COMPARATIVE SPERM PROTEOMICS OF CANCER PATIENTS AND FERTILE HEALTHY MEN USING LC-MS/MS

Ashok Agarwal¹, Manesh Kumar Panner Selvam¹, Peter N. Pushparaj²

¹Cleveland Clinic, American Center for Reproductive Medicine - Department of Urology, Cleveland, U.S.A.
²Center of Excellence in Genomic Medicine, King Abdulaziz University, Jeddah, Saudi Arabia.

Introduction

• Cancer affects the reproductive health of males. Sperm banking prior to cancer treatment is the recommended procedure to preserve their fertility.
• The effect of cancer on the fertility status of these men cannot be determined using conventional semen analysis.
• The objective was to compare the sperm proteome of cancer patients before initiating cancer therapy with healthy fertile men.

Study Design and Participants

• Pooled cryopreserved semen samples from patients in the cancer group (n=5) and fertile healthy men (n=5) were run in triplicates on 1D-SDS PAGE.
• Global proteomic analysis was performed using liquid chromatography tandem mass spectrometry (LC-MS/MS).
• Proteins and peptides were identified using search programs MASCOT and SEQUEST.
• Functional pathway analysis of the differentially expressed sperm proteins (DEPs) was done using the Ingenuity Pathway Analysis (IPA) software.

Results

Study Design and Participants

Cryopreserved Semen Samples

- Cancer group:
  - Testicular cancer (n=28)
  - Hodgkin’s disease (n=20)
  - Lymphoma (n=8)
  - Leukemia (n=5)
- Control group:
  - Fertile healthy men (n=7)

Table 1: The top five canonical pathways associated with male cancer patients

<table>
<thead>
<tr>
<th>S.No</th>
<th>Canonical Pathway</th>
<th>-log(B-H p-value)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mitochondrial Dysfunction</td>
<td>20.6</td>
<td>7.41E-24</td>
</tr>
<tr>
<td>2</td>
<td>Oxidative Phosphorylation</td>
<td>12.7</td>
<td>1.31E-15</td>
</tr>
<tr>
<td>3</td>
<td>Sirtuin Signaling Pathway</td>
<td>7.38</td>
<td>3.86E-10</td>
</tr>
<tr>
<td>4</td>
<td>Protein Ubiquitination Pathway</td>
<td>6.91</td>
<td>3.86E-10</td>
</tr>
<tr>
<td>5</td>
<td>TCA Cycle II (Eukaryotic)</td>
<td>6.25</td>
<td>8.66E-09</td>
</tr>
</tbody>
</table>

- The rapamycin-insensitive companion of mammalian target of rapamycin (RICTOR) function was activated in cancer patients which in turn affected spermatogenic process.
- Sperm function in cancer patients is compromised as a result of mitochondrial dysfunction and defective ubiquitination system.

Conclusion

• Pathways associated with normal physiological sperm function are compromised in cancer patients.
• Fertility of these patients are at risk due to altered expression of certain critical sperm proteins.