SILAC-BASED PROTEOMICS OF PRIMARY HUMAN RENAL CELLS REVEALS A NOVEL LINK BETWEEN MALE SEX HORMONES AND IMPAIRED ENERGY METABOLISM IN DIABETIC KIDNEY DISEASE

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INTRODUCTION

- **Male sex** predisposes to **chronic kidney disease** (CKD)[1].
- Since **androgens** have shown to exert **deleterious effects** in a variety of **kidney cells**[2], we hypothesized that **dihydrotestosterone** (DHT) would alter the biology of the renal tubular cell by inducing changes in the **proteome**.
- By employing stable isotope labeling with amino acids (**SILAC**)[3] in an indirect **spike-in** fashion[4], we aimed to accurately quantify the proteome in DHT- and 17β-estradiol (EST)-treated **human primary proximal tubular epithelial cells (PTEC)**[5].

AIMS

SHOTGUN PROTEOMICS

BIOINFORMATIC ANALYSIS

VERIFICATION AND VALIDATION

AIM 1: Perform an in-depth analysis of the sex hormone-regulated proteome in human PTEC after stimulation with DHT or EST.

AIM 2: Identify the key molecular pathways and biological processes that DHT and EST signature proteins play a role in.

AIM 3: Verify and validate in vitro and in vivo top candidate proteins discovered in aims 1 and 2.

METHODS

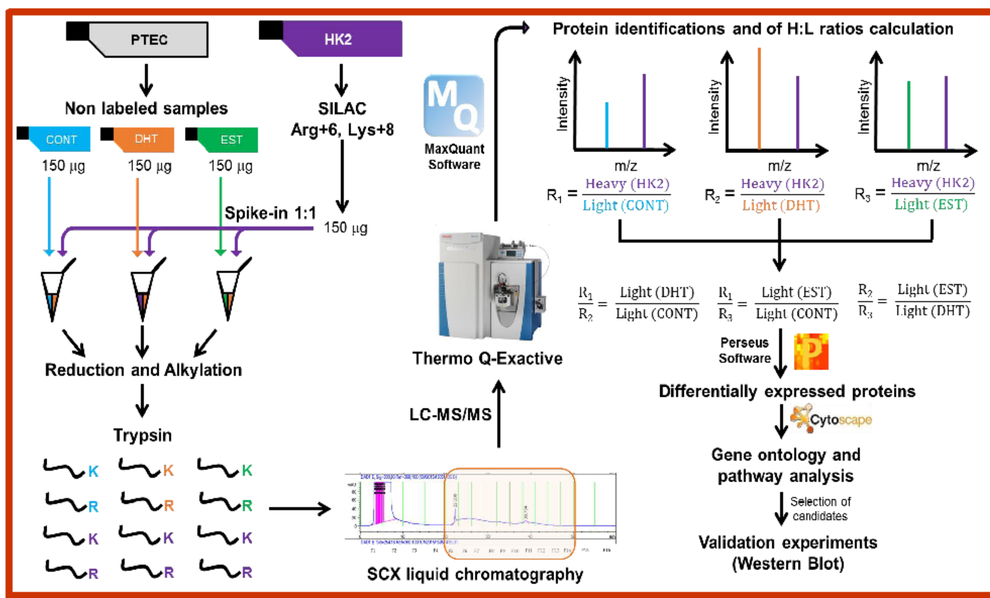

Figure 1. Experimental scheme. The figure shows a simplified workflow, including sex hormone 8h-treatment to PTEC, SILAC labeling of HK-2 cells, 1:1 mixing of labeled and non-labeled proteins, protein digestion, SCX fractionation followed by LC-MS/MS, data analysis by MaxQuant, assignment of heavy/light protein ratios, calculation of DHT/CONT, EST/CONT and EST/DHT ratios, selection of differentially regulated proteins, validation studies and bioinformatic analysis.

