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ORIGINAL ARTICLE

Male Fertility

Reduced semen quality in patients with testicular cancer seminoma is associated with alterations in the expression of sperm proteins

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Testicular cancer seminoma is one of the most common types of cancer among men of reproductive age. Patients with this condition usually present reduced semen quality, even before initiating cancer therapy. However, the underlying mechanisms by which testicular cancer seminoma affects male fertility are largely unknown. The aim of this study was to investigate alterations in the sperm proteome of men with seminoma undergoing sperm banking before starting cancer therapy, in comparison to healthy proven fertile men (control group). A routine semen analysis was conducted before cryopreservation of the samples (n = 15 per group). Men with seminoma showed a decrease in sperm motility (P = 0.019), total motile count (P = 0.001), concentration (P = 0.003), and total sperm count (P = 0.001). Quantitative proteomic analysis identified 393 differentially expressed proteins between the study groups. Ten proteins involved in spermatogenesis, sperm function, binding of sperm to the oocyte, and fertilization were selected for validation by western blot. We confirmed the underexpression of heat shock-related 70 kDa protein 2 (P = 0.041), ubiquinol-cytochrome C reductase core protein 2 (P = 0.026), and testis-specific sodium/potassium-transporting ATPase subunit alpha-4 (P = 0.016), as well as the overexpression of angiotensin I converting enzyme (P = 0.005) in the seminoma group. The altered expression levels of these proteins are associated with spermatogenesis dysfunction, reduced sperm kinematics and motility, failure in capacitation and fertilization. The findings of this study may explain the decrease in the fertilizing ability of men with seminoma before starting cancer therapy.

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INTRODUCTION

Germ cell tumors (GCTs) represent the most common type of testicular cancer, accounting for about 90%–95% of all cases. The principal types of GCTs are nonseminomas and seminomas; the latter usually grows and spreads more slowly. In the last decades, there is a growing trend in the proportion of seminomas.[1] The survival rate of men with seminoma is very high (over 95%); thus, it is generally not seen as a threat to public health. However, its impact on male fertility represents a major concern for reproductive medicine as it frequently affects men in reproductive age (20–44 years).[2]

Men with seminoma present impaired fertilizing ability, even before diagnosis.[3] Testicular cancer seminoma affects the hypothalamic-pituitary-gonadal (HPG) axis and consequently disturbs spermatogenesis.[4] These deleterious effects are dependent on the stage and type of seminoma, resulting in poor semen quality or even azoospermia.[5] The treatment for this type of cancer, usually performed by surgery, chemotherapy, or radiotherapy, further affects semen quality[5] and hormonal function,[6] thus highly impairing male fertility. In fact, after cancer therapy, patients may become temporarily or permanently infertile.[7] For that reason, it is strongly recommended that men diagnosed with seminoma undergo sperm banking to increase the probability to father a child in the future.[8] The chances to establish a pregnancy by natural conception are 30% lower after the cancer therapy and the recovery of fertilizing ability usually takes several years.[9] Therefore, in many surviving patients with seminoma, assisted reproductive technology (ART) with cryopreserved samples is the only option for having children.[10] Still, sperm banking is not possible for many patients due to the high cost or lack of facilities, urgency to initiate the treatment, impaired spermatogenesis, and/or poor semen quality at the time of specimen collection.[11]

Proteomics studies have been recently used as a valuable tool to explore how certain health conditions affect male reproductive potential, especially by evaluating spermatozoa and seminal plasma proteome.[12,13] Although spermatozoa are transcriptionally and translationally silent after being produced in the testis, the acquisition of sperm function occurs during maturation in the epididymis and transit through the female reproductive tract.[14] Therefore, the sperm proteome is highly susceptible to alterations according to the health status of

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the individual, and this impacts the quality of sperm parameters. The deleterious effects of seminoma treatment represent a challenge to understand the mechanisms behind the impairment of male fertility caused by the disease. In this study, we used semen samples from men with testicular cancer seminoma that were cryopreserved before starting cancer therapy, to investigate alterations in the sperm proteome in comparison with healthy proven fertile men.

PARTICIPANTS AND METHODS

Semen analysis and cryopreservation

This study was conducted after approval by the Institutional Review Board (IRB) of Cleveland Clinic, Cleveland, OH, USA. Semen samples were obtained from healthy volunteers with proven fertility (control, n = 15) and patients with seminoma (n = 15). All the participants signed informed written consent to allow the use of their samples in this study. The inclusion criteria were as follows: (1) control group, healthy fertile men who had fathered a child in the last 2 years; (2) seminoma group, patients diagnosed with seminoma and undergoing sperm banking before starting cancer therapy. Following 2–3 days of abstinence, semen samples were collected at the Andrology Center, Cleveland Clinic. Samples were liquefied for 20–30 min in an incubator (Panasonic, Newark, NJ, USA) at 37°C, and a routine semen analysis was conducted according to the World Health Organization (WHO) 2010 guidelines.[15] Semen volume, sperm motility, and sperm concentration were recorded. Total sperm count and total motile count were also calculated and the results were expressed as mean ± standard error of the mean (s.e.m.). Whole ejaculate samples were immediately cryopreserved in TEST-yolk buffer (TYB; Irvine Scientific, Santa Ana, CA, USA) in a ratio of 1:1 as previously described[16] and finally labeled and stored in liquid nitrogen at −196°C.

Protein extraction and estimation

Samples were thawed on ice and centrifuged at 4000*g* for 10 min (Eppendorf, Hauppauge, NY, USA). To remove the freezing medium (TYB) as much as possible, the sperm pellet was washed four times in phosphate-buffered saline (PBS; Sigma-Aldrich, St. Louis, MO, USA) and centrifuged at 4000*g* for 10 min at 4°C. Total sperm protein was extracted overnight at 4°C with radioimmunoprecipitation assay (RIPA) buffer (Sigma-Aldrich). Subsequently, samples were centrifuged at 10 000*g* for 30 min at 4°C, to recover the protein fraction (supernatant). Pierce BCA Protein Assay kit (Thermo Fisher Scientific, Waltham, MA, USA) was used to estimate the protein concentration, according to the manufacturer's instructions.

Quantitative proteomic analysis

Three samples from the control or seminoma group were randomly selected for the proteomic analysis by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Samples were pooled (n = 3) using the same amount of protein from each sample. Each pool was then evaluated as an individual sample in the proteomic analysis. The system used was a Finnigan LTQ-Orbitrap Elite hybrid mass spectrometer (Thermo Fisher Scientific) using the previously described conditions and software.[17] Scaffold (version 4.0.6.1, Proteome Software Inc., Portland, OR, USA) was used for the identification of differentially expressed proteins (DEPs) between the control and seminoma groups. The spectral counts were used to determine the abundance of each protein (very low, low, medium, or high). The identified DEPs were categorized as underexpressed, overexpressed, or unique to one of the groups, based on the normalized spectral abundance factor (NSAF) ratio according to previously reported criteria.[17]

Bioinformatic analysis

Bioinformatic analysis of DEPs identified by LC-MS/MS was carried out using the Ingenuity Pathway Analysis software (IPA; Qiagen, Hilden, Germany). IPA was used to evaluate the canonical pathways, top diseases and bio-functions, and upstream regulators related to the identified DEPs. Proteins were selected for validation by western blot considering the following criteria: (1) proteins involved in reproductive system development and function; (2) proteins involved in the top canonical pathways; (3) proteins with a higher difference of abundance between the experimental groups; and (4) proteins with a well-described function in the literature. Only proteins that met all the above-mentioned criteria were subjected to western blot.

Western blot

Western blot was performed using individual samples from the control and seminoma groups (n = 6 per group). A total of 25 μg protein per sample was mixed with 4 × Laemmli sample buffer (Bio-Rad, Hercules, CA, USA) in a ratio of 1:3 and made up to 25 μl with PBS. Samples were boiled at 95°C for 10 min and immediately loaded into a 4%–15% (*w/v*) polyacrylamide gel (Bio-Rad). Electrophoresis was performed with constant voltage (90 V) for 2 h. Precision Plus Protein™ Dual Xtra Standard (Thermo Fisher Scientific) was used as the molecular weight marker. The resolved proteins were transferred (20 V for 30 min) to methanol-activated polyvinylidene difluoride (PVDF) membranes (GE Healthcare, Marlborough, MA, USA) and blocked for 90 min at room temperature, with a 5% (*w/v*) nonfat milk (Bio-Rad) solution prepared in tris-buffered saline with tween-20 (TBST; Sigma-Aldrich). Membranes were incubated overnight (4°C) with specific primary antibodies followed by the respective secondary antibodies at room temperature, for 90 min **(Supplementary Table 1)**. Membranes were incubated with enhanced chemiluminescence (ECL) reagent (GE Healthcare) for 5 min, and the chemiluminescence signals were read in the ChemiDoc™ MP Imaging System (Bio-Rad). Densities from each band were quantified with Image Lab™ Software (version 6.0.1, Bio-Rad) and divided by the corresponding total protein lane density. Total protein density was obtained by incubation of the membranes with total colloidal gold protein stain (BioRad). The results were expressed as fold variation relative to the control group.

Statistical analyses

After testing normal distribution by the Kolmogorov–Smirnov test, semen parameters and western blot results were analyzed by Mann–Whitney U test for independent samples, using the MedCalc Software (version 17.8; MedCalc Software, Ostend, Belgium). All data are presented as mean ± s.e.m., and differences with $P < 0.05$ were considered statistically significant.

RESULTS

Semen quality in patients with testicular cancer seminoma

The average volume of the ejaculates was very similar between the control and seminoma groups (**Table 1**). However, there was a decrease in sperm

Table 1: Semen parameters of fertile men (control) and patients with testicular cancer seminoma

Parameter	*Control*	*Seminoma*	*P*
Semen volume (ml)	3.53±0.35	3.33±0.42	0.541
Sperm motility (%)	67±3	54±5	0.019
Sperm concentration (10^6 ml^{-1})	95.49±7.79	46.72±12.19	0.003
Total sperm count (10^6)	316.92±45.41	136.11±41.55	0.001
Total motile count (10^6)	211.88±30.09	75.63±22.44	0.001

Results are presented as mean±s.e.m. (n=15 per group). Statistical significance was considered for $P < 0.05$. s.e.m.: standard error of the mean

motility ($P = 0.019$), sperm concentration ($P = 0.003$), total sperm count ($P = 0.001$), and total motile count ($P = 0.001$) in patients with seminoma relative to control (**Table 1**). Nevertheless, all the samples were considered normozoospermic according to the WHO 2010 criteria.[15]

Differentially expressed proteins

Proteomic analysis identified 1149 proteins in the control group and 911 in the seminoma group. After comparative analysis between the experimental groups, a total of 1192 proteins were quantified and 393 were found to be differentially expressed (**Supplementary Table 2**). More than half (52.7%) of the DEPs were underexpressed, while 20.1% were overexpressed in spermatozoa of patients with seminoma. Furthermore, 4.1% of the DEPs were unique to the seminoma group and 23.1% unique to the control group (**Figure 1**).

