation in both three dimensional structures and functional domains.

Conclusion: In conclusion, this study demonstrates that zebrafish has the appropriate genetic characteristics to be further considered as model to investigate bone diseases associated with RTT.

P1-20
Functional study of OPTN promoter: new OPTN regulators and effect of genetic variants

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Background: Optineurin (*OPTN*) is a gene located in chromosome 10 that has been associated with several pathologies, including Paget's disease of bone (PDB). Using DNA samples from our cohort of PDB patients we have found two SNPs in *OPTN* promoter (rs3829923 and RV -9906) that could alter *OPTN* expression. However little is known about the role of this gene in bone and how this gene is regulated.

Materials and methods: Using bioinformatics, we analyzed the *OPTN* promoter to look for NFkB putative binding sites and performed several deletion constructs to identify the most important regulatory region within the *OPTN* promoter. By transfection and co-transfection assays, we analyzed the activity of each construct in the presence of NFkB. Moreover, we also assessed the effect in the promoter activity of the variants identified for rs3829923 and RV -9906.

Results: Our results showed that rs3829923 *T* allele increases *OPTN* promoter activity due to a gain of E47 and E2F1 inductive effect, and the rare variant RV -9906 was responsible for an increase of *OPTN* promoter activity due to a loss of SP1 inhibitory effect. Our results also showed that NFkB and E2F3 regulate positively and that RXR regulates negatively *OPTN* promoter activity.

Conclusions: Our work clarified the functional effect of these variants found in *OPTN* gene of PDB patients and how these variants could contribute to PDB pathophysiology. Also with this work we were able to define genomic regions important for NFkB, E47, SP1 and E2F1 regulatory effects, unraveling new OPTN regulators.

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