RESULTS (I): PROTEOMICS

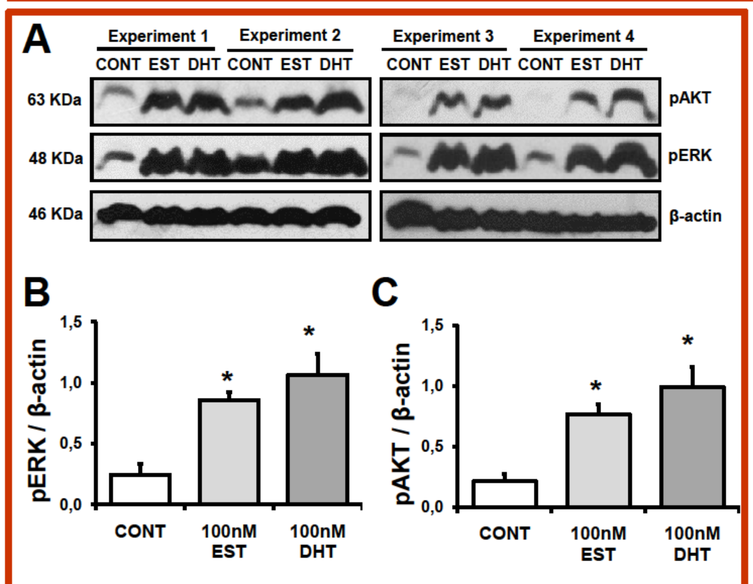

Figure 2. Control experiments. Panel A shows a representative Western blot demonstrating phosphorylation of AKT and ERK after 10min-stimulation with EST and DHT, but not in cells exposed to ethanol alone (CONT). Densitometry analysis of each band was performed using Image J software. Intensities for pERK (B) and pAKT (C) were calculated and normalized to β-actin. Data are expressed as means ± SEM. *P<0.05 compared to control cells.

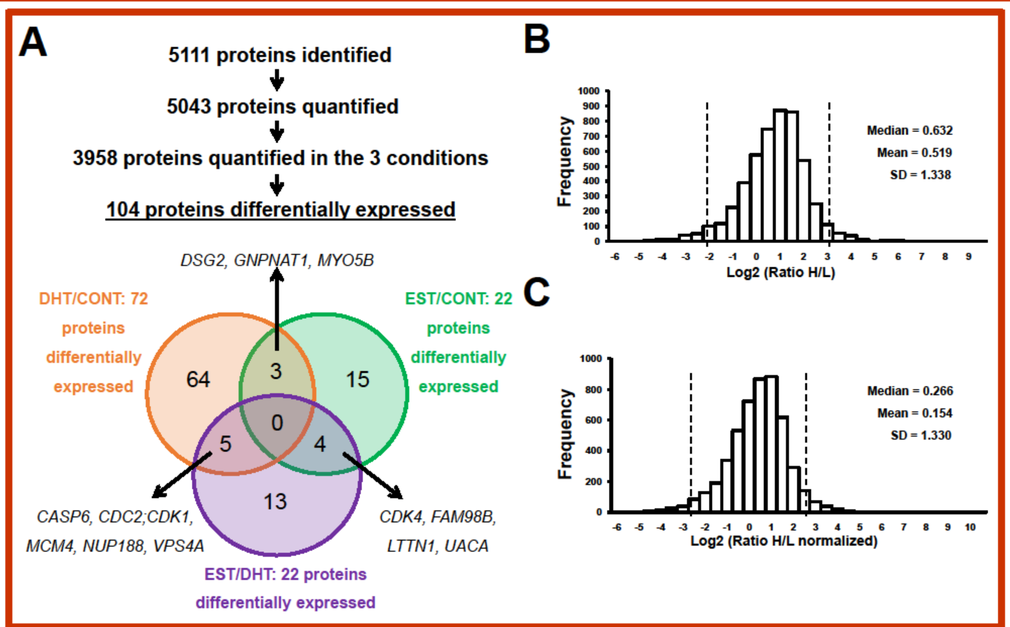

Figure 3. Identified, quantified and differentially expressed proteins in our spike-in SILAC approach. The scheme illustrates the number of identified and quantified proteins, followed by a Venn diagram representing the number and overlap of significantly regulated proteins according to significance A for DHT/CONT, EST/CONT and EST/DHT ratio values (A). Histograms depict the distributions of Log2 transformed heavy to light (H/L, B) and H/L normalized (C) ratios. Vertical lines represent 1.96·SD.

