SPERMATOZOA PROTEIN PROFILES IN CRYOBANKED SEMEN SAMPLES FROM TESTICULAR CANCER PATIENTS BEFORE TREATMENT

Ashok Agarwal, PhD.,1 Eva Tvrdova, PhD.,1,2 Rakesh Sharma, PhD.,1 Sajal Gupta, MD.,1 Gulfam Ahmad, PhD.,1,3 and Edmund S. Sabanegh, MD.4

1American Center For Reproductive Medicine, Cleveland Clinic, Cleveland, OH; 2Department of Animal Physiology, Faculty of Biotechnology and Food Sciences, Nitra, Slovakia; 3Physiology and Cell Biology, University of Health Sciences, Lahore, Pakistan; 4Department of Urology, Cleveland Clinic, Cleveland, OH

INTRODUCTION
Testicular cancer (TC) is the most common malignant diagnosis in the reproductive age group. Current attention focuses on the activity of the tumor and whether the treatment has a significant impact on the reproductive function of the patient. The cancer can be curable with early detection and decreased male fertility may not be present even before the start of cancer treatment. Endocrine imbalances and altered spermatogenesis leading to azoospermia or oligospermia, as well as the sperm motility and mobility ability may be observed in pre-treatment oncological patients. Presence of a malignancy may lead to glandular dysfunction through a variety of mechanisms, which may be commonly observed in males with general infertility as well.

Cancer is also believed to be one of the most important causes of male subfertility. Numerous reports also suggest that patients with a decreased fertility of unknown etiology may be at a higher risk for developing TC. Both cancer and sterile infertile patients exhibit common features of testicular infertility. Unfortunately, the sperm count, leukocytospermia and a high percentage of non-spermatozoa are usually the first observations in sterile infertile patients. It is therefore suggested that the development of testicular cancer is a result of the interaction of paracrine and endocrine factors leading to testicular degeneration and eventual infertility.

Ecto ACP
Expressed ubiquitously with the highest levels in the prostate and testes. Essential for the maintenance of sperm motility.

OE/M
Detected in testis. Functions during spermatogenesis as a chaperone for a wide range of proteins.

UE/M
Highly expressed in testis. Possesses single nucleotide polymorphism (SNP).

OE/H
Expressed in testis. Force generating protein of respiratory system.

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CONCLUSIONS
1. Testicular cancer of the reproductive cells affected by testicular cancer vary significantly than normal male reproductive systems.

2. Overexpression and underexpression of DEPs in the testicular cancer group only may be a contributory factor in the severity of the disease and sperm dysfunction in these patients.

3. Alterations in the sperm proteins of testicular cancer patients may provide an insight into the underlying processes, quality and quantity of testicular cancer patients’ sperm.

4. DEPs identified may serve as useful biomarkers in the diagnosis of testicular cancer and useful non-invasive tool to assist endocrinology specialists in a better identification and management of fertility in patients suffering from testicular cancer.

RESULTS

Table 1: Differentially expressed proteins compared to the infertile control group that are involved in reproductive functions

<table>
<thead>
<tr>
<th>Protein Name</th>
<th>Expression Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Semenogelin</td>
<td>Overexpressed</td>
</tr>
</tbody>
</table>
| Intron 
| cell 
| cancer | proteins |
| (n=51) |
| Intron 
| cell 
| cancer | proteins |
| (n=51) |
| VL |
| M |
| L |
| 50 |
| 0 |
| 50 |
| 100 |
| 150 |
| 200 |
| 250 |
| 300 |
| 350 |
| 400 |
| 0 5 10 15 20 25 30 35 40 |
| Overexpressed |
| Underexpressed |
| 24.94% |
| (134/389; 34.45%), small molecule metabolic processes (132/389; 34.76%), immune system processes (57/389; 14.77%), biological regulation (223/389; 57.45%), developmental processes. Among the biological processes, the majority of the DEPs were involved in protein metabolism. Of the proteins that were underexpressed in the IF group, 47 (33.3%) showed very low abundance, 26 low abundance, 34 medium abundance and 2 very high abundance. Of the DEPs unique to the cancer patients, 5 displayed very low abundance (VL), 7 low abundance (L), 31 medium abundance (M) and 4 very high abundance (H).

