INFERTILITY IN MEN WITH TESTICULAR CANCER NON-SEMINOMA IS ASSOCIATED WITH SPERM MITOCHONDRIAL DYSFUNCTION AND DEFECTS IN MOTILITY AND SPERM-OCYTE BINDING

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Introduction

The National Cancer Institute (NCI) reported 8,850 new testicular cancer in 2017 and this number is estimated to increase to 9,310 new cases in 2018. TC can be classified into germ cell tumors, non-germ cell tumors, and embryonal tumors. About 90 to 95 percent of TCs originate from germ cells and the highly prevalent germ cell tumors (GCT) are further classified as either seminoma or nonseminomas, based on the pathological and clinical manifestations. GCTs represent the most common type of TC, accounting for about 90-95% of all cases. The principal types of GCTs are the testicular cancer non-semionomas (TCNS) and testicular cancer seminomas (TCS) and the former is usually growing and spreading faster. The survival rate of men with TC is well over 95%. However, its impact on male fertility represents a major concern for reproductive medicine as it frequently affects men in reproductive age (20-44 yrs). Several studies reveal that even prior to TC diagnosis, there is a decrease in the fertilizing capacity of these men.

In the present study, we have analyzed the sperm proteome of patients with TCNS before cancer treatment to unravel the proteins responsible for the altered reproductive function.

Materials and Methodology

After getting institutional approval, banked semen samples from men with proven fertility (n=15) and TCNS before start of cancer treatment (n=15) were obtained for the proteomic analysis using Freigein LTQ Orbitrap Elite Hybrid Mass Spectrometry (Figure 1). Scaffold (Proteome Software Inc., Portland, OR, USA, version 4.0.6.1) was used for the identification of the differentially expressed proteins (DEPs) between the TCNS and control groups. The spectral counts were used to determine the abundance of each protein and identified DEPs were categorized as upregulated, downregulated or unique to one of the groups, based on the normalized spectral abundance factor (NSAF) ratio.

Functional Bioinformatics and Western Blot analysis

Bioinformatic analysis of DEPs identified by LC-MS/MS was carried out using the Ingenuity Pathway Analysis (IPA, QIAGEN, USA) software. Proteins were selected for validation by WB considering the following criteria: 1) sperm mitochondrial dysfunction, deficits in motility and sperm-oocyte-binding; 2) proteins involved in the top canonical pathways; 3) proteins with a higher difference of abundance relative to the control group; 4) proteins with a well-described function in the literature.

Western blot (WB) was performed using individual samples from the normal control and TCNS groups (n=6/group). Results were expressed as fold variation relative to the control group.

Results

Table 1. Comparison of parameters of fertile donors and patients with TCNS before banking.

Table 2. Specific functions of the DEPs related to reproductive system development and function identified by the bioinformatic analysis when comparing the sperm proteome of patients with TCNS relative to fertile men.

Table 3. Proteomic data of DEPs identified in the spermatozoa samples of fertile men (control) and men with TCNS before cancer therapy, which were selected for validation by Western Blot.

Figure 1. Experimental Design

Healthy Volunteers with Proven Fertility

Table 4. Western Blot Data showing expression of selected proteins in TCNS and control group.

Conclusion

1. The downregulation of proteins in the spermatozoa of TCNS patients like ACR, AKTP4, ANTP, PBP, ZPBP and other functionally related proteins could be responsible for reduced fertility, fertilization, formation of flagella, binding of sperm with zona pellucida, and abnormal morphology of sperm leading subsequently to subfertility/infertility androgenesis.

2. Underexpression of NDUFS1, UQCR and OXCR2 indicate sperm mitochondrial dysfunction, while underexpression of ATP1A4 exploring the decrease in the number of motile sperm in TCNS patients.

3. Decreased expression of ANTP2 may lead to impaired sperm-oocyte binding.

4. These DEPs may serve as potential biomarkers for the development of new diagnostic or treatment approaches for infertile patients with TCNS.

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