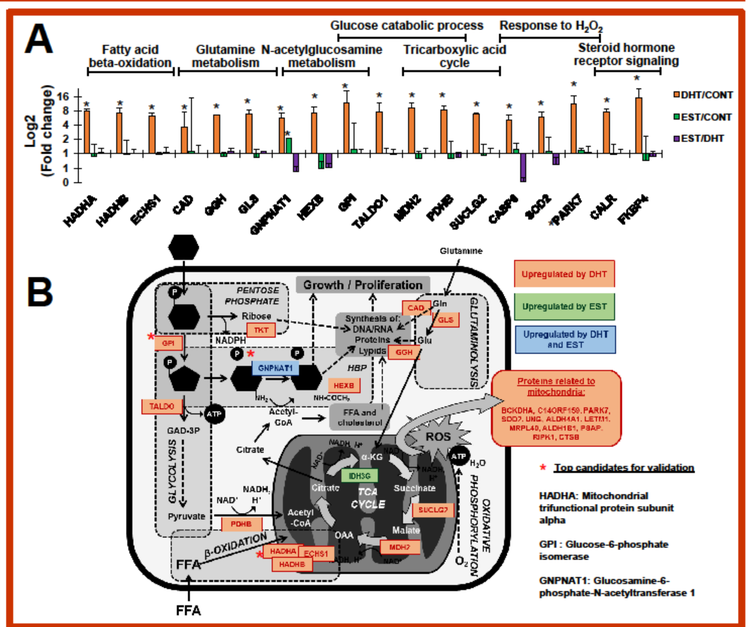

Figure 4. Enriched biological processes among the 72 proteins differentially regulated by DHT. Log2 transformed DHT/CONT, EST/CONT and EST/DHT ratios of several DHT-regulated proteins involved in the most representative, informative and significantly enriched biological processes according to gene ontology analysis using BinGO plugin in Cytoscape (A). An asterisk indicate that these protein was significant by significance A with p<0.01 in at least 2 experiments (A). Scheme of several processes related to energy metabolism and mitochondria (B).

RESULTS (II): VALIDATION STUDIES

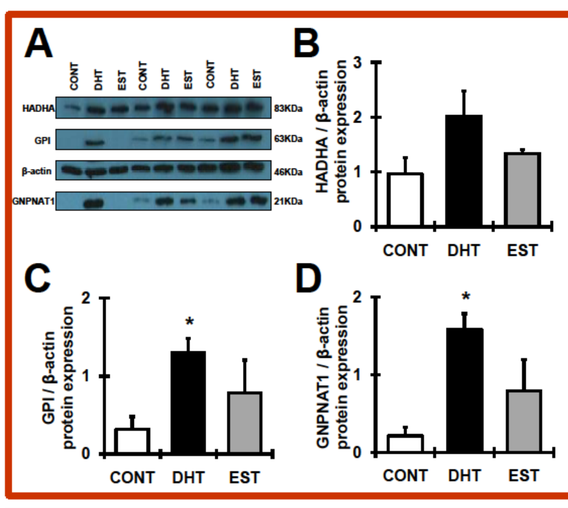

Figure 5. In vitro validation studies for HADHA, GPI, and GNPNAT1. Panel A shows the immunoblots representing HADHA, GPI, and GNPNAT1 protein expression in the same treated PTEC used for the spike in SILAC study. Intensities for HADHA (B), GPI (C) and GNPNAT1 (D) were calculated and normalized to β-actin (B, C, D). Data are expressed as means ± SEM. *P<0.05 compared to control cells.