Selection of proteins for validation

According to the IPA analysis, among the top diseases and bio-functions related to "physiological system development and function," the category with the highest *P* value was "reproductive system development and function." Within this category, we selected seven proteins involved in specific reproductive processes (**Table 2**): angiotensin-converting enzyme (ACE), acrosin precursor (ACR), T-complex protein 1 subunit gamma (CCT3), sperm surface protein Sp17 (SPA17), sodium/potassium-transporting ATPase subunit alpha-4 (ATP1A4), heat shock-related 70 kDa protein 2 (HSPA2), and proteasome activator complex subunit 4 (PSME4). Some of these proteins were also involved in the top canonical pathways identified in this dataset. While HSPA2 participates in the "protein ubiquitination pathway" and "unfolded protein response," ACE is related to "phagosome maturation." Other top five canonical pathways included "mitochondrial dysfunction" and "oxidative phosphorylation." Among the proteins involved in those pathways were NADH-ubiquinone oxidoreductase 75 kDa subunit (NDUFS1), cytochrome b-c1 complex subunit 2 (UQCRC2), and ATP synthase subunit alpha (ATP5A), which are subunits of the mitochondrial complexes I, III, and V, respectively. These three proteins were also selected for analysis by western blot. The abundance and expression pattern of the ten selected proteins obtained by the proteomic analysis is presented in **Table 3**.

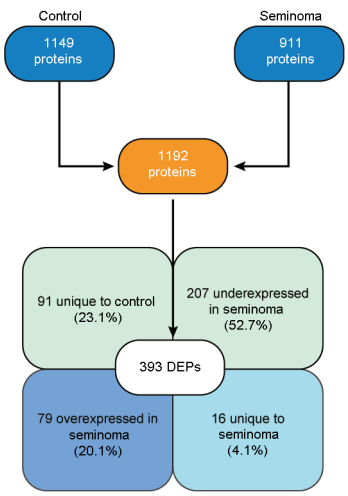

Figure 1: Number of proteins identified by proteomic analysis of spermatozoa samples obtained from fertile men (control) and men with testicular cancer seminoma, and expression profile of the DEPs identified after comparative analysis between the experimental groups. DEPs: differentially expressed proteins.

Prediction of the upstream regulators

The IPA analysis predicted the activation or inhibition of several proteins, which could be responsible for the altered expression in the sperm proteome of men with seminoma. The rapamycin-insensitive companion of mammalian target of rapamycin (RICTOR) was predicted to be activated, thus leading to the underexpression of NDUFS1, UQCRC2, ATP5A1, and PSME4. Moreover, it was predicted that the underexpression of ATP5A1 and ATP1A4 may involve the activation of the amyloid-beta A4 protein (APP). On the other hand, the inhibition of the heat shock factor protein 2 (HSF2) was predicted to regulate the underexpression of CCT3, as well as six other chaperonins of the T-complex protein-1 (TCP-1) family (CCT2, CCT4, CCT5, CCT6A, CCT7, and CCT8).

Western blot analysis

All proteins selected for western blot analysis were identified. There was an increase in the protein expression of ACE ($P = 0.005$) and ACR ($P = 0.009$) in the seminoma group (2.61 ± 0.38 and 2.02 ± 0.26-fold variation to control, respectively) in comparison with the

Table 2: Specific functions of the differentially expressed proteins related to reproductive system development and function identified by the bioinformatic analysis when comparing the sperm proteome of patients with testicular cancer seminoma with fertile men

Process	*Protein*	*P*
Binding of sperm	ACE, ACR, CCT3, SPA17	<0.0001
Fertilization	ACE, ACR, ATP1A4, SPA17	<0.0001
Cell movement of sperm	ATP1A4	<0.0001
Spermatogenesis	ACE, ATP1A4, HSPA2, PSME4, SPA17	0.0003
Function of sperm	ATP1A4	0.0028
Acrosome reaction	ACR	0.0037
Fertility	ACE, ACR, PSME4	0.0067
Morphology of male germ cells	ACR, PSME4	0.0089
Morphology of sperm	ACR	0.0120
Hyperactivation of sperm	ATP1A4	0.0133

ACE: angiotensin-converting enzyme; ACR: acrosin precursor; ATP1A4: sodium/potassium-transporting ATPase subunit alpha-4; CCT3: T-complex protein 1 subunit gamma; HSPA2: heat shock-related 70 kDa protein 2; PSME4: proteasome activator complex subunit 4; SPA17: sperm surface protein Sp17

Table 3: Proteomic data of the differentially expressed proteins identified in the spermatozoa samples of fertile men (control) and men with testicular cancer seminoma before cancer therapy, which were selected for validation by western blot

Protein	*Abundance*		*NSAF ratio*	*Expression profile*	*P*
	Control	*Seminoma*			
ACE	High	High	1.62	Overexpressed in seminoma	0.0131
ACR	High	Medium	0.34	Underexpressed in seminoma	0.0001
ATP1A4	Medium	Very low	0.07	Underexpressed in seminoma	0.0001
ATP5A1	High	Medium	0.18	Underexpressed in seminoma	<0.0001
CCT3	High	Very low	0.09	Underexpressed in seminoma	<0.0001
HSPA2	High	High	0.53	Underexpressed in seminoma	<0.0001
NDUFS1	Medium	Very low	0.42	Underexpressed in seminoma	0.0307
PSME4	Medium	Very low	0.13	Underexpressed in seminoma	0.0006
SPA17	Medium	Very low	0.02	Underexpressed in seminoma	0.0001
UQCRC2	High	Low	0.23	Underexpressed in seminoma	0.0001

ACE: angiotensin-converting enzyme; ACR: acrosin; ATP1A4: sodium/potassium-transporting ATPase subunit alpha-4; ATP5A: ATP synthase subunit alpha; CCT3: T-complex protein 1 subunit gamma; HSPA2: heat shock-related 70 kDa protein 2; NDUFS1: NADH-ubiquinone oxidoreductase 75 kDa subunit; NSAF: normalized spectral abundance factor; PSME4: proteasome activator complex subunit 4; SPA17: sperm surface protein Sp17; UQCRC2: cytochrome b-c1 complex subunit 2

control (1.00 ± 0.25 and 1.00 ± 0.19, respectively) (**Figure 2**). On the other hand, there was a decrease in the protein levels of ATP1A4 ($P = 0.016$) and HSPA2 ($P = 0.041$) in men with seminoma (0.53 ± 0.03 and 0.32 ± 0.11-fold variation to control, respectively) when compared with the control group (1.00 ± 0.25 and 1.00 ± 0.22, respectively) (**Figure 2**). The protein levels of CCT3, SPA17, and PSME4 were similar between the study groups. There was also a decrease ($P = 0.026$) in the protein expression levels of UQCRC2 (0.34 ± 0.14-fold variation to control) in the seminoma group relative to the control (1.00 ± 0.14) (**Figure 3**). No differences were found in the protein levels of NDUFS1 or ATP5A1 between the experimental groups.

DISCUSSION

The present study is the first attempt to identify alterations in spermatozoa proteome of patients with seminoma before initiating cancer therapy, using fertile donors as control group. Our goal was to evaluate the expression levels of proteins involved in reproductive function from spermatogenesis to sperm function and fertilization. This may provide new insights on the underlying mechanisms responsible for the reduced sperm quality in men with seminoma.

Spermatogenesis consists of a complex process of spermatozoa production that involves several steps of germ cell differentiation. The bioinformatic analysis identified an underexpression of PSME4 in spermatozoa of patients with seminoma. PSME4 plays a role in the morphology of male germ cells; it is particularly important for histone replacement during chromatin remodeling and DNA double-strand break repair.[18] It has been reported that mice lacking this protein present impaired spermatogenesis and reduced fertility.[19] Thus, the downregulation of this protein may contribute to reduced fertility in men with seminoma. Although we were not able to confirm the underexpression of PSME4 by western blot in our dataset, we observed the underexpression of the molecular chaperone HSPA2 by both proteomics and western blot analysis. Molecular chaperones are essential for normal sperm production and functional transformation. HSPA2 acts as a protein quality control system as it ensures the correct folding/refolding of proteins and activates the degradation of misfolded proteins.[20] It has been described that HSPA2 participates in the stability of the microtubules during the meiotic process of germ cell differentiation.[21] In fact, animal studies show that knockout mice for *Hspa2* exhibit an enormous number of apoptotic germ cells, resulting in infertility.[22] Men with abnormal spermatogenesis frequently present a reduced *hspA2* mRNA expression.[23] Thus, the downregulation of HSPA2 protein in men with seminoma may contribute to the decreased production of normal spermatozoa during spermatogenesis, which is in accordance with the observed reduction in sperm concentration and total sperm count in seminoma group.

The protein ATP1A4 was identified as downregulated in seminoma group by the proteomic analysis, and this result was confirmed by the western blot technique. IPA analysis revealed that ATP1A4 participates in several reproductive processes, including spermatogenesis, function of sperm, cell movement of sperm, hyperactivation, and fertilization. ATP1A4 is the catalytic subunit of the Na^+/K^+-ATPase membrane protein, which controls the exchange of sodium and potassium ions across the plasma membrane in an ATP-dependent reaction.[24] The regulation of ions in spermatozoa is essential for the acquisition of motility and fertilizing ability. ATP1A4 plays a key role in maintaining human sperm motility.[25] It has been shown that male mice lacking this subunit are completely sterile and their spermatozoa present not only reduced motility but also impaired hyperactivation and inability to fertilize *in vitro*.[26] These studies highlight the importance of ATP1A4 for male fertility, and the underexpression of this protein in spermatozoa of men with seminoma may explain the decrease in sperm motility and total motile count relative to proven fertile men (control group). The downregulation of ATP1A4 was related to the activation of APP. In fact, this protein has been identified in human spermatozoa and suggested to play an important role in sperm function, especially in signaling events involved in sperm motility.[27]

Another important process crucial for sperm function is mitochondrial function. It is required for energy production necessary for spermatozoa movement and production of reactive oxygen species (ROS) in physiological amounts to trigger capacitation and regulate hyperactivation and acrosome reaction.[28] Mitochondrial function relies on the expression of the mitochondrial complexes I–IV for oxidative phosphorylation (OXPHOS) and complex V for ATP production.[29] Our proteomic data showed a downregulation of NDUFS1, UQCRC2, and ATP5A1 in the seminoma group, which are subunits of complex I, III, and V, respectively. The downregulation of these three proteins was predicted to be induced by the activation of RICTOR, which plays a key role in spermatogenesis and sperm maturation signaling

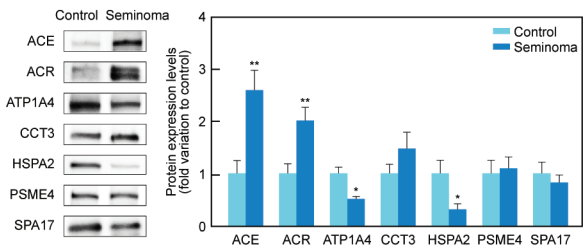

Figure 2: Graphical representation of the expression levels of proteins involved in reproductive functions (ACE, ACR, ATP1A4, CCT3, HSPA2, PSME4, and SPA17) in spermatozoa samples obtained from fertile men (control) and men with testicular cancer seminoma. Results are presented as fold variation to control and expressed as mean±standard error of the mean ($n = 15$ per group). Statistical significance is indicated as: $^*P < 0.05$, $^{**}P < 0.01$, seminoma versus control. Representative blots for each protein are also presented. ACE: angiotensin-converting enzyme; ACR: acrosin precursor; ATP1A4: sodium/potassium-transporting ATPase subunit alpha-4; CCT3: T-complex protein 1 subunit gamma; HSPA2: heat shock-related 70 kDa protein 2; PSME4: proteasome activator complex subunit 4; SPA17: sperm surface protein Sp17.

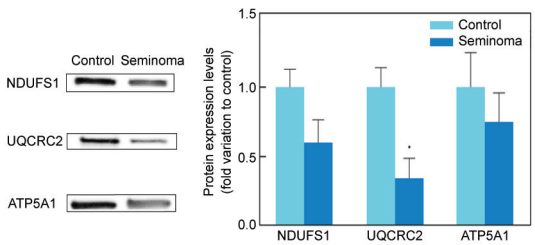

Figure 3: Graphical representation of the protein expression levels of mitochondrial complex subunits NDUFS1, UQCRC2, and ATP5A1 in spermatozoa samples obtained from fertile men (control) and men with testicular cancer seminoma. Results are presented as fold variation to control and expressed as mean ± standard error of the mean ($n = 15$ per group). Statistical significance is indicated as: $^*P < 0.05$, seminoma versus control. Representative blots for each protein are also presented. NDUFS1: NADH-ubiquinone oxidoreductase 75 kDa subunit; UQCRC2: cytochrome b-c1 complex subunit 2; ATP5A: ATP synthase subunit alpha.

pathways.[28] The mitochondrial subunits are essential for the proper assembly of the complexes; thus, alterations in their protein expression in spermatozoa are indicative of mitochondrial dysfunction, as reported by the IPA canonical pathways.[30] Although the western blot analysis demonstrated a tendency of reduced expression of the three mitochondrial subunits, only the UQCRC2 was decreased in patients with seminoma. Downregulation of UQCRC2 was associated with reduced sperm kinematics, ATP production, and capacitation, which ultimately compromises sperm binding and fertilization.[31] In fact, an underexpression of UQCRC2 was observed in infertile men with varicocele.[32]

The acquisition of sperm fertilizing ability involves a timed triggering of events in the female reproductive system, culminating in sperm–oocyte binding. SPA17 and CCT3 are two sperm proteins involved in this function, which were identified as downregulated in the seminoma group by the proteomic analysis. SPA17 is a mannose-binding protein that binds to zona pellucida carbohydrates during fertilization.[33] It also plays an important role in germ cell differentiation during spermatogenesis, as its expression increases from early to late stages.[34] CCT3 is one of the subunits of the TCP-1 complex. Although we selected to evaluate the expression levels of this subunit, six other subunits of this complex (CCT2, CCT4, CCT5, CCT6A, CCT7, and CCT8) were also downregulated in men with seminoma. These subunits mediate capacitation-dependent binding of spermatozoa to the zona pellucida.[35] Thus, the downregulation of this system may compromise sperm fertilization.[36] The downregulation of TCP-1 complex subunits was predicted to be due to HSF2 inhibition. In fact, disruption of *hsf2* in mice affected testicular size[37] and induced spermatogenic defects.[38] When active, HSF2 is likely to induce the upregulation of HSPA2.[39] Thus, the predicted inhibition of HSF2 in men with seminoma is in accordance with the downregulation of HSPA2. Although the underexpression of SPA17 and CCT3 was not confirmed by the western blot, the downregulation of HSPA2 in men with seminoma may contribute to the loss of sperm function. In fact, this protein is known to regulate the formation of zona pellucida-binding sites in spermatozoa during spermatogenesis.[40] In addition, it regulates fertilization by mediating the function of sperm surface receptors, such as sperm adhesion molecule 1 (SPAM1) and arylsulfatase A (ARSA), during sperm-egg recognition.[41] Previous proteomic studies have shown low expression levels of HSPA2 in men with asthenozoospermia[42] and primary or secondary infertility.[43] Another study also reported a downregulation of HSPA2, ATP1A4, and SPA17 in infertile varicocele patients.[32] Our results suggest that the altered expression levels of these proteins in men with seminoma may contribute to the impairment of male fertility.

The proteomic analysis also identified ACE as overexpressed in the seminoma group, and this result was confirmed by western blot. This protein is a zinc metallopeptidase responsible for the conversion of angiotensin I to angiotensin II.[44] The role of ACE in male reproductive function is not completely understood. Studies with ACE-deficient mice reported that these animals produce a normal number of spermatozoa and present normal motility and morphology. However, the spermatozoa were unable to bind and fertilize the egg.[45,46] A negative correlation between sperm-bound ACE activity and sperm motility has also been observed.[47] The testis-specific isoform of this protein (tACE) is believed to be released from functional spermatozoa during capacitation and acrosome reaction to increase the fertilizing ability.[48] In fact, a lower tACE activity was detected in spermatozoa from normozoospermic men relative to those with oligoasthenozoospermia.[47] Thus, the overexpression of this protein in spermatozoa from men with seminoma may be responsible for the decrease in sperm motility observed in this group, and possibly explains why some men with seminoma are not able to have children even before the treatment.

Finally, the protein ACR, in its precursor form (proacrosin), was identified as underexpressed in the seminoma group by the proteomic analysis. This protein is activated and converted to its active form during acrosome reaction, playing a role in sperm–oocyte binding.[49] In contrast, using western blot, we found a high overexpression of this protein in men with seminoma. Although we cannot clearly infer about the molecular mechanisms, any of the scenarios (underexpression/overexpression) could lead to a defective acrosome reaction and impaired fertilization. The difference on these results may be due to the sensitivity of each technique and to the sample size. Further studies to assess acrosin activity in men with seminoma are needed to clarify the impact of this condition in acrosome reaction.

Overall, our study points toward important alterations in sperm proteins with a key role in male fertility in men with seminoma. As of today, no specific sperm markers have been identified for the clinical diagnosis and monitoring of testicular cancer seminoma development. The expression levels of HSPA2, ATP1A4, UQCRC2, and ACE can be helpful sperm biomarkers when evaluating the fertility status of a man, which may allow the early diagnosis of seminomas in a noninvasive approach. Although there is still a lot to explore in the pathophysiology of male subfertility/infertility in men with seminoma, our results represent a step forward in understanding the molecular mechanisms behind the reduced sperm quality in these patients. Future advances in mass spectrometry and bioinformatics will improve our understanding on human sperm function in healthy and disease conditions.

AUTHOR CONTRIBUTIONS

AA and RS were responsible for the conception and design of the study. TRD was responsible for the acquisition and interpretation of data, as well as writing the first draft. GA helped in samples processing and PNP performed the bioinformatic analysis. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declared no competing interests.

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Supplementary Information is linked to the online version of the paper on the *Asian Journal of Andrology* website.

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Supplementary Table 1: List of the primary and secondary antibodies used in this study

Antibody	*Source*	*KDa*	*Dilution*	*Vendor*	*Catalog Number*
ACE	Rabbit	200	1:1000	Abcam	ab85955
ACR	Rabbit	46	1:1000	Abcam	ab203289
ATP1A4	Rabbit	100	1:10000	Abcam	ab76020
ATP5A	Mouse	54	1:1000	Abcam	ab110411
CCT3	Rabbit	61	1:2000	Abcam	ab225878
HSPA2	Mouse	70	1:500	Abcam	ab89130
NDUFS1	Rabbit	79	1:10000	Abcam	ab157221
PSME4	Rabbit	211	1:500	Abcam	ab181203
SPA17	Rabbit	17	1:1000	Abcam	ab172626
UQCRC2	Mouse	48	1:1000	Abcam	ab110411
Mouse*	Rabbit	-	1:10000	Abcam	ab6728
Rabbit*	Goat	-	1:10000	Abcam	ab97051

*Secondary antibody. ACE: angiotensin-converting enzyme; ACR: acrosin precursor; CCT3: T-complex protein 1 subunit gamma; SPA17: sperm surface protein Sp17; ATP1A4: sodium/potassium-transporting ATPase subunit alpha-4; HSPA2: heat shock-related 70 kDa protein 2; PSME4: proteasome activator complex subunit 4; NDUFS1: NADH-ubiquinone oxidoreductase 75 kDa subunit; UQCRC2: cytochrome b-c1 complex subunit 2; ATP5A: ATP synthase subunit alpha

Supplementary Table 2: List of the differentially expressed proteins identified by the bioinformatic analysis when comparing the sperm proteome of fertile men (control) and patients with testicular cancer seminoma

	Protein	*Accession*	*Average SC*		*Abundance*		*NSAF ratio*	*t-test*	*Expression*
			Control	*Seminoma*	*Control*	*Seminoma*	*Seminoma/Control*	*P*	
1	Transmembrane and coiled-coil domain-containing protein 2	56847610	23.3	0	M	ni	0.00	0.00000	Unique to Control
2	Isocitrate dehydrogenase (NAD) subunit alpha, mitochondrial precursor	5031777	48.0	0	M	ni	0.00	0.00000	Unique to Control
3	Succinyl-CoA ligase (ADP-forming) subunit beta, mitochondrial precursor	11321583	25.7	0	M	ni	0.00	0.00001	Unique to Control
4	Short-chain specific acyl-CoA dehydrogenase, mitochondrial precursor	4557233	50.0	0	M	ni	0.00	0.00001	Unique to Control
5	Probable serine carboxypeptidase CPVL isoform X1	530384848	27.0	0	M	ni	0.00	0.00006	Unique to Control
6	ATP synthase subunit O, mitochondrial precursor	4502303	33.7	0	M	ni	0.00	0.00018	Unique to Control
7	Doublecortin domain-containing protein 2C	566006166	21.7	0	M	ni	0.00	0.00021	Unique to Control
8	Bifunctional glutamate/proline-tRNA ligase	62241042	21.0	0	M	ni	0.00	0.00088	Unique to Control
9	Exportin-7	154448892	27.3	0	M	ni	0.00	0.00197	Unique to Control
10	Uncharacterized protein KIAA1683 isoform X1	530415216	23.3	0	M	ni	0.00	0.00606	Unique to Control
11	Leucine-rich repeat-containing protein 37A3 isoform X14	578840218	12.3	0	L	ni	0.00	0.00000	Unique to Control
12	Heme oxygenase 2 isoform a	555943918	11.3	0	L	ni	0.00	0.00001	Unique to Control
13	Actin-related protein T3	221139714	17.7	0	L	ni	0.00	0.00001	Unique to Control
14	Ubiquitin carboxyl-terminal hydrolase 7 isoform 1	150378533	18.3	0	L	ni	0.00	0.00001	Unique to Control
15	Tetratricopeptide repeat protein 25	13899233	12.7	0	L	ni	0.00	0.00003	Unique to Control
16	Actin-like protein 7A	5729720	16.7	0	L	ni	0.00	0.00005	Unique to Control
17	Dynein intermediate chain 2, axonemal isoform X4	530412670	15.7	0	L	ni	0.00	0.00008	Unique to Control
18	Four and a half LIM domains protein 1 isoform 5	228480205	18.7	0	L	ni	0.00	0.00008	Unique to Control
19	Putative lipoyltransferase 2, mitochondrial precursor	221554520	9.0	0	L	ni	0.00	0.00011	Unique to Control

Contd...

Supplementary Table 2: Contd...

	Protein	Accession	Average SC		Abundance		NSAF ratio	t-test	Expression
			Control	Seminoma	Control	Seminoma	Seminoma/Control	P	
20	Tubulin polymerization-promoting protein family member 2	226491350	16.3	0	L	ni	0.00	0.00012	Unique to Control
21	Isocitrate dehydrogenase (NAD) subunit beta, mitochondrial isoform a precursor	28178821	19.3	0	L	ni	0.00	0.00019	Unique to Control
22	Protein DPCD	39930355	18.3	0	L	ni	0.00	0.00028	Unique to Control
23	Long-chain-fatty-acid-CoA ligase 3	42794754	9.7	0	L	ni	0.00	0.00031	Unique to Control
24	Sodium/potassium-transporting ATPase subunit alpha-3 isoform 1	22748667	17.3	0	L	ni	0.00	0.00047	Unique to Control
25	26S proteasome non-ATPase regulatory subunit 4	5292161	9.0	0	L	ni	0.00	0.00047	Unique to Control
26	ATP synthase subunit g, mitochondrial	51479156	9.0	0	L	ni	0.00	0.00049	Unique to Control
27	Acyl-CoA dehydrogenase family member 9, mitochondrial	21361497	18.0	0	L	ni	0.00	0.00061	Unique to Control
28	Transcription factor A, mitochondrial isoform 1 precursor	4507401	15.0	0	L	ni	0.00	0.00065	Unique to Control
29	Elongation factor Tu, mitochondrial precursor	34147630	10.7	0	L	ni	0.00	0.00066	Unique to Control
30	Eukaryotic translation elongation factor 1 epsilon-1 isoform 2	208879470	8.0	0	L	ni	0.00	0.00068	Unique to Control
31	Voltage-dependent calcium channel subunit alpha-2/delta-2 isoform X1	530373385	8.0	0	L	ni	0.00	0.00075	Unique to Control
32	Armadillo repeat-containing protein 12 isoform X1	530381603	12.0	0	L	ni	0.00	0.00079	Unique to Control
33	Deoxyuridine 5'-triphosphate nucleotidohydrolase, mitochondrial isoform 3	70906444	13.0	0	L	ni	0.00	0.00081	Unique to Control
34	Probable inactive serine protease 37 isoform 1 precursor	285394164	9.0	0	L	ni	0.00	0.00087	Unique to Control
35	26S proteasome non-ATPase regulatory subunit 14	5031981	9.0	0	L	ni	0.00	0.00088	Unique to Control
36	Mitochondria-eating protein isoform X4	530376736	16.7	0	L	ni	0.00	0.00097	Unique to Control
37	Mitochondrial fission 1 protein	151108473	8.7	0	L	ni	0.00	0.00110	Unique to Control
38	Alpha-soluble NSF attachment protein	47933379	8.0	0	L	ni	0.00	0.00137	Unique to Control
39	Maleylacetoacetate isomerase isoform 1	22202624	9.0	0	L	ni	0.00	0.00179	Unique to Control
40	40S ribosomal protein S15	4506687	10.0	0	L	ni	0.00	0.00184	Unique to Control
41	Aladin isoform 2	291045307	8.3	0	L	ni	0.00	0.00186	Unique to Control
42	Ubiquitin carboxyl-terminal hydrolase isozyme L1	21361091	14.3	0	L	ni	0.00	0.00195	Unique to Control
43	Stomatin-like protein 2, mitochondrial isoform a	7305503	12.3	0	L	ni	0.00	0.00200	Unique to Control
44	Protein FAM209B isoform X2	578835992	8.0	0	L	ni	0.00	0.00205	Unique to Control
45	Putative protein FAM71E2	223972704	12.3	0	L	ni	0.00	0.00229	Unique to Control
46	Acyl-protein thioesterase 1 isoform 1	5453722	11.7	0	L	ni	0.00	0.00240	Unique to Control
47	Histone H1t	20544168	8.0	0	L	ni	0.00	0.00244	Unique to Control
48	Armadillo repeat-containing protein 4 isoform X3	578818430	18.7	0	L	ni	0.00	0.00324	Unique to Control
49	Dnaj homolog subfamily B member 1 isoform X1	578833210	13.3	0	L	ni	0.00	0.00375	Unique to Control
50	Calcium-binding mitochondrial carrier protein Aralar2 isoform 1	237649019	14.0	0	L	ni	0.00	0.00450	Unique to Control

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Supplementary Table 2: Contd...

	Protein	Accession	Average SC		Abundance		NSAF ratio	t-test	Expression
			Control	Seminoma	Control	Seminoma	Seminoma/Control	P	
51	Long-chain-fatty-acid-CoA ligase ACSBG2 isoform a	574584557	17.7	0	L	ni	0.00	0.00479	Unique to Control
52	Methionine-tRNA ligase, cytoplasmic	14043022	10.0	0	L	ni	0.00	0.00512	Unique to Control
53	60S acidic ribosomal protein P0	4506667	13.3	0	L	ni	0.00	0.00722	Unique to Control
54	Cytoplasmic dynein 1 heavy chain 1	33350932	11.3	0	L	ni	0.00	0.00728	Unique to Control
55	ADP-ribosylation factor 6	4502211	9.0	0	L	ni	0.00	0.00763	Unique to Control
56	Glycine-tRNA ligase precursor	116805340	15.0	0	L	ni	0.00	0.00770	Unique to Control
57	BAG family molecular chaperone regulator 5 isoform b	6631077	9.3	0	L	ni	0.00	0.00818	Unique to Control
58	60S ribosomal protein L7a	4506661	5.3	0	VL	ni	0.00	0.00000	Unique to Control
59	Isobutyryl-CoA dehydrogenase, mitochondrial	7656849	7.0	0	VL	ni	0.00	0.00000	Unique to Control
60	cAMP-dependent protein kinase catalytic subunit gamma	15619015	7.0	0	VL	ni	0.00	0.00000	Unique to Control
61	Vitamin K epoxide reductase complex subunit 1-like protein 1 isoform 1	46309463	3.7	0	VL	ni	0.00	0.00000	Unique to Control
62	Translocation protein SEC63 homolog	6005872	2.0	0	VL	ni	0.00	0.00001	Unique to Control
63	UDP-N-acetylhexosamine pyrophosphorylase	156627575	3.0	0	VL	ni	0.00	0.00001	Unique to Control
64	Guanine nucleotide-binding protein subunit beta-2-like 1	5174447	2.0	0	VL	ni	0.00	0.00003	Unique to Control
65	Dynein intermediate chain 1, axonemal isoform 2	526479830	7.0	0	VL	ni	0.00	0.00003	Unique to Control
66	Fibronectin type III domain-containing protein 8	8922138	2.0	0	VL	ni	0.00	0.00009	Unique to Control
67	40S ribosomal protein S26-like	530438702	3.0	0	VL	ni	0.00	0.00009	Unique to Control
68	Mitochondrial import receptor subunit TOM22 homolog	9910382	6.0	0	VL	ni	0.00	0.00009	Unique to Control
69	Cation channel sperm-associated protein subunit beta precursor	51339295	2.0	0	VL	ni	0.00	0.00009	Unique to Control
70	Maestro heat-like repeat-containing protein family member 7	223278410	3.3	0	VL	ni	0.00	0.00010	Unique to Control
71	ADP-ribosylation factor-like protein 2 isoform 1	148612885	2.7	0	VL	ni	0.00	0.00015	Unique to Control
72	protein NDRG1 isoform 1	207028748	4.0	0	VL	ni	0.00	0.00016	Unique to Control
73	Speriolin isoform 1	197276668	6.3	0	VL	ni	0.00	0.00017	Unique to Control
74	Radial spoke head protein 6 homolog A	13540559	3.3	0	VL	ni	0.00	0.00018	Unique to Control
75	DCN1-like protein 1	36030883	4.7	0	VL	ni	0.00	0.00025	Unique to Control
76	dnaJ homolog subfamily C member 3 precursor	5453980	3.7	0	VL	ni	0.00	0.00025	Unique to Control
77	Sialic acid synthase	12056473	3.0	0	VL	ni	0.00	0.00028	Unique to Control
78	Glutamine-tRNA ligase isoform b	441478305	3.7	0	VL	ni	0.00	0.00028	Unique to Control
79	Mimitin, mitochondrial	29789409	4.3	0	VL	ni	0.00	0.00031	Unique to Control
80	60S ribosomal protein L22 proprotein	4506613	5.0	0	VL	ni	0.00	0.00032	Unique to Control
81	EF-hand calcium-binding domain-containing protein 14	7662160	6.7	0	VL	ni	0.00	0.00033	Unique to Control

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Supplementary Table 2: Contd...

