We analyzed the gene and protein expression of serologically defined colon cancer antigen 8. Gene expression was upregulated in the maturing rat testis, and was localized to the spermatocytes. Protein was detected in the spermatids and at the sites of mRNA expression. Specific expression of colon cancer antigen 8 was observed in the maturing rat testis.

Key words: centrosomal colon cancer autoantigen protein; immunohistochemistry; in situ hybridization; serologically defined colon cancer antigen 8; spermatogenesis

Spermatogenesis is a complex process during which many genes are systematically expressed. Male reproductive cells are arranged in the seminiferous tubules from the basement membrane as spermatogonia, spermatocytes, and spermatids. Spermatogonia undergo mitosis to form primary spermatocytes, which undergo meiosis to form spermatids. We have screened the genes expressed during this process using differential display (DD). The genes classified and characterized so far include (i) molecular motor proteins that transfer cytoplasmic cellular substances (e.g., kinesin); (ii) cytoplasmic regulators of membrane trafficking (e.g., RabGAP/TBC); (iii) molecular chaperones (e.g., the small heat shock protein Hsp20, which is a member of the αB crystallin-related protein family); (iv) the sperm flagellum-movement associated protein ODF1 and an adenylate kinase domain-containing protein that catalyzes ATP synthesis; and (v) tumor suppressor gene products in relation to Ha-ras (e.g., hraslS5).

In addition to these genes, we identified a novel class of gene that was specifically expressed during spermatogenesis. This gene product was identified as serologically defined colon cancer antigen 8 (SDCCAG8) or centrosomal colon cancer autoantigen protein (CCCAP), and its C-terminal portion was identified as serologically defined human colon cancer autoantigen (NY-CO-8). These cancer autoantigens were initially screened by serological analysis of a recombinant cDNA expression library (SEREX) derived from human tumors. During this process, a range of antigens (NY-CO-1 to 48) was screened. The Sdccag8 gene product was determined to be a component of the centrosome. It possessed a coiled-coil domain in its C-terminus. Coiled-coil domains are found in many proteins (e.g., structural proteins, motor proteins, and transcription factors) that interact with other proteins to perform specific functions. In the case of the bacteriophage Mnt repressor, dimerization of two anti-parallel coiled-coil structures is required to bind DNA and regulate gene expression controlling the switch between the lysogenic and lytic growth cycles of bacteria. Mouse CCCAP has also been determined to be capable of homo-oligomerization using the yeast 2-hybrid system. These observations suggest that this gene product has certain specific functions related to spermatogenesis.

Since the function of the Sdccag8 gene product is largely unknown in relation to spermatogenesis, we determined its mRNA and protein expression in maturing rat tissues.

Gene expression in 7-week-old rat testes was compared with the expression observed in 3-week-old rat testes by DD. We identified several differentially regulated gene fragments and analyzed the expression of rat Sdccag8 (accession no. NM_177929). Gene expression was determined by Northern blotting. Five μg of RNA was electrophoresed in a formaldehyde-containing agarose gel, and blotted onto a Hybond N + membrane (GE Healthcare, Buckinghamshire, UK). The 582-bp PCR product containing the 5′ non-coding and coding cDNA fragments of Sdccag8 was used as a probe in the Northern blot experiments. Probe DNA was labeled using a random primer [α-32P] dCTP labeling system (GE Healthcare). The membrane was rehybridized with rat β-actin cDNA as an internal control for the amount and integrity of RNA. Gene expression in the testis was determined by in situ hybridization (ISH), as previously reported, except for the following modifications: Riboprobes were synthesized using the T7 promoter attached to a cDNA fragment from the +308 to the +733 region of the gene as a template using DIG RNA Labeling kit (Roche, Basel, Switzerland). The
We electrophoresed 3 µg of each protein on a sodium dodecyl sulphate containing 7.5% polyacrylamide gel, and transferred it to a Hybond C nylon membrane. The positions of the protein standards are indicated on the left by arrows.

probe was hybridized to 5-µm sections of 8-week-old rat testis, and the signal was developed using the DIG Nucleic Acid Detection kit (Roche) with methyl-green as a counterstain, following the manufacturer’s protocol.

Protein expression was determined by Western blotting. Two synthetic oligo-peptides, C + SSLAEAQ-ERETSAFK and DQLRAQLPSPMQSDC, were used to immunize rabbits, and sera were collected as a polyclonal antibody by a custom antibody producing service immunizing rabbits, and sera were collected as a polyclonal antibody by a custom antibody producing service. The SDCCAG8 mRNA signal position is indicated by an arrowhead on the right. The protein expression level of SDCCAG8 was determined by Western blotting. Antisera were raised by immunizing rabbits with two synthesized oligopeptides designed to a rat-specific region of the protein, excluding the coiled-coil domain. We analyzed the expression of SDCCAG8 in the testis, ovary, and colon, and detected a testis-specific band for SDCCAG8 of approximately 80 kDa (Fig. 1C). Since the mouse CCCAP protein is reported to be 83 kDa in size, we concluded that the antisera raised from the two synthesized oligo-peptides can be used to detect rat SDCCAG8, and we used the antisera in a subsequent immunohistochemical experiment.

Since the expression of Sdccag8 was specific to the testis and increased during maturation, we determined its expression in the testis using ISH on 8-week-old rat testis sections. We observed the individual stages of the seminiferous tubules. Gene expression was widespread in the spermatocytes (Fig. 2).

Protein expression in the testis sections was determined by immunohistochemistry using the antisera that were used for Western blotting. Signals were detected in round, elongated spermatids, as well as in the spermatocytes (Fig. 3), in agreement with the observations from ISH. These results confirm that the SDCCAG8 translated in spermatocytes is stably maintained in spermatids, and has specific functions during the meiotic process and the subsequent morphological changes in sperm cells.

SDCCAG8 has been identified as CCCAP in mice and humans. It contains a typical C-terminal coiled-coil domain, and its molecular size in the mouse was predicted to be 83 kDa. Its C-terminal region is identical to NY-CO-8, a colon cancer autoantigen. CCCAP is a component of the centrosome and it can homo-oligomerize. Expression of murine CCCAP and of human NY-CO-8 has been reported to be low but ubiquitous in organs examined, although relatively high expression was observed in the human testis using an NY-CO-8 probe in Northern blotting. In agreement
with this observation, we observed high, specific expression of rat Sdccag8 in the maturing testis. Hence, we predict a specific function of SDCCAG8 in the maturing rat testis, in addition to its centrosome-associated function.

More than 100 cancer/testis (CT) antigen genes have been identified.9,10 Their expression is restricted to normal adult testicular germ cells, and various types of cancer cells. These candidates, including NY-CO-8, have been analyzed using SEREX.7) Gene expression is regulated by the abundance of methylation in the promoter sequences of the genes, including CT antigen, such as mouse maelstrom11) and human threonine protease genes.12) In particular, the roles of CT antigens in cancer cells and testicular germ cells remain largely unknown. Nevertheless, CT antigens can be used not only as a diagnostic tool in cancer treatment, but also as promising target molecules in cancer immunotherapy.

The importance of the gene expression of Sdccag8 in relation to meiosis and spermatid differentiation should be determined in future work, since the finding that the CT antigen gene has a role in the development of spermatocytes and spermatids is novel.

Fig. 2.  Cellular Localization of the Rat Sdccag8 Transcript in 8-Week-Old Rat Testis.
In situ hