In vitro evidence of the promoting effect of testosterone in kidney stone disease : A proteomics approach and functional validation

Channarong Changtong[#], Paleerath Peerapen[#], Supaporn Khamchun, Kedsarin Fong-ngern, Somchai Chutipongtanate, and Visith Thongboonkerd*

Medical Proteomics Unit, Office for Research and Development, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, THAILAND; Center for Research in Complex Systems Science, Mahidol University, Bangkok, THAILAND

*[#]Equal contributions by these authors, *Correspondence: vthongbo@yahoo.com*

Introduction

Incidence of kidney stone disease in males is 2-4 fold greater than in females. Testosterone has been proposed as the key factor responsible for the male preference of this disease. Nevertheless, mechanisms of promoting effect of testosterone on calcium oxalate monohydrate (COM) kidney stone formation remained unclear. This study aimed to determine effects of testosterone on kidney stone disease using a proteomics approach.

Materials and Methods

MDCK renal tubular cells

↓

With or without 20 nM Testosterone (7 days)

↓

2-DE analysis

↓

MS/MS identification of differentially expressed proteins

→

- **Functional classification of proteins**
  - PANTHER database
- **Global protein network analysis**
  - STRING database
- **Validation of proteome data**
  - Western blotting analysis of α-enolase in whole cell lysate
- **Localization of the increased α-enolase**
  - Immunofluorescence staining
  - Western blotting analysis
- **Functional investigation of α-enolase**
  - COM crystal adhesion assay with specific antibody neutralization

Results

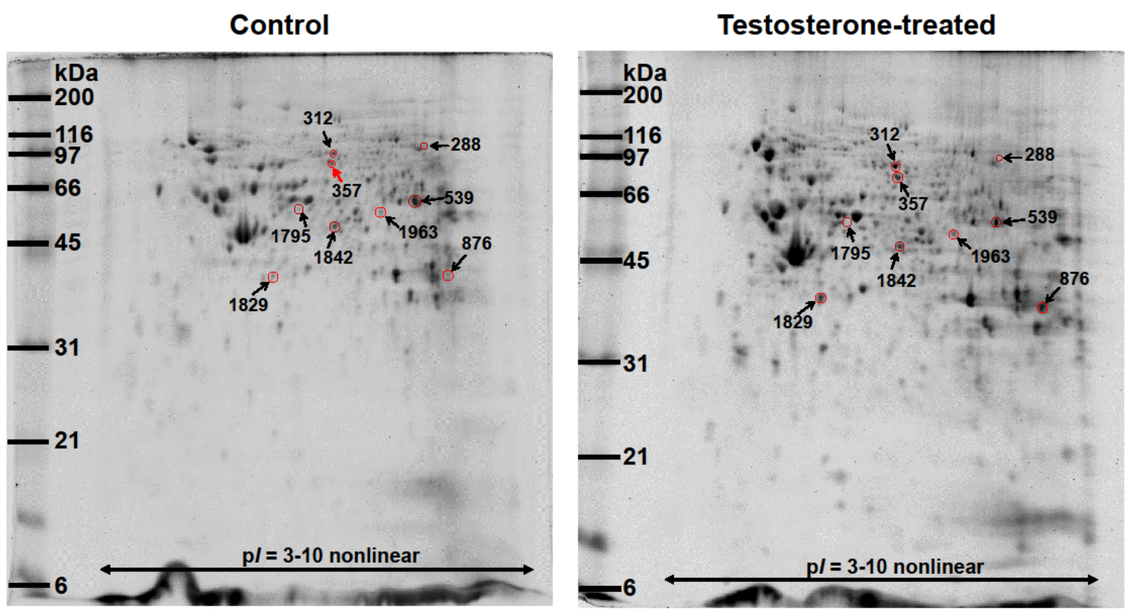
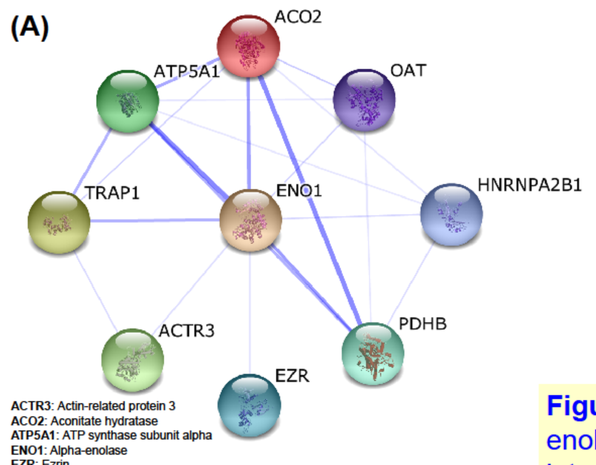


Figure 1: 2-D proteome maps of differentially expressed proteins in controlled vs. testosterone-treated MDCK cells.

Table 1: Summary of differentially expressed proteins identified by MS/MS analyses.

Protein name	Spot no.	Alteration	Function	Subcellular localization
Ezrin	312	Increased	• Cellular component organization	Cytoplasm and cell membrane
Heat shock protein 75 kDa, mitochondrial	357	Increased	• Chaperone-mediated protein folding • Response to stress	Mitochondria
Heterogeneous nuclear ribonucleoproteins A2/B1 isoform A2	876	Increased	• Cell cycle • Gene expression • RNA splicing	Nucleus and cytoplasm
Pyruvate dehydrogenase E1 component subunit beta, mitochondrial	1829	Increased	• Cellular metabolic process	Mitochondria
Alpha-enolase	1963	Increased	• Glucose metabolic process	Cytoplasm and cell membrane
Aconitate hydratase	288	Decreased	• Cellular metabolic process	Mitochondria
ATP synthase subunit alpha	539	Decreased	• Cellular metabolic process	Mitochondria and cell membrane
Ornithine aminotransferase	1842	Decreased	• Cellular amino acid biosynthetic process	Mitochondria
Actin-related protein 3	1795	Absent	• Cellular component organization	Cytoplasm and cell membrane



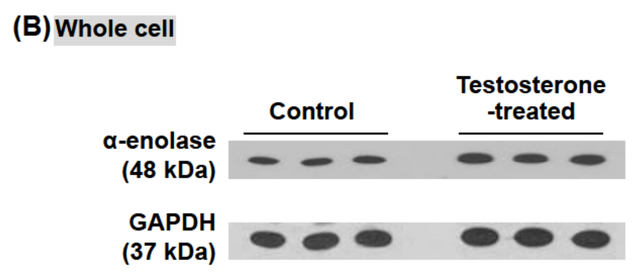


Figure 2: Protein network analysis revealed α-enolase as the central node of protein-protein interactions **(A)**. The increased level of α-enolase was confirmed by Western blot analysis **(B)**.

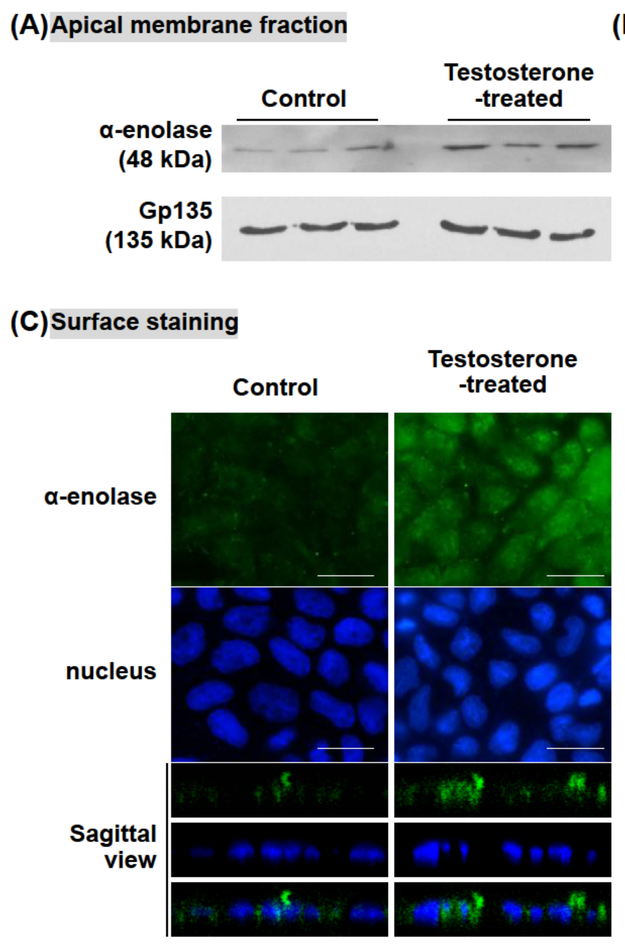


Figure 3: Western blot analysis revealed the increased α-enolase level in both apical membrane **(A)** and cytosolic **(B)** fractions of testosterone-treated cells. Immunofluorescence study showed the increased α-enolase both on cell surface **(C)** and intracellularly **(D)** induced by testosterone. Green = α-enolase staining, Blue = nuclear staining, scale bar = 20 μm.

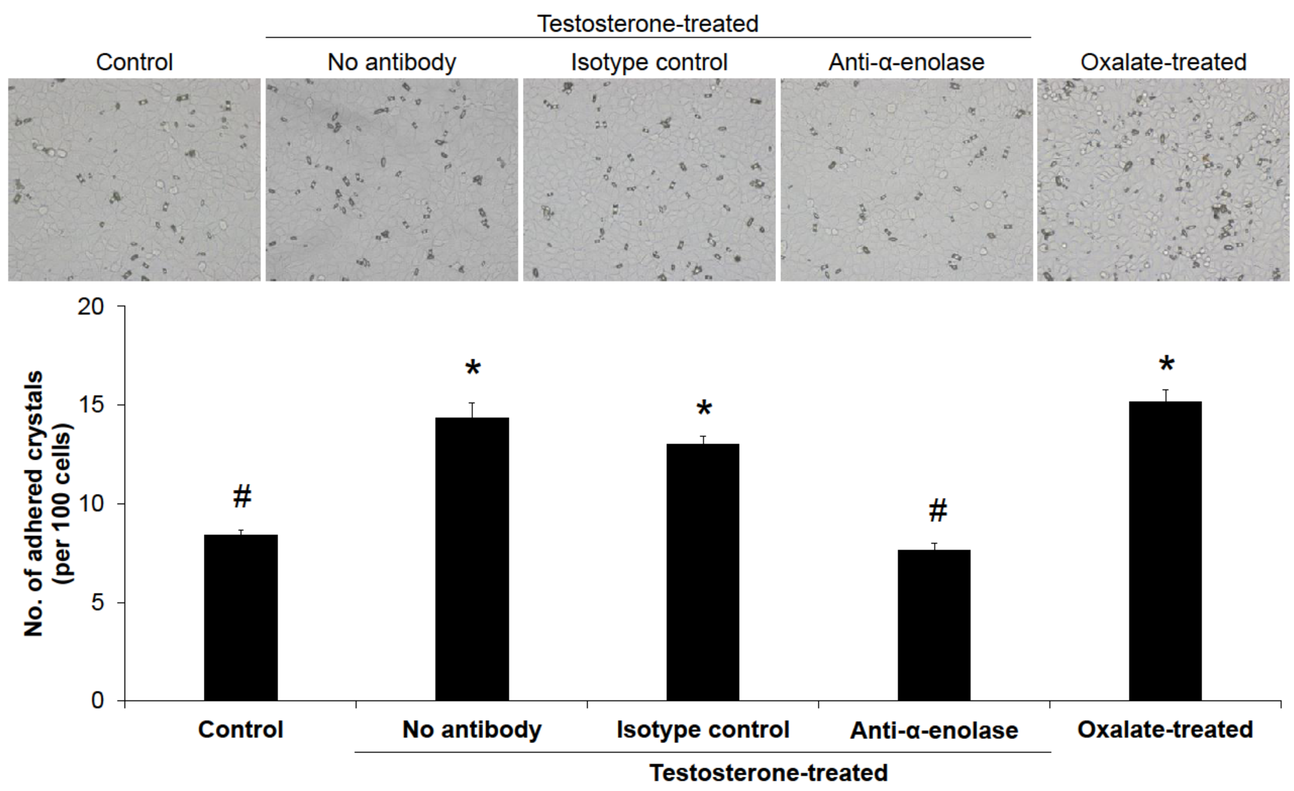


Figure 4: Calcium oxalate monohydrate (COM) crystal adhesion assay revealed the increased COM crystal-binding capacity in testosterone-treated cells and high oxalate-induced α-enolase overexpressed cells. The increase of COM binding capacity of the testosterone-treated cells was reduced by neutralizing surface α-enolase using anti-α-enolase antibody. * = $p < 0.05$ vs. control and testosterone-treated cells neutralized with anti-α-enolase antibody, # = $p < 0.05$ vs. other three groups. N=3 for each group.

Conclusions

Our data provided an *in vitro* evidence demonstrating a promoting effect of testosterone on kidney stone disease via enhanced COM crystal-cell adhesion by the increased surface alpha-enolase.

This study was supported by Mahidol University research grant, Office of the Higher Education Commission and Mahidol University under the National Research Universities Initiative, and the Thailand Research Fund (RTA5680004). VT is supported by "Chalermphrakiat" and "Research Staff" Grants from Faculty of Medicine Siriraj Hospital.