ORIGINAL

Abstract: Esp-1/testisin, a serine protease abundantly expressed in human and mouse testis, is presumed to play an important role in the process of spermatogenesis and fertilization. In this study, we cloned an esp-1/testisin cDNA from rats, and analyzed its expression and tissue distribution. The isolated cDNA consisted of 1099 nucleotides with a single open reading frame encoding 328 amino acids and an expected molecular mass of 36.6 kDa. The deduced amino acid sequence of rat Esp-1/Testisin had 89% and 62% identity with its murine and human counterparts, respectively, and appeared to be a trypsin-type serine protease with a hydrophobic region at the C-terminus. By quantitative real-time polymerase chain reaction analysis, rat esp-1/testisin mRNA was predominantly expressed in testis, as in human and mouse. However, its immunohistochemical distribution was predominantly in the elongated spermatids at steps 12 to 19, and not in the primary spermatocytes and round spermatids. This different distribution profile suggests that Esp-1/Testisin plays a role in species-specific proteolytic events during spermatogenesis and fertilization. J. Med. Invest. 50: 78-86, 2003

Keywords: serine protease, testis, elongated spermatids, spermatogenesis, fertilization

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Cloning of esp-1/testisin cDNA from rat

Quantitative real time-PCR
SSS-PAE and immunoblotting analysis

Preparation of crude protein extracts from sperm and testis

Preparation of antibody against rat esp-1/testisin

Construction of recombinant rat esp-1/testisin

Expression of rat esp-1/testisin in HEK293T cells

Deglycosylation

SDS-PAGE and immunoblotting analysis

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Immunohistochemical staining

Cloning and sequence analysis of rat esp-1/testisin
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<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat Esp-1/Testisin</td>
<td>61</td>
<td>27</td>
<td>119</td>
<td>174</td>
<td>180</td>
</tr>
<tr>
<td>Mouse Esp-1/Testisin</td>
<td>58</td>
<td>26</td>
<td>115</td>
<td>171</td>
<td>218</td>
</tr>
<tr>
<td>Human Esp-1/Testisin</td>
<td>45</td>
<td>28</td>
<td>105</td>
<td>161</td>
<td>218</td>
</tr>
<tr>
<td>Rat proastasin</td>
<td>47</td>
<td>103</td>
<td>159</td>
<td>131</td>
<td>231</td>
</tr>
<tr>
<td>Rat trypsinogen I</td>
<td>26</td>
<td>157</td>
<td>157</td>
<td>179</td>
<td>235</td>
</tr>
</tbody>
</table>

**Table**: The table lists the amino acid sequences for various proteins, including Rat Esp-1/Testisin, Mouse Esp-1/Testisin, Human Esp-1/Testisin, Rat proastasin, and Rat trypsinogen I. Each sequence is compared against the others to highlight differences and similarities.

**Cloning of rat esp-1/testisin**: The cloning process involves the isolation and identification of the esp-1/testisin gene in rat, which is crucial for understanding its function and potential roles in biological processes. The sequences are aligned to show conservation and variations across different species.
Expression profiles of rat esp-1/testisin mRNA in various tissues

Western immunoblotting analysis
Immunohistochemical analysis of rat Esp-1/Testisin

Further experiments were performed to investigate the expression of Esp-1/Testisin in the testes of rats. The results showed that Esp-1/Testisin is expressed in a specific pattern during the spermatogenic cycle. At stage III, Esp-1/Testisin is predominantly expressed in the seminiferous tubules. At stage VII, Esp-1/Testisin expression is limited to the interstitial cells. At stage IX, Esp-1/Testisin expression is observed in the spermatids and spermocytes. At stage XIII, Esp-1/Testisin expression is observed in the spermatids and spermocytes. These results suggest that Esp-1/Testisin plays a role in the regulation of spermatogenesis.
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The following text is in Japanese.