	Protein	Accession	Average SC		Abundance		NSAF ratio	t-test	Expression
			Control	Seminoma	Control	Seminoma	Seminoma/Control	P	
82	Iron-sulfur cluster assembly enzyme ISCU, mitochondrial isoform X1	530400013	4.7	0	VL	ni	0.00	0.00036	Unique to Control
83	Growth hormone-inducible transmembrane protein	118200356	4.7	0	VL	ni	0.00	0.00037	Unique to Control
84	S-phase kinase-associated protein 1 isoform b	25777713	4.0	0	VL	ni	0.00	0.00040	Unique to Control
85	Calcium-binding mitochondrial carrier protein Aralar1	21361103	3.3	0	VL	ni	0.00	0.00050	Unique to Control
86	diphosphomevalonate decarboxylase	4505289	2.3	0	VL	ni	0.00	0.00051	Unique to Control
87	V-type proton ATPase subunit E 2 isoform X1	530368260	4.0	0	VL	ni	0.00	0.00052	Unique to Control
88	Nucleosome assembly protein 1-like 1	21327708	4.3	0	VL	ni	0.00	0.00056	Unique to Control
89	26S protease regulatory subunit 4	24430151	6.0	0	VL	ni	0.00	0.00056	Unique to Control
90	Mitochondrial ornithine transporter 1	7657585	5.3	0	VL	ni	0.00	0.00057	Unique to Control
91	60S ribosomal protein L5	14591909	3.7	0	VL	ni	0.00	0.00097	Unique to Control
92	Dynein heavy chain 17, axonemal	256542310	88.0	1.0	H	VL	0.01	0.00001	UE in Seminoma
93	L-amino-acid oxidase isoform 2 precursor	384381475	76.0	0.3	M	VL	0.01	0.00000	UE in Seminoma
94	Sperm-associated antigen 6 isoform X1	530392552	58.0	0.3	M	VL	0.01	0.00000	UE in Seminoma
95	Nuclear pore complex protein Nup93 isoform X1	530424559	37.7	0.3	M	VL	0.01	0.00129	UE in Seminoma
96	Valine-tRNA ligase	5454158	87.7	1.7	H	VL	0.01	0.00004	UE in Seminoma
97	Sperm surface protein Sp17	8394343	31.3	0.3	M	VL	0.02	0.00010	UE in Seminoma
98	Exportin-2 isoform 1	29029559	16.7	0.3	L	VL	0.02	0.00026	UE in Seminoma
99	26S proteasome non-ATPase regulatory subunit 13 isoform 1	157502193	19.7	0.3	L	VL	0.02	0.00142	UE in Seminoma
100	Cathepsin F precursor	6042196	21.0	0.3	M	VL	0.03	0.00007	UE in Seminoma
101	26S proteasome non-ATPase regulatory subunit 7	25777615	13.7	0.3	L	VL	0.03	0.00230	UE in Seminoma
102	Uncharacterized protein C7orf61	51972226	14.3	0.3	L	VL	0.03	0.00109	UE in Seminoma
103	Vacuolar protein sorting-associated protein 13A isoform C	66346672	19.7	0.3	L	VL	0.03	0.00122	UE in Seminoma
104	Mitochondrial pyruvate carrier 1-like protein	306922396	18.0	0.3	L	VL	0.03	0.00012	UE in Seminoma
105	Plasma membrane calcium-transporting ATPase 4 isoform 4b	48255957	52.3	2.7	M	VL	0.03	0.00001	UE in Seminoma
106	Presequence protease, mitochondrial isoform 2 precursor	41352061	50.3	1.0	M	VL	0.03	0.00003	UE in Seminoma
107	Exportin-1 isoform X1	530368070	8.3	0.3	L	VL	0.03	0.00004	UE in Seminoma
108	Ras-related protein Rab-11B	190358517	15.7	0.3	L	VL	0.03	0.00012	UE in Seminoma
109	Phosphatidylethanolamine-binding protein 4 precursor	116812622	15.0	0.3	L	VL	0.04	0.00029	UE in Seminoma
110	Protein FAM71A	282721094	12.3	0.3	L	VL	0.04	0.00258	UE in Seminoma
111	Puromycin-sensitive aminopeptidase	158937236	45.0	1.3	M	VL	0.04	0.00124	UE in Seminoma
112	Epimerase family protein SDR39U1 isoform 1	116812630	13.3	0.3	L	VL	0.04	0.00151	UE in Seminoma

Contd...

Supplementary Table 2: Contd...

	Protein	Accession	Average SC		Abundance		NSAF ratio	t-test	Expression
			Control	Seminoma	Control	Seminoma	Seminoma/Control	P	
113	V-type proton ATPase catalytic subunit A	19913424	15.7	0.3	L	VL	0.04	0.00313	UE in Seminoma
114	Cullin-associated NEDD8-dissociated protein 1	21361794	143.3	7.0	H	VL	0.05	0.00000	UE in Seminoma
115	Low molecular weight phosphotyrosine protein phosphatase isoform c	4757714	8.7	0.3	L	VL	0.05	0.00005	UE in Seminoma
116	Dynein heavy chain 8, axonemal isoform X1	578811443	132.3	6.3	H	VL	0.05	0.00003	UE in Seminoma
117	Heat shock protein 75, mitochondrial isoform 1 precursor	155722983	8.3	0.3	L	VL	0.05	0.00081	UE in Seminoma
118	Cullin-3 isoform 3	380714665	58.3	2.3	M	VL	0.05	0.00012	UE in Seminoma
119	Lysosomal alpha-glucosidase isoform X1	530411863	5.0	0.3	VL	VL	0.05	0.00074	UE in Seminoma
120	Isoleucine-tRNA ligase, mitochondrial precursor	46852147	40.7	1.7	M	VL	0.05	0.00001	UE in Seminoma
121	Protein FAM71B	222418633	46.7	1.3	M	VL	0.05	0.00050	UE in Seminoma
122	Actin-related protein T2	29893808	45.7	1.7	M	VL	0.06	0.00004	UE in Seminoma
123	Thioredoxin domain-containing protein 3	148839372	18.3	1.0	L	VL	0.06	0.00016	UE in Seminoma
124	Carnitine O-palmitoyltransferase 1, muscle isoform isoform a	4758050	11.3	1.0	L	VL	0.06	0.00107	UE in Seminoma
125	Phosphoglycolate phosphatase	108796653	15.3	0.3	L	VL	0.06	0.00014	UE in Seminoma
126	Ecto-ADP-ribosyltransferase 3 isoform X8	530377706	38.3	1.7	M	VL	0.06	0.00004	UE in Seminoma
127	EF-hand calcium-binding domain-containing protein 1 isoform a	13375787	11.3	0.3	L	VL	0.07	0.00341	UE in Seminoma
128	Izumo sperm-egg fusion protein 2 isoform X1	578833932	9.0	0.3	L	VL	0.07	0.00273	UE in Seminoma
129	Sodium/potassium-transporting ATPase subunit alpha-4 isoform 1	153946397	59.7	5.3	M	VL	0.07	0.00006	UE in Seminoma
130	Enoyl-CoA delta isomerase 2, mitochondrial isoform 2	260274832	25.3	1.0	M	VL	0.07	0.00081	UE in Seminoma
131	Casein kinase II subunit beta isoform 1	23503295	9.3	0.3	L	VL	0.07	0.00181	UE in Seminoma
132	Small membrane A-kinase anchor protein	110349742	9.3	0.3	L	VL	0.07	0.00193	UE in Seminoma
133	60S ribosomal protein L12	4506597	14.3	0.7	L	VL	0.07	0.00044	UE in Seminoma
134	Leucine-rich repeat-containing protein 37A3 precursor	75677612	20.3	1.3	M	VL	0.07	0.00021	UE in Seminoma
135	NADH dehydrogenase (ubiquinone) iron-sulfur protein 8, mitochondrial isoform X1	530396818	8.0	0.3	L	VL	0.07	0.00305	UE in Seminoma
136	Heat shock 70 protein 4L	31541941	93.3	2.3	H	VL	0.08	0.00012	UE in Seminoma
137	Sperm equatorial segment protein 1 precursor	21717832	100.7	5.0	H	VL	0.08	0.00000	UE in Seminoma
138	Pyruvate dehydrogenase E1 component subunit beta, mitochondrial isoform 1 precursor	156564403	67.3	3.3	M	VL	0.08	0.00002	UE in Seminoma
139	Choline transporter-like protein 5 isoform B	194239633	8.0	1.3	L	VL	0.08	0.00340	UE in Seminoma
140	6-Phosphofructokinase type C isoform 1	11321601	131.3	10.7	H	L	0.09	0.00000	UE in Seminoma
141	26S proteasome non-ATPase regulatory subunit 12 isoform 1	4506221	10.7	0.7	L	VL	0.09	0.00427	UE in Seminoma
142	ruvB-like 2	5730023	137.3	7.7	H	VL	0.09	0.00007	UE in Seminoma
143	T-complex protein 1 subunit gamma isoform a	63162572	128.7	7.7	H	VL	0.09	0.00000	UE in Seminoma

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Supplementary Table 2: Contd...

	Protein	Accession	Average SC		Abundance		NSAF ratio	t-test	Expression
			Control	Seminoma	Control	Seminoma	Seminoma/Control	P	
144	ATP synthase subunit beta, mitochondrial precursor	32189394	354.3	21.7	H	M	0.09	0.00000	UE in Seminoma
145	Phosphatidylglycerophosphatase and protein-tyrosine phosphatase 1 isoform 1	148224884	11.7	0.7	L	VL	0.09	0.00097	UE in Seminoma
146	26S proteasome non-ATPase regulatory subunit 3	25777612	35.3	3.0	M	VL	0.09	0.00001	UE in Seminoma
147	Importin-5 isoform X2	530423350	24.7	1.7	M	VL	0.10	0.00181	UE in Seminoma
148	Mitochondrial dicarboxylate carrier isoform 2	20149598	56.0	3.7	M	VL	0.10	0.00026	UE in Seminoma
149	TMEM189-UBE2V1 fusion protein	40806190	8.3	0.7	L	VL	0.10	0.00394	UE in Seminoma
150	Dynein heavy chain 7, axonemal	151301127	18.0	1.0	L	VL	0.11	0.00033	UE in Seminoma
151	Lysozyme-like protein 1	73390143	9.7	0.7	L	VL	0.11	0.00451	UE in Seminoma
152	Importin subunit alpha-1	4504897	54.7	3.3	M	VL	0.11	0.00004	UE in Seminoma
153	Nuclear pore complex protein Nup155 isoform 1	24430149	86.0	8.3	H	L	0.12	0.00002	UE in Seminoma
154	Mitochondrial 2-oxoglutarate/malate carrier protein isoform 1	21361114	39.0	2.7	M	VL	0.12	0.00399	UE in Seminoma
155	Hyaluronidase PH-20 isoform 2	23510418	35.3	2.3	M	VL	0.12	0.00063	UE in Seminoma
156	40S ribosomal protein S16	4506691	10.7	0.7	L	VL	0.12	0.00186	UE in Seminoma
157	26S proteasome non-ATPase regulatory subunit 11	28872725	13.0	1.0	L	VL	0.12	0.00032	UE in Seminoma
158	26S proteasome non-ATPase regulatory subunit 6 isoform 2	7661914	18.7	1.7	L	VL	0.13	0.00236	UE in Seminoma
159	T-complex protein 1 subunit zeta-2 isoform X1	578830267	36.7	3.3	M	VL	0.13	0.00001	UE in Seminoma
160	Bifunctional ATP-dependent dihydroxyacetone kinase/FAD-AMP lyase (cyclizing) isoform X1	530396576	29.0	3.0	M	VL	0.13	0.00104	UE in Seminoma
161	Ropporin-1B	59891409	92.7	7.3	H	VL	0.13	0.00003	UE in Seminoma
162	Dynactin subunit 2 isoform 3	387527974	15.7	1.7	L	VL	0.13	0.00423	UE in Seminoma
163	ras-related protein Rab-14	19923483	19.0	1.3	L	VL	0.13	0.00925	UE in Seminoma
164	Proteasome activator complex subunit 4	163644283	52.7	5.7	M	VL	0.13	0.00058	UE in Seminoma
165	T-complex protein 1 subunit alpha isoform a	57863257	132.3	13.0	H	L	0.13	0.00002	UE in Seminoma
166	Pyruvate dehydrogenase E1 component subunit alpha, testis-specific form, mitochondrial precursor	4885543	50.0	3.7	M	VL	0.13	0.00018	UE in Seminoma
167	Dynein light chain roadblock-type 2	18702323	8.7	0.7	L	VL	0.13	0.00338	UE in Seminoma
168	Nuclear transport factor 2	5031985	9.7	0.7	L	VL	0.13	0.00959	UE in Seminoma
169	Metalloreductase STEAP4 isoform 1	100815815	13.3	1.7	L	VL	0.13	0.00942	UE in Seminoma
170	Prenylated Rab acceptor protein 1	222144309	8.3	1.0	L	VL	0.13	0.00233	UE in Seminoma
171	Heat shock protein 105 isoform 1	42544159	8.7	0.7	L	VL	0.14	0.00595	UE in Seminoma
172	ATP synthase subunit gamma, mitochondrial isoform L (liver) precursor	50345988	48.7	3.3	M	VL	0.14	0.00014	UE in Seminoma
173	3-Hydroxyisobutyryl-CoA hydrolase, mitochondrial isoform 1 precursor	37594471	11.7	1.0	L	VL	0.14	0.00055	UE in Seminoma
174	Transmembrane protein 89 precursor	56847630	12.7	1.0	L	VL	0.14	0.00529	UE in Seminoma

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Supplementary Table 2: Contd...

