DIFFERENTIALLY EXPRESSED PROTEINS INVOLVED IN ACETYLATION OF SPERMATOZOA IN INFERTILE MEN WITH UNILATERAL AND BILATERAL VARICOCELE

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ABSTRACT

Objective: Differentially expressed proteins (DEP) have been identified in spermatozoa of infertile men with unilateral and bilateral varicoceles. To maintain protein stability, transcriptionally silent spermatozoa depend on the post-translational modifications (PTMs) such as acetylation, phosphorylation and ubiquitination. Acetylation involves the transfer of acetyl groups from acetyl-CoA to a free amino group of a target protein. The objective of our study was to examine global proteomic data from men with unilateral and bilateral varicocele and identify functional proteins involved in sperm acetylation process.

Database searching and protein identification

Results: Utilizing the raw data files published in our previous studies on unilateral varicocele (Agarwal et al., 2015), we performed protein identification. All MS/MS raw files were analyzed using Mascot, Sequest and X! Tandem to search the human reference database (33292 entries) assuming the digestion enzyme trypsin. We identified 9699 proteins in the proteome of spermatozoa. Quantitative proteomics

RESULTS

5. STRING analysis displayed the DEPs present in the proteasome complex interact with each other to form binding entities (Figure 4b). These DEPs were under expressed and present as low abundance molecules in bilateral varicocele.

6. Protein-protein interaction identified functionally active binding mechanism between the proteins involved in mitochondrial dysfunction and heat shock proteins involved in DNA damage (Figure 4c).

7. Based on the protein interaction network and their functional importance, nine proteins were identified as potential biomarkers to differentiate clinical bilateral varicocele from unilateral varicocele (Table 1).

INTRODUCTION

Varicocele is the most common treatable cause of male infertility. It is accompanied by poor semen quality, increased seminal oxidative stress and sperm DNA fragmentation. Alteration in their expression has deleterious effect on the sperm function in varicocoele patients. Recently, we examined the differential expression of proteins involved in the acetylation process, apoptosis, DNA damage and oxidative stress in spermatozoa of infertile men with unilateral and bilateral varicocele. Hence it is essential to determine the differential expression and abundance of these proteins pertaining to spermatogenesis, spermatid differentiation or development, acrosome reaction, mitochondrial dysfunction, energy metabolism, apoptosis, DNA damage, DNA methylation and oxidative stress.

The goal of this study was to re-examine our published proteomic data on unilateral and bilateral varicocele and identify proteins that are involved in the acetylation process.

REFERENCES

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Materials and Methods: We compared the sperm proteome in pooled unilateral (n=5) and bilateral (n=3) varicocele patients using a LTQ-Orbitrap Elite hybrid mass spectrometer system. Protein abundance was quantified based on the normalized spectral abundance factor. DEPs involved in the acetylation process were identified using UniProtKB, IPA, and MetaCore. Protein-protein interaction network data was done using TopNet and STRING software. Cytoplasm was used for plotting results.

Results: 135 DEPs were involved in the acetylation process, 62 DEPs were overexpressed and 32 underexpressed in unilateral group compared to bilateral varicocele group. Bioinformatic analysis revealed low abundance and underexpressed proteasome complex proteins in the bilateral varicocele group. Transcription factors of androgen receptor, p53 and NRF2 were the key interlinked molecules regulated by the proteasome complex may increase sperm DNA damage and apoptosis in patients with bilateral varicocele due to aggregation of the misfolded proteins.

RESULTS

1. 135 differentially expressed proteins were involved in the acetylation process. Among these DEPs 62 were overexpressed and 32 were underexpressed in unilateral varicocele. Apart from these DEPs, 13 proteins were uniquely expressed and 28 were expressed in bilateral varicocele group respectively (Figure 1a). Most of these uniquely expressed proteins present in low and very low abundance in unilateral varicocele group (Figure 1b).

2. GO enrichment analysis and IPA annotation identified 84.48% proteins involved in the acetylation process derived from the proteome. Functional analysis was done using IPA (Ingenuity Pathway Analysis) and STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) software. STRING was used to display the functional link between the protein-protein interaction among the proteins involved in acetylation process with respect to spermatogenesis, spermatid differentiation or development, sperm motility, capacitation, acrosome reaction, mitochondrial dysfunction, energy metabolism, apoptosis, DNA damage, DNA methylation and oxidative stress.

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