Expression of the Ha-ras Suppressor Family Member 5 Gene in the Maturing Rat Testis

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We analyzed the gene expression of Ha-ras suppressor family member 5 (Hrasls5), which is considered to modulate the Ha-ras signaling cascade, from maturing rat testis. Expression was detected primarily in the spermatocytes in the maturing rat testis. The Hrasls5 gene product might function as a tumor suppressor as well as in spermatogenesis, as deduced from its amino acid sequence.

Key words: Hrasls5; in situ hybridization; RLP-1; spermatogenesis; tumor suppressor

Spermatogenesis is a complex process, during which many associated genes are expressed systematically. Many of the genes involved in this process have been cloned and characterized. These gene products are thought to cooperate to produce mature sperm cells from spermatogonia to spermatocytes during the meiotic process, to regulate cell division, and to complete the morphological construction of the sperm cell. Small G-protein family member proteins (Ras proteins) have been deduced to be involved in these processes.1) Ras proteins are classified as members of the proto-oncogene family, and are subdivided into Ha-ras, Ki-ras, and N-ras, controlling the cell cycle, proliferation, and differentiation via the mitogen activating kinase (MAPK) signaling cascade in the cytoplasm.2,3) It has been observed that Ras proto-oncogene family genes are expressed in developing mouse male germ cells.4) Ras protein forms a complex with PI3-kinase and PKCzeta, and is found in spermatozoa. The complex is involved in sperm motility as well as the signaling cascade, as described previously.5) NIH3T3 cells expressing Ras protein show transforming properties when a specific mutation is introduced into the protein,5) and orchietomy reduces hepatotumorigenesis in H-ras12V transgenic mice.6) Based on this knowledge, certain testis-related factors may be involved in MAPK cascade activation. Thus the Ras mediated MAPK signaling cascade is essential for normal spermatogenesis. Taking all this together, it is interesting to note that Ha-ras proto-oncogene might be closely associated with spermatogenesis. In addition to this, the frequency observed of testis cancer is not higher than in other cancers. Furthermore, tumor cells are usually derived from spermatocytes.

The existence of tumor suppressor molecules is therefore hypothesized, and candidates have been screened, for example by subtraction hybridization. H-rev107, which is specifically expressed in a phenotypic revertant of Ras-transformed rat fibroblasts, has been cloned as a candidate tumor suppressor gene.7–11) Tumor suppressor genes are recognized as class I and II. Expression of a class II suppressor protein is down-regulated by a class I suppressor protein, and its mutation coordinately inhibits the expression of several class II genes.7) Strong expression of H-rev107, a class II suppressor protein, is observed in human testis, particularly in round spermatids, but expression is significantly reduced in testicular germ cell tumors.12) Another Ha-ras suppressor gene (A-C1) has been cloned from mouse by differential display, and the product also modulates the Ras-mediated signaling pathway.13) Gene expression has been determined using the human homolog as a probe, and strongly observed in human testis.14) Recently, Jin et al. cloned a novel gene that encodes a rat lecithin-retinol acyltransferase-like protein (RLP-1).15) This gene is identical to Hrasls5, which we cloned, and the product shows phosphatidylethanolamine (PE) N-acetylation enzyme activity, as well as deduced tumor suppressor activity, and is highly

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Note

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homologous with the deduced amino acid sequences of both A-C1 and H-rev107. Expression of the gene has been detected in adult rat testis, yet the importance of enzyme activity in the development of the testis is not certain.

In this experiment, we determined the gene expression of Hrasls5 during the developmental stage in rat testis and other organs. Expression in the testis was confirmed through histological examination, and the importance of the gene product during spermatogenesis is discussed.

The cDNA fragment, which encodes rat Hrasls5, was synthesized by reverse transcription polymerase chain reaction (RT-PCR) using an RNA template prepared from 7-week-old Sprague-Dawley (SD) rat testis. PCR was conducted to produce a probe of about 1.0 kbp in length, which contained the open reading frame (ORF) of the gene, using the following 20 mer primers: TCG CTC ACC TCC TAT TCT AG as upstream primer, and TGT TTA ACA GGG CCA CTG AG as downstream primer. Total RNA was prepared from the testis and from the individual organs of a 7-week-old animal. The individual RNA (3 μg) was electrophoresed in a gel containing formaldehyde, and then transferred to a nylon membrane, Hybond N\(^+\) (GE Healthcare, Buckinghamshire, UK). The cDNA encoding Hrasls5 was labeled by a random primer labeling system (GE Healthcare) using \(\text{[α-}^{32}\text{P]}\)dCTP, and was used as a probe. Hybridization was performed, and the blot was rehybridized with rat β-actin cDNA as an internal standard. Testicular expression of Hrasls5 was determined by \textit{in situ} hybridization following a procedure described previously.\(^{16}\)

Briefly, digoxigenin (DIG)-labeled sense and antisense riboprobes were hybridized with a 5 μm testicular section from an 8-week-old rat. The signals were detected using a BlueMap NBT/BCIP kit (Ventana, Yokohama, Japan).

We screened the genes expressed after sexual maturation in rat testis by differential display. One of the genes we cloned was identical to Hrasls5, and the sequence data were deposited in the DNA database (accession no. BC099084). This gene product was identified as RLP-1.\(^{15}\) Gene expression of Hrasls5 was detected in 7-week-old rat testis, and the levels increased until the rats reached 15 weeks of age (Fig. 1A). Strong expression was detected in the testis at 7 weeks, and the position was a little lower than the 18S rRNA size expected from the DNA deposited in the database (accession no. BC099084), in which the mRNA size is reported as 1,540 bp. Expression was not detected in any of the organs we examined, including the ovary, thyroid, and heart (Fig. 1B). The expression of Hrasls5 was specific to the testis during sexual maturation of the animals, and was not expressed in the ovary. The gene product is closely involved in the maturation process from spermatocyte to spermatid, since expression was up-regulated in 7-week-old rat testis. Since a testis-specific function of the gene product was indicated, expression in a section of the testis was determined by \textit{in situ} hybridization. We observed and compared the hybridization profiles at each stage in the seminiferous tubules. Expression was primarily detected in spermatocytes and round spermatids, and also sparsely in elongated spermatids, during the haploidgenesis process in each of the seminiferous tubules examined, corresponding to stages I to XI (Fig. 2).

It has been reported that Ha-ras gene expression is constitutive in rat testis, even at relatively low levels, during sexual maturation from newborn through adult.\(^{17}\) Among the Ras suppressor gene family members and in contrast to expression of the Ha-ras gene, human A-C1, H-rev107, and rat RLP-1 genes are strongly and primarily expressed in adult testis.\(^{12,14,15}\) Furthermore, even higher expression of rat H-rev107 gene is observed.
in most adult tissues, and this is especially localized to epithelium.\textsuperscript{10} By observing each seminiferous stage in the tubules, we detected the expression of \textit{Hrasls5} from spermatocytes through spermatids, but no expression was detected in the ovary, as a female reproductive system, by Northern blot analysis. It is hypothesized that Ras suppressor gene products act as negative regulators of Ras signaling molecules, by coordinating the regulation of cell proliferation during normal meiotic processes, especially in spermatogenesis. It would be interesting to determine the reason for the consistently lower level of Ha-ras gene expression during sexual maturation in the testis, as compared with that of Ras suppressor family members, such as A-C1, H-rev107, RLP-1, and \textit{Hrasls5}.

In future work, it will be necessary to determine how the \textit{Hrasls5} gene product regulates the Ha-ras signaling cascade, especially in relation to the process of spermatogenesis.

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