	Protein	Accession	Average SC		Abundance		NSAF ratio	t-test	Expression
			Control	Seminoma	Control	Seminoma	Seminoma/Control	P	
175	T-complex protein 1 subunit beta isoform 1	5453603	120.7	12.0	H	L	0.14	0.00005	UE in Seminoma
176	T-complex protein 1 subunit zeta isoform a	4502643	71.7	7.3	M	VL	0.15	0.00013	UE in Seminoma
177	Inactive serine protease 54 precursor	122937420	19.0	1.7	L	VL	0.15	0.00060	UE in Seminoma
178	Nucleoporin p54 isoform 1	26051237	21.7	2.3	M	VL	0.16	0.01278	UE in Seminoma
179	T-complex protein 1 subunit theta isoform 1	48762932	77.7	8.3	M	L	0.16	0.00032	UE in Seminoma
180	Sperm protein associated with the nucleus on the X chromosome B/F	190570192	22.0	2.7	M	VL	0.16	0.00428	UE in Seminoma
181	Histone H2A-Bbd type 2/3	63029935	21.7	2.0	M	VL	0.16	0.00688	UE in Seminoma
182	Transcription elongation factor B polypeptide 2 isoform a	6005890	11.0	1.3	L	VL	0.16	0.00017	UE in Seminoma
183	Protein MENT isoform X1	578801150	97.7	11.0	H	L	0.17	0.00005	UE in Seminoma
184	ATP synthase subunit d, mitochondrial isoform a	5453559	33.0	3.0	M	VL	0.17	0.00091	UE in Seminoma
185	Ropporin-1A isoform X1	530374814	55.7	5.7	M	VL	0.17	0.00001	UE in Seminoma
186	ATP synthase F (0) complex subunit B1, mitochondrial precursor	21361565	35.3	3.7	M	VL	0.17	0.00076	UE in Seminoma
187	NADH dehydrogenase (ubiquinone) flavoprotein 1, mitochondrial isoform 1 precursor	20149568	14.0	1.7	L	VL	0.17	0.00400	UE in Seminoma
188	Apolipoprotein O isoform X1	578837961	40.3	4.3	M	VL	0.17	0.00011	UE in Seminoma
189	26S proteasome non-ATPase regulatory subunit 1 isoform 1	25777600	49.0	8.3	M	L	0.17	0.00016	UE in Seminoma
190	Elongation factor 1-delta isoform 1	304555581	32.3	3.7	M	VL	0.18	0.00006	UE in Seminoma
191	26S proteasome non-ATPase regulatory subunit 8	156631005	25.7	2.3	M	VL	0.18	0.00001	UE in Seminoma
192	ATP synthase subunit alpha, mitochondrial isoform a precursor	50345984	265.3	33.0	H	M	0.18	0.00001	UE in Seminoma
193	Heat shock 70 protein 1-like isoform X1	530381921	207.0	24.3	H	M	0.19	0.00038	UE in Seminoma
194	Nitrilase homolog 1 isoform 3	297632348	18.7	2.0	L	VL	0.19	0.00095	UE in Seminoma
195	T-complex protein 1 subunit eta isoform a	5453607	129.7	16.0	H	L	0.19	0.00010	UE in Seminoma
196	Calcium-binding tyrosine phosphorylation-regulated protein isoform a	24797108	63.3	9.0	M	L	0.20	0.00012	UE in Seminoma
197	Tricarboxylate transport protein, mitochondrial isoform b	374717343	15.3	1.7	L	VL	0.20	0.00049	UE in Seminoma
198	T-complex protein 1 subunit epsilon	24307939	78.7	11.3	M	L	0.20	0.00001	UE in Seminoma
199	Tissue alpha-L-fucosidase precursor	119360348	19.0	1.7	L	VL	0.20	0.00008	UE in Seminoma
200	GTP-binding nuclear protein Ran	5453555	22.0	2.7	M	VL	0.20	0.00007	UE in Seminoma
201	Dipeptidyl peptidase 2 isoform X1	530426726	17.7	1.7	L	VL	0.20	0.00216	UE in Seminoma
202	3'(2'),5'-Bisphosphate nucleotidase 1 isoform X3	530365931	19.3	2.3	L	VL	0.20	0.00871	UE in Seminoma
203	Lysine-tRNA ligase isoform 1	194272210	30.0	3.0	M	VL	0.20	0.00462	UE in Seminoma
204	Mitochondrial thiamine pyrophosphate carrier isoform X1	530412630	16.3	2.0	L	VL	0.21	0.00139	UE in Seminoma
205	Vesicle-fusing ATPase isoform X1	578831007	16.7	2.3	L	VL	0.21	0.00045	UE in Seminoma

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Supplementary Table 2: Contd...

	Protein	Accession	Average SC		Abundance		NSAF ratio	t-test	Expression
			Control	Seminoma	Control	Seminoma	Seminoma/Control	P	
206	FUN14 domain-containing protein 2	24371248	60.3	8.0	M	L	0.22	0.00023	UE in Seminoma
207	Mitochondrial pyruvate carrier 2	219521872	25.7	5.0	M	VL	0.22	0.00370	UE in Seminoma
208	Dynein light chain 1, axonemal isoform 1	164607156	14.3	2.0	L	VL	0.23	0.00000	UE in Seminoma
209	Cytochrome b-c1 complex subunit 2, mitochondrial precursor	50592988	111.0	14.0	H	L	0.23	0.00006	UE in Seminoma
210	ADP/ATP translocase 4	13775208	140.3	25.3	H	M	0.23	0.00180	UE in Seminoma
211	26S protease regulatory subunit 7 isoform 1	4506209	9.7	1.7	L	VL	0.23	0.00149	UE in Seminoma
212	Uncharacterized protein C9orf9	33285006	45.0	6.7	M	VL	0.23	0.00005	UE in Seminoma
213	ADP/ATP translocase 2	156071459	35.7	8.3	M	L	0.24	0.00081	UE in Seminoma
214	Synaptojanin-2-binding protein	157388993	28.3	5.0	M	VL	0.24	0.00057	UE in Seminoma
215	Heat shock 70 protein 1A/1B	167466173	54.7	8.0	M	L	0.24	0.00086	UE in Seminoma
216	26S protease regulatory subunit 6B isoform 1	5729991	16.0	2.3	L	VL	0.24	0.00222	UE in Seminoma
217	Mannose-6-phosphate isomerase isoform 1	4505235	9.0	1.3	L	VL	0.25	0.00220	UE in Seminoma
218	Nuclear pore membrane glycoprotein 210 precursor	27477134	23.3	3.3	M	VL	0.25	0.00026	UE in Seminoma
219	Arylsulfatase A isoform a precursor	313569791	28.0	3.3	M	VL	0.25	0.00335	UE in Seminoma
220	Leucine-rich repeat-containing protein 37A isoform X5	530413292	165.7	27.7	H	M	0.26	0.00002	UE in Seminoma
221	Solute carrier family 2, facilitated glucose transporter member 5 isoform X2	578799621	31.0	7.7	M	VL	0.26	0.00472	UE in Seminoma
222	Protein-glutamine gamma-glutamyltransferase 4	156627577	232.3	44.0	H	M	0.26	0.00006	UE in Seminoma
223	Protein FAM162A	49355721	9.0	1.3	L	VL	0.26	0.00288	UE in Seminoma
224	26S protease regulatory subunit 6A	21361144	22.3	4.0	M	VL	0.27	0.00780	UE in Seminoma
225	Myosin regulatory light chain 12B	15809016	11.7	2.3	L	VL	0.27	0.00058	UE in Seminoma
226	Hexokinase-1 isoform X2	530393498	345.3	64.0	H	M	0.27	0.00001	UE in Seminoma
227	NADH dehydrogenase (ubiquinone) iron-sulfur protein 7, mitochondrial	187281616	9.7	2.0	L	VL	0.27	0.00101	UE in Seminoma
228	Cytochrome b-c1 complex subunit Rieske, mitochondrial	163644321	27.0	4.7	M	VL	0.27	0.00004	UE in Seminoma
229	Leucine-rich repeat-containing protein 37B precursor	53829385	176.3	40.3	H	M	0.27	0.00007	UE in Seminoma
230	ES1 protein homolog, mitochondrial-like isoform X1	578797780	35.0	5.7	M	VL	0.27	0.00001	UE in Seminoma
231	Lysosomal Pro-X carboxypeptidase isoform 1 preproprotein	4826940	13.3	1.7	L	VL	0.27	0.00077	UE in Seminoma
232	Transmembrane protein 190 precursor	21040263	33.3	5.7	M	VL	0.28	0.00004	UE in Seminoma
233	UTP-glucose-1-phosphate uridylyltransferase isoform a	48255966	20.3	5.0	M	VL	0.28	0.00603	UE in Seminoma
234	26S protease regulatory subunit 10B	195539395	21.3	4.3	M	VL	0.29	0.00659	UE in Seminoma
235	Dynactin subunit 1 isoform 4	205277396	21.7	3.7	M	VL	0.29	0.00497	UE in Seminoma
236	26S protease regulatory subunit 8 isoform 1	24497435	17.0	3.7	L	VL	0.29	0.00479	UE in Seminoma

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Supplementary Table 2: Contd...

	Protein	Accession	Average SC		Abundance		NSAF ratio	t-test	Expression
			Control	Seminoma	Control	Seminoma	Seminoma/Control	P	
237	Ethanolamine-phosphate cytidylyltransferase isoform 6	532524977	16.0	3.0	L	VL	0.29	0.00018	UE in Seminoma
238	60 heat shock protein, mitochondrial isoform X1	530370277	125.7	16.3	H	L	0.30	0.00029	UE in Seminoma
239	Beta-galactosidase-1-like protein isoform X1	530370954	35.3	4.7	M	VL	0.30	0.00099	UE in Seminoma
240	Adenylate kinase isoenzyme 1 isoform X1	530390694	45.3	7.7	M	VL	0.30	0.00114	UE in Seminoma
241	Dynein light chain Tctex-type 1	5730085	10.0	1.7	L	VL	0.30	0.00600	UE in Seminoma
242	Chitinase domain-containing protein 1 isoform X2	530395670	17.3	3.3	L	VL	0.31	0.00482	UE in Seminoma
243	A-kinase anchor protein 4 isoform 1	21493037	156.3	28.0	H	M	0.31	0.00000	UE in Seminoma
244	Hypoxia up-regulated protein 1 isoform X2	530397761	177.0	37.0	H	M	0.31	0.00002	UE in Seminoma
245	Diablo homolog, mitochondrial isoform 1 precursor	9845297	27.3	6.7	M	VL	0.32	0.00223	UE in Seminoma
246	Zona pellucida-binding protein 2 isoform 2 precursor	40556389	45.7	9.0	M	L	0.32	0.00009	UE in Seminoma
247	Ubiquitin-like modifier-activating enzyme 1 isoform X1	530421539	75.0	11.3	M	L	0.32	0.00035	UE in Seminoma
248	40S ribosomal protein S15a	14165469	12.7	2.3	L	VL	0.33	0.00231	UE in Seminoma
249	Prohibitin isoform 1	527498279	22.7	4.7	M	VL	0.33	0.00192	UE in Seminoma
250	Long-chain-fatty-acid-CoA ligase 6 isoform e	327412327	39.0	6.0	M	VL	0.33	0.00042	UE in Seminoma
251	Importin subunit beta-1 isoform 1	19923142	54.7	11.7	M	L	0.34	0.00045	UE in Seminoma
252	Long-chain-fatty-acid-CoA ligase 1 isoform X3	530377352	170.7	45.7	H	M	0.34	0.00024	UE in Seminoma
253	AP-1 complex subunit beta-1 isoform b	260436860	12.0	2.3	L	VL	0.34	0.00257	UE in Seminoma
254	Acrosin precursor	148613878	255.7	65.3	H	M	0.34	0.00011	UE in Seminoma
255	Glutathione S-transferase omega-2 isoform 2	300360567	10.3	2.0	L	VL	0.34	0.00400	UE in Seminoma
256	Carboxypeptidase D isoform 1 precursor	22202611	36.3	7.0	M	VL	0.34	0.02105	UE in Seminoma
257	Phosphate carrier protein, mitochondrial isoform b precursor	4505775	38.3	14.0	M	L	0.35	0.00176	UE in Seminoma
258	Heat shock 70 protein 4	38327039	44.7	7.7	M	VL	0.35	0.00303	UE in Seminoma
259	Fatty acid-binding protein, epidermal	4557581	30.7	6.0	M	VL	0.35	0.00328	UE in Seminoma
260	Ras-related protein Rab-2A isoform a	4506365	156.7	37.3	H	M	0.36	0.00002	UE in Seminoma
261	3-hydroxyacyl-CoA dehydrogenase type-2 isoform 1	4758504	43.0	9.3	M	L	0.36	0.00138	UE in Seminoma
262	T-complex protein 1 subunit delta isoform a	38455427	108.3	25.7	H	M	0.36	0.00075	UE in Seminoma
263	cAMP-dependent protein kinase type II-alpha regulatory subunit isoform X1	530372834	109.7	29.0	H	M	0.36	0.00000	UE in Seminoma
264	Glutamine synthetase isoform X1	578800828	23.3	5.3	M	VL	0.36	0.01626	UE in Seminoma
265	Calmodulin isoform X1	578826144	75.0	20.7	M	M	0.37	0.00004	UE in Seminoma
266	Elongation factor 1-beta	4503477	10.0	2.3	L	VL	0.37	0.00022	UE in Seminoma
267	ruvB-like 1	4506753	99.7	20.3	H	M	0.37	0.00684	UE in Seminoma

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Supplementary Table 2: Contd...

	Protein	Accession	Average SC		Abundance		NSAF ratio	t-test	Expression
			Control	Seminoma	Control	Seminoma	Seminoma/Control	P	
268	Elongation factor 1-alpha 1	4503471	155.0	51.3	H	M	0.38	0.00001	UE in Seminoma
269	hsc70-interacting protein isoform 1	19923193	30.3	7.3	M	VL	0.38	0.01230	UE in Seminoma
270	Transmembrane protein 126A isoform 1	14150017	22.0	6.0	M	VL	0.38	0.00020	UE in Seminoma
271	26S proteasome non-ATPase regulatory subunit 2 isoform 1	25777602	56.0	9.0	M	L	0.39	0.00049	UE in Seminoma
272	Arachidonate 15-lipoxygenase B isoform d	85067501	23.7	4.3	M	VL	0.40	0.00236	UE in Seminoma
273	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex subunit 9, mitochondrial precursor	6681764	9.7	2.3	L	VL	0.40	0.00078	UE in Seminoma
274	electron transfer flavoprotein subunit beta isoform 1	4503609	22.0	5.0	M	VL	0.41	0.00000	UE in Seminoma
275	Tubulin alpha-3C/D chain	156564363	242.3	60.7	H	M	0.41	0.00001	UE in Seminoma
276	Fumarylacetoacetate hydrolase domain-containing protein 2B	40786394	31.0	7.7	M	VL	0.41	0.00018	UE in Seminoma
277	Peroxiredoxin-5, mitochondrial isoform a precursor	6912238	78.0	20.7	M	M	0.41	0.00039	UE in Seminoma
278	NADH-ubiquinone oxidoreductase 75 subunit, mitochondrial isoform 4	316983156	21.0	4.0	M	VL	0.42	0.03074	UE in Seminoma
279	Probable C-mannosyltransferase DPY19L2 isoform X1	578823598	101.0	26.0	H	M	0.42	0.00052	UE in Seminoma
280	L-lactate dehydrogenase A-like 6B	15082234	145.0	42.7	H	M	0.42	0.00021	UE in Seminoma
281	Acrosin-binding protein precursor	17999524	288.3	74.7	H	M	0.43	0.00003	UE in Seminoma
282	Tubulin beta-4B chain	5174735	287.3	81.3	H	L	0.43	0.00004	UE in Seminoma
283	Electron transfer flavoprotein subunit alpha, mitochondrial isoform a	4503607	46.0	12.0	M	L	0.44	0.00065	UE in Seminoma
284	Serpin B6 isoform d	425876768	79.0	33.3	M	M	0.46	0.00013	UE in Seminoma
285	Lysozyme-like protein 4 isoform X2	578805633	29.3	8.0	M	L	0.46	0.00114	UE in Seminoma
286	cAMP-dependent protein kinase type I-alpha regulatory subunit isoform a	443497964	42.0	12.0	M	L	0.47	0.00245	UE in Seminoma
287	Vesicle-associated membrane protein-associated protein A isoform 2	94721252	38.7	12.0	M	L	0.47	0.00220	UE in Seminoma
288	Acrosomal protein SP-10 isoform a precursor	4501879	64.3	20.0	M	M	0.48	0.00064	UE in Seminoma
289	Carnitine O-acetyltransferase isoform 2	383209673	41.0	11.7	M	L	0.48	0.00294	UE in Seminoma
290	Endoplasmin precursor	4507677	543.7	146.0	H	H	0.49	0.00066	UE in Seminoma
291	Izumo sperm-egg fusion protein 4 isoform 1 precursor	89903025	119.3	39.0	H	M	0.53	0.00058	UE in Seminoma
292	Heat shock-related 70 protein 2	13676857	442.0	126.0	H	H	0.53	0.00000	UE in Seminoma
293	Elongation factor 2	4503483	84.3	30.0	H	M	0.56	0.00021	UE in Seminoma
294	Clathrin heavy chain 1 isoform X2	530411491	122.7	51.3	H	M	0.58	0.00014	UE in Seminoma
295	Sperm acrosome membrane-associated protein 1 precursor	13569934	176.0	83.7	H	H	0.60	0.00073	UE in Seminoma
296	Zona pellucida-binding protein 1 isoform 1 precursor	229577313	278.7	110.3	H	H	0.61	0.00002	UE in Seminoma
297	Phosphoglycerate kinase 2	31543397	204.3	79.0	H	M	0.65	0.00034	UE in Seminoma
298	2,4-Dienoyl-CoA reductase, mitochondrial precursor	4503301	180.7	70.0	H	M	0.65	0.00211	UE in Seminoma

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Supplementary Table 2: Contd...

	Protein	Accession	Average SC		Abundance		NSAF ratio	t-test	Expression
			Control	Seminoma	Control	Seminoma	Seminoma/Control	P	
299	Aminopeptidase N isoform X1	530407092	218.0	186.3	H	H	1.54	0.00040	OE in Seminoma
300	Calreticulin precursor	4757900	125.0	106.7	H	H	1.55	0.00511	OE in Seminoma
301	Dipeptidyl peptidase 4	18765694	138.0	122.0	H	H	1.61	0.00164	OE in Seminoma
302	Plastin-2 isoform X2	530402335	116.7	100.0	H	H	1.62	0.00036	OE in Seminoma
303	Angiotensin-converting enzyme isoform 1 precursor	4503273	141.3	124.7	H	H	1.62	0.01310	OE in Seminoma
304	Transitional endoplasmic reticulum ATPase	6005942	145.0	97.0	H	H	1.75	0.00238	OE in Seminoma
305	Neprilysin	116256327	85.3	59.3	H	H	2.01	0.00052	OE in Seminoma
306	Adipocyte plasma membrane-associated protein	24308201	54.0	66.7	M	M	2.01	0.00011	OE in Seminoma
307	Carboxypeptidase Z isoform 1 precursor	62388877	29.3	28.0	M	M	2.06	0.00040	OE in Seminoma
308	Annexin A5	4502107	41.0	48.0	M	M	2.08	0.00049	OE in Seminoma
309	Annexin A2 isoform 2	50845386	46.7	57.7	M	M	2.09	0.00126	OE in Seminoma
310	Lysosome-associated membrane glycoprotein 1 precursor	112380628	18.0	20.0	L	M	2.14	0.00275	OE in Seminoma
311	Plasma serine protease inhibitor preproprotein	194018472	40.7	45.7	M	M	2.15	0.00208	OE in Seminoma
312	Dehydrogenase/reductase SDR family member 7 isoform X1	530403978	22.3	35.7	M	M	2.46	0.00033	OE in Seminoma
313	Cysteine-rich secretory protein 1 isoform 1 precursor	25121982	24.0	35.7	M	M	2.53	0.00587	OE in Seminoma
314	Annexin A4	4502105	22.3	31.3	M	M	2.56	0.01438	OE in Seminoma
315	Calnexin precursor	66933005	34.3	51.3	M	M	2.56	0.00094	OE in Seminoma
316	Clusterin isoform X1	578815184	116.7	175.3	H	H	2.71	0.00034	OE in Seminoma
317	Gastricsin isoform 1 preproprotein	4505757	18.0	29.7	L	M	2.76	0.00296	OE in Seminoma
318	Metalloproteinase inhibitor 1 precursor	4507509	9.3	14.7	L	L	2.80	0.00553	OE in Seminoma
319	Lactotransferrin isoform 1 precursor	54607120	702.3	996.7	H	H	2.87	0.00000	OE in Seminoma
320	Protein S100-A9	4506773	23.7	45.0	M	M	3.35	0.00011	OE in Seminoma
321	Glyceraldehyde-3-phosphate dehydrogenase, testis-specific	7657116	48.7	106.7	M	H	3.41	0.00000	OE in Seminoma
322	Protein disulfide-isomerase precursor	20070125	85.0	185.0	H	H	3.53	0.00012	OE in Seminoma
323	Histone H4	4504301	13.7	27.7	L	M	3.56	0.00040	OE in Seminoma
324	Maltase-glucoamylase, intestinal isoform X1	578814724	16.7	33.0	L	M	3.58	0.00342	OE in Seminoma
325	Alpha-actinin-4	12025678	44.0	48.7	M	M	3.62	0.00001	OE in Seminoma
326	Lysozyme C precursor	4557894	7.7	16.3	VL	L	3.76	0.00087	OE in Seminoma
327	Alpha-1-antichymotrypsin precursor	50659080	18.7	33.7	L	M	3.82	0.00044	OE in Seminoma
328	Protein S100-A8	21614544	15.0	33.3	L	M	3.98	0.00483	OE in Seminoma
329	Thioredoxin-dependent peroxide reductase, mitochondrial isoform b	32483377	3.3	8.3	VL	L	4.38	0.00328	OE in Seminoma

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Supplementary Table 2: Contd...

	Protein	Accession	Average SC		Abundance		NSAF ratio	t-test	Expression
			Control	Seminoma	Control	Seminoma	Seminoma/Control	P	
330	Semenogelin-2 precursor	4506885	261.3	682.7	H	H	4.41	0.00044	OE in Seminoma
331	Prosaposin isoform a preproprotein	11386147	20.0	48.0	M	M	4.73	0.00704	OE in Seminoma
332	Olfactomedin-4 precursor	32313593	15.3	35.7	L	M	4.90	0.00039	OE in Seminoma
333	Lactadherin isoform a preproprotein	167830475	8.3	25.0	L	M	4.91	0.00144	OE in Seminoma
334	Mucin-5B precursor	301172750	22.0	65.0	M	M	4.97	0.00001	OE in Seminoma
335	Prolactin-inducible protein precursor	4505821	238.3	849.0	H	H	4.99	0.00045	OE in Seminoma
336	Alpha-1-antitrypsin precursor	189163528	13.7	34.7	L	M	5.01	0.00000	OE in Seminoma
337	Histone H3.3	4885385	5.7	18.7	VL	L	5.70	0.00001	OE in Seminoma
338	Annexin A11 isoform X1	530393508	4.0	16.0	VL	L	6.08	0.00010	OE in Seminoma
339	Ectonucleotide pyrophosphatase/phosphodiesterase family member 3	111160296	7.0	24.3	VL	M	6.24	0.00214	OE in Seminoma
340	Cathepsin D preproprotein	4503143	3.3	11.7	VL	L	6.46	0.00352	OE in Seminoma
341	BPI fold-containing family B member 1 precursor	40807482	4.7	15.3	VL	L	6.64	0.00033	OE in Seminoma
342	Fibronectin isoform 1 preproprotein	47132557	112.7	505.0	H	H	7.15	0.00001	OE in Seminoma
343	Nucleobindin-2 isoform X1	578820554	13.0	51.3	L	M	7.44	0.00026	OE in Seminoma
344	Semenogelin-1 preproprotein	4506883	94.0	422.3	H	H	7.78	0.00187	OE in Seminoma
345	Cytoskeleton-associated protein 4	19920317	1.3	8.0	VL	L	8.24	0.00084	OE in Seminoma
346	Ribonuclease pancreatic precursor	38201684	0.7	4.7	VL	VL	8.79	0.00074	OE in Seminoma
347	Transketolase isoform 1	205277463	3.7	15.3	VL	L	8.84	0.00097	OE in Seminoma
348	Neutrophil defensin 1 precursor	124248516	8.7	36.0	L	M	9.32	0.00010	OE in Seminoma
349	Neutrophil gelatinase-associated lipocalin precursor	38455402	9.3	58.7	L	M	9.97	0.00000	OE in Seminoma
350	Myeloperoxidase precursor	4557759	69.3	368.7	M	H	10.30	0.00000	OE in Seminoma
351	Myeloblastin precursor	71361688	6.0	34.0	VL	M	10.33	0.00005	OE in Seminoma
352	Catalase	4557014	1.7	8.3	VL	L	10.41	0.00009	OE in Seminoma
353	Azurocidin preproprotein	11342670	15.7	95.0	L	H	11.52	0.00000	OE in Seminoma
354	Carcinoembryonic antigen-related cell adhesion molecule 1 isoform 1 precursor	19923195	2.0	14.0	VL	L	12.42	0.00321	OE in Seminoma
355	Erythrocyte band 7 integral membrane protein isoform a	38016911	9.3	58.7	L	M	13.46	0.00055	OE in Seminoma
356	Apolipoprotein B-100 precursor	105990532	1.7	12.0	VL	L	13.65	0.00031	OE in Seminoma
357	Cysteine-rich secretory protein 3 isoform 1 precursor	300244560	0.7	5.3	VL	VL	14.12	0.00098	OE in Seminoma
358	Mucin-6 isoform X1	578840955	3.7	29.3	VL	M	14.30	0.00214	OE in Seminoma
359	ERO1-like protein alpha precursor	7657069	0.7	8.7	VL	L	15.45	0.00087	OE in Seminoma
360	Annexin A3	4826643	4.0	45.7	VL	M	17.62	0.00001	OE in Seminoma

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Supplementary Table 2: Contd...

	Protein	Accession	Average SC		Abundance		NSAF ratio	t-test	Expression
			Control	Seminoma	Control	Seminoma	Seminoma/Control	P	
361	Neutrophil elastase preproprotein	4503549	2.3	23.0	VL	M	17.84	0.00006	OE in Seminoma
362	Phospholipase B-like 1 precursor	110227598	1.7	22.7	VL	M	22.14	0.00036	OE in Seminoma
363	Laminin subunit alpha-5 precursor	21264602	4.3	39.0	VL	M	22.30	0.00613	OE in Seminoma
364	Moesin isoform X1	530421753	0.3	4.0	VL	VL	25.18	0.00003	OE in Seminoma
365	Eosinophil cationic protein precursor	45243507	1.0	20.3	VL	M	27.03	0.00057	OE in Seminoma
366	Carcinoembryonic antigen-related cell adhesion molecule 6 precursor	40255013	2.0	24.3	VL	M	27.08	0.00170	OE in Seminoma
367	Syntenin-1 isoform X1	530388518	0.3	5.7	VL	VL	30.17	0.00085	OE in Seminoma
368	CD63 antigen isoform A	383872447	1.0	14.7	VL	L	31.84	0.00518	OE in Seminoma
369	Collagen alpha-1 (XVIII) chain isoform 1 precursor	110611235	1.0	27.0	VL	M	33.62	0.00154	OE in Seminoma
370	Laminin subunit gamma-1 precursor	145309326	1.0	22.0	VL	M	40.70	0.00062	OE in Seminoma
371	Integrin alpha-M isoform 1 precursor	224831239	5.3	176.0	VL	H	50.91	0.00002	OE in Seminoma
372	Laminin subunit beta-2 isoform X1	530372442	1.3	45.0	VL	M	61.08	0.00000	OE in Seminoma
373	Alpha-1-acid glycoprotein 1 precursor	167857790	0.7	20.7	VL	M	64.68	0.00005	OE in Seminoma
374	Integrin beta-2 precursor	188595677	2.3	124.0	VL	H	71.24	0.00000	OE in Seminoma
375	Carcinoembryonic antigen-related cell adhesion molecule 8 precursor	21314600	0.3	18.0	VL	L	103.73	0.00001	OE in Seminoma
376	Cytochrome b-245 heavy chain	6996021	0.3	15.0	VL	L	112.04	0.00002	OE in Seminoma
377	Bactericidal permeability-increasing protein precursor	157276599	0.3	49.0	VL	M	300.57	0.00002	OE in Seminoma
378	Matrix metalloproteinase-9 preproprotein	74272287	0.0	90.0	ni	H	Seminoma only	0.00000	Unique to Seminoma
379	Leukocyte elastase inhibitor	13489087	0.0	23.0	ni	M	Seminoma only	0.00006	Unique to Seminoma
380	Arachidonate 5-lipoxygenase isoform 2	371877525	0.0	12.7	ni	L	Seminoma only	0.00000	Unique to Seminoma
381	Prostate and testis expressed protein 4 precursor	221554530	0.0	8.0	ni	L	Seminoma only	0.00002	Unique to Seminoma
382	Chitinase-3-like protein 1 precursor	144226251	0.0	10.3	ni	L	Seminoma only	0.00003	Unique to Seminoma
383	ADP-ribosyl cyclase 2 precursor	168229159	0.0	13.7	ni	L	Seminoma only	0.00009	Unique to Seminoma
384	Peptidoglycan recognition protein 1 precursor	4827036	0.0	10.7	ni	L	Seminoma only	0.00059	Unique to Seminoma
385	Neutrophil collagenase preproprotein	4505221	0.0	16.3	ni	L	Seminoma only	0.00120	Unique to Seminoma
386	Haptoglobin isoform 2 preproprotein	186910296	0.0	11.3	ni	L	Seminoma only	0.00207	Unique to Seminoma
387	Resistin precursor	301129180	0.0	6.0	ni	VL	Seminoma only	0.00001	Unique to Seminoma
388	Matrilin-2 isoform a precursor	62548860	0.0	3.0	ni	VL	Seminoma only	0.00002	Unique to Seminoma
389	Immunoglobulin alpha Fc receptor isoform a precursor	4503673	0.0	3.7	ni	VL	Seminoma only	0.00018	Unique to Seminoma
390	Vascular non-inflammatory molecule 2 isoform X1	578813045	0.0	6.0	ni	VL	Seminoma only	0.00025	Unique to Seminoma
391	Integrin beta-2 isoform X1	578836536	0.0	6.3	ni	VL	Seminoma only	0.00029	Unique to Seminoma

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Supplementary Table 2: Contd...

	Protein	*Accession*	*Average SC*		*Abundance*		*NSAF ratio*	*t-test*	*Expression*
			Control	*Seminoma*	*Control*	*Seminoma*	*Seminoma/Control*	*P*	
392	Flotillin-2 isoform X1	530410971	0.0	6.3	ni	VL	Seminoma only	0.00055	Unique to Seminoma
393	Cathepsin G preproprotein	4503149	0.0	3.3	ni	VL	Seminoma only	0.00065	Unique to Seminoma

H: high; L: low; M: medium; ni: not identified; NSAF: normalized spectral abundance factor; OE: overexpressed; SC: spectral counts; UE: underexpressed; VL: very low