Molecular interactions and endeavours were performed using available databases such as TCDB, Reactome, KEGG Pathways and BioCarta. Software tools such as IPA (Ingenuity Pathway Analysis) and Reactome database were used to identify pathways and molecules involved in the DEPs.

Proteins were compared using the T-test (with significance set at a P-value of 0.05). Proteins were grouped into categories based on their ratios: ≥2.5 for overexpressed, ≤0.4 for underexpressed proteins. Statistical analysis was performed using the Scaffold software package. The average number of peptide counts per protein was used as a measure of protein abundance. The analysis was done in triplicate using the label-free quantification method and used the ScanCount plug-in to count the number of images that matched the features of the protein. The ratios of peptide counts were calculated for each protein. A protein was considered to be significantly differentially expressed if the ratio was greater than 2 or less than 0.4. Prognostic factors were identified using a combination of statistical analysis and biological knowledge. Significant differences were considered to be present if the P-value was less than 0.05.

Pacta bioinformatics analysis

Functional annotation and enrichment analysis were performed using the GO Term Finder, GO Term Mapper, UniProt, Software Tools for Researching Annotations and databases such as Reactome, KEGG Pathways, BioCarta, and IPA (Ingenuity Pathway Analysis) to determine the enriched GO terms and pathways for the DEPs. The biological processes, cellular component and molecular function were analyzed to identify significant pathways and processes involved in the DEPs.

Proteomic profiling

Semen was obtained from 16 patients diagnosed with testicular cancer prior to any cancer treatment. Endocrine imbalance and disturbed spermatogenesis may lead to gonadal dysfunction through a variety of mechanisms which may be commonly observed in males with general infertility as well.

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Proteomic analysis and Liquid chromatography mass spectrometry analysis (LC-MS)

Global proteomic analysis was performed using the label-free quantification method. Following isolation and disruption, protein samples were extracted and purified. The extracted proteins were then fractionated using size-exclusion chromatography and reconsolidated. The purified and reconsolidated proteins were subjected to LC-MS analysis. The extracted proteins were fractionated using size-exclusion chromatography and reconsolidated. The purified and reconsolidated proteins were subjected to LC-MS analysis.

Criteria for protein identification and Quantitative analysis

Scalable was used to identify MS/MS-based peptide and protein identifications. Proteins were assigned with gene ontology (GO) terms from National Center for Biotechnology Information (NCBI). Normalization of spectral counts using the NCBI (normalised spectral abundance factor) approach was applied prior to relative protein quantification. Differentially expressed proteins (DEPs) were separated into 4 categories:

1. Very low abundance (VL), spectral count range 1-7.7, p<0.001 and (NASP) ratio ≥2.5 for overexpressed, ≤0.4 for underexpressed proteins.

2. Low abundance (L), spectral count range 8-19.5, p<0.01 and (NASP) ratio ≥2.5 for overexpressed, ≤0.4 for underexpressed proteins.

3. Medium abundance (M), spectral count range 20-79.5, p<0.05 and (NASP) ratio ≥2.5 for overexpressed, ≤0.4 for underexpressed proteins.

4. High abundance (H), spectral count range ≥80, p<0.05 and (NASP) ratio ≥1.5 for overexpressed, ≤0.67 for underexpressed proteins.

Overall, 79 proteins were associated with disease state, 36 proteins with increased function and 2 proteins with reproduction.

Protein name

Testis specific protein, only expressed in round spermatids. Major structural component of sperm fibrous sheath. Plays a role in sperm motility.

RUVBL1
Testis specific. Alterations in its expression are linked to non-obstructive azoospermia.

CRISP1
Involved in the binding of capacitated spermatozoa to the egg’s zona pellucida.

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