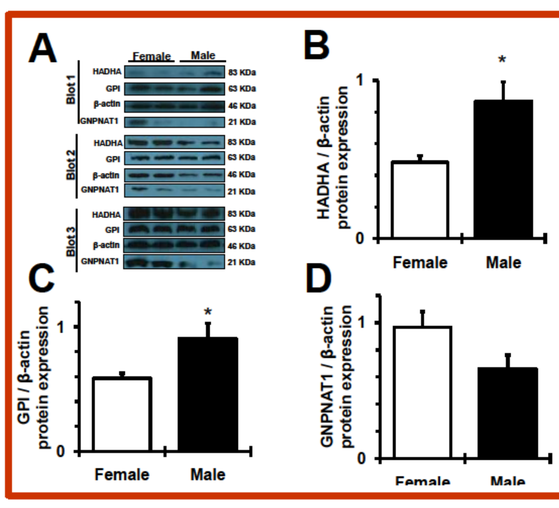

Figure 6. In vivo validation studies for HADHA, GPI, and GNPNAT1. Panel A shows the immunoblots representing HADHA, GPI, and GNPNAT1 protein expression in the kidney of 16-week healthy female (n=6) and male (n=6) mice. Intensities for HADHA (B), GPI (C) and GNPNAT1 (D) were calculated and normalized to β-actin (B, C, D). Data are expressed as means ± SEM. *P<0.05 compared to female.

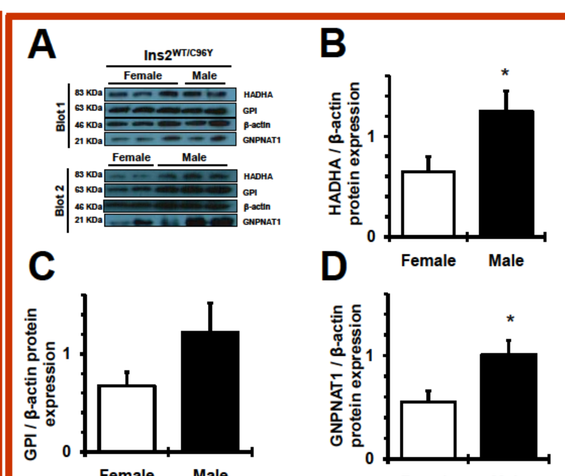

Figure 7. Protein expression of HADHA, GPI, and GNPNAT1 in the mouse diabetic kidney. The expression of the 3 candidate proteins was evaluated in the renal cortex of 16-week female (n=5) and male (n=5) diabetic $Ins2^{WT/C96Y}$ (Akita) mice (A). Intensities for HADHA (B), GPI (C) and GNPNAT1 (D) were calculated and normalized to β-actin (B, C, D). Data are expressed as means ± SEM. *P<0.05 compared to female.

CONCLUSIONS

- To our knowledge, this is the first effort to date to characterize proteomic responses of human kidney cells to DHT and EST stimulation. In addition, we are the first to use a spike-in SILAC quantitative proteomic approach between two different renal cell lines.
- DHT alone was able to dysregulate processes related to energy metabolism that are also altered by diabetes[6].
- SILAC ratios of 3 candidates representing glycolysis, N-acetylglucosamine metabolism and fatty acid β-oxidation, namely GPI, GNPNAT1 and HADHA, were verified in vitro.
- In vivo, renal GPI and HADHA protein expression was increased in males. Furthermore, male sex was associated to higher GPI, GNPNAT1 and HADHA protein expression in diabetic Akita mice.
- In this discovery-based study, we provide a detailed portrait of the key biological processes impaired by DHT and not EST and, in combination with bioinformatics analysis and the corresponding verification and validation experiments, we demonstrate sex-specific regulation of three candidate proteins related to energy metabolism that may play a critical role in CKD, especially in DKD.

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