OBJECTIVE

Our earlier proteomic research on oxidative stress in infertile men demonstrated 10 differentially expressed proteins involved in three different cellular networks. These proteins [especially angiotensin-converting enzyme (ACE) and mitogen-activated protein kinase (MAPK3K3)] are localized either in the center of the network or function as interlink protein between the networks. Testicular isoform of ACE (tACE) is a ~80kDa protein located in the periacrosomal region of the spermatozoa and is involved in capacitation and acrosomal reaction. MAPK3K3 is one of the interlink proteins that regulates flagellar movement and hyperactivation during capacitation and acrosome reaction. The objective of our study was to validate whether these two proteins can be potential biomarkers in spermatozoa under oxidative stress characterized by high and low levels of reactive oxygen species (ROS).

INTRODUCTION

• Oxidative stress is due to an imbalance between the excessive production of reactive oxygen species (ROS) and antioxidant defense system in favor of oxidation. Evidence of oxidative stress on male reproduction has been the subject of numerous publications, and its role is well established in the evaluation of male infertility cases. Excess cellular ROS has a detrimental effect on biologically significant molecules such as lipids, nucleic acids and especially proteins. In our previous global proteomics analysis identified the testicular form of Angiotensin IACE and Mitogen-activated protein kinase kinase kinase 3 (MAPK3K3) expression level were altered in male infertility cases with oxidative stress.

• Angiotensin-converting enzyme (ACE) encodes two isoforms: somatic (sACE) and testicular (tACE) exclusively expressed in spermatids and mature spermatozoa. Several studies reported that IACE activity is associated with male infertility. Previous reports demonstrated male reproductive function is modulated by mitogen activated protein kinase (MAPK) pathway. MAPK3K3 is a component of a protein kinase signal transduction that mediates activation of the NF-kappa-B, AP1, and DDIT3 transcriptional regulators. MAPK has been detected in the tail of ejaculated sperm and the regulation of forwarding and hyperactivated sperm motility. Also, MAPK is phosphorylated to inhibit sperm motility.

• In this study, protein expression levels of IACE and MAPK3K3 have been validated by western blot and molecular localization of these were shown by immunocytochemistry staining. These proteins are associated with oxidative stress induced male infertility cases and may serve as a potential biomarker for improved diagnosis.

RESULTS

1. Bioinformatics analysis showed activated cellular networks interactions involved in the cellular network. IACE and MAPK3K3 are potential proteins for sperm function in acrosome and motility respectively.

2. Expression profile of IACE and MAPK3K3 confirmed global LC-MS proteomics analysis. Elevated IACE level refers acne dysfunction, while overexpression of MAPK3K3 shows hyperactivity.

3. Sperm immunocytochemistry staining detected IACE in the post-acrosomal region while MAPK3K3 in the tail.

4. These proteins play important roles for critical sperm function like acrosome activity and motility and have potentials to be biomarkers for specific male infertility cases.

REFERENCES


Figure 1: Activated cellular network including core proteins (red) and interlink proteins (blue) in patients with oxidative stress. Lines between the proteins indicates protein-protein interaction.

Table 1: Fold change in IACE and MAPK3K3 in ROS (-) and ROS (+) patients in comparison to control group.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Donor</th>
<th>Control</th>
<th>Patient</th>
<th>Fold Change</th>
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<tbody>
<tr>
<td>IACE</td>
<td></td>
<td></td>
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<tr>
<td>ROS (-)</td>
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Figure 2: Western blot gel image shows protein bands referring protein expression. Tubulin was housekeeping protein that was used for internal control.

Figure 3: Cellular localization of IACE and MAPK3K3 proteins by immunocytochemistry.

CONCLUSIONS