ABSTRACT

Vasculitis appears to affect various stages of spermatogenesis. It causes scrotal hypotension, hypogastric, hormonai abnormalities, and block of motility from local and/or androgen glands leading to oxidative stress. The objective was to study the effect of vasculitis on the distribution of spermatogenic proteins in infertile vasculitis compared to fertile men.

MATERIALS AND METHODS: Proteins were extracted from fertile and bilateral vasculitis patients (20 and with fertile protein(s)). 10 gel electrophoresis followed by LC-MS/MS to identify the vasculitis group. The overall vasculitis group was anatomically characterized (NCBI, UQ). Functional and pathway database.

RESULTS: 15-20% of the adult male population and is implicated as a factor in about 40% of infertile men. The recommendation of the American Society of Reproductive Medicine is to treat a vasculitis when it is palpable and present with at least one abnormal semen parameter in couples presenting with infertility or when the female partner is normal. Numerous factors have been proposed to explain the occurrence of this multifactorial disease including viral, heat stress, testicular hypoxia, increased metal toxicity, increased oxidative stress and increased DNA damage. However, the underlying molecular mechanisms of vasculitis associated testicular dysfunction remain unclear.

High thoroughput integrated proteomic analysis of spermatozoal proteins in pathophysiology of varicocoele associated male infertility.

INTRODUCTION

Vasculitis is diagnosed in about 15-20% of the adult male population and is implicated as a factor in about 40% of infertile men. The recommendation of the American Society of Reproductive Medicine is to treat a vasculitis when it is palpable and present with at least one abnormal semen parameter in couples presenting with infertility or when the female partner is normal. Numerous factors have been proposed to explain the occurrence of this multifactorial disease including viral, heat stress, testicular hypoxia, increased metal toxicity, increased oxidative stress and increased DNA damage. However, the underlying molecular mechanisms of vasculitis associated testicular dysfunction remain unclear.

Proteomics is a rapidly emerging technology that allows the simultaneous detection of thousands of proteins. A few studies have looked at altered protein profiles in vasculitis patients before and after vasculitis repair. However, irrespective of the type (unilateral or bilateral) and/or grade of vasculitis, a cohort of patients remains infertile.

In this study, we aim at comparing the protein profile of spermatozoa from infertile men with vasculitis with that of fertile men. We also examined proteins involved in key sperm functions related to spermatogenesis, sperm motility, and mitochondrial dysfunction.

MATERIALS AND METHODS

This study was approved by the Institutional Review Board. Semen samples were collected from 50 infertile varicocele patients and 10 proven fertile men without a vasculitis. Semen analysis was conducted and 5 samples from vasculitis and 5 from the fertile group were pooled and prepared for proteomic analysis.

Liquid Chromatography Mass Spectrometer analysis (LC-MS) 5 samples from each group were normalized for protein concentration and pooled. Equal amounts of proteins were diluted in the SDS-PAGE sample buffer and fractionated using 10% SDS-PAGE. The gels bands were reduced and alkylated followed by trypsinization. The samples were then assigned to MS analysis. The digest was analyzed using the data dependent multi-protein mode of the instrument accessing full scan mass spectra to determine peptide molecular weights and product ion spectra (MS/MS) to determine amino sequences in subsequent sequence databases.

Database Searching and Protein Identification

Tandem mass spectra were extracted by Proteome Discoverer version 1.4.288. All MS/MS samples were analyzed using Mascot, Sequest and Tandem. To validate MS/MS-based peptide and protein identifications, Scaffold was used. Proteins were annotated with gene ontology (GO) terms from NCBI.

Quantitative Proteomics

To account for the sample-to-sample variation seen during the replicate analysis of the sample, normalized spectral abundance factor (NSAF) approach was applied prior to quantification of the relative amount of protein present. The abundance of the proteins was classified according to the average spectral count, identified on the basis of a fold change (log2) greater than 1.67 and a value of p<0.05.

RESULTS

1. Very low abundance: Spectral count range 0.3 ≤ 1.6 and NSAF ratio ≤ 2.0 for underexpressed proteins

2. Low abundance: Spectral count range 2.0 ≤ 5.0 and NSAF ratio ≤ 2.0 for overexpressed proteins

3. Medium abundance: Spectral count range between 5.0 ≤ 10.0 and NSAF ratio > 2.0 for overexpressed proteins

4. High abundance: Spectral count >10.0 and NSAF ratio > 2.0 for overexpressed proteins

Bioinformatics analysis

Functional annotation and enrichment analysis were performed using publicly available bioinformatics annotation tools and databases (GO Term Finder, GO Term Mapper, UniProt, STRING). Database for Annotation, Visualization and Integrated Discovery (DAVID) and proprietary software packages such as IPA (Ingenuity Pathway Analysis) and Search Tool for the identification of Interacting Genes/Proteins (STRING).

CONCLUSIONS

1. In fertile men with varicocoele there are a large number of spermatogenic proteins that are underexpressed compared to fertile men.

2. Many of the underexpressed proteins are in the mitochondria, endoplasmic reticulum and in metabolic pathways, transport, protein folding and protein pumps.

3. Many of these proteins are involved in spermatogenesis, sperm motility and mitochondrial dysfunction.

4. Fertile men exhibit unique proteins that are completely missing in infertile men with varicocoele.

5. The underexpression of these proteins in infertile men is indicative of development of vasculitis and sperm dysfunction that ultimately results in male infertility.

6. Validation of some of these DEP proteins in this study will narrow down the key proteins involved in the development of vasculitis. This may help the clinicians identify patients who are more likely to benefit from varicocoele surgery and have improvement in sperm quality, and consequently, an increased likelihood of a successful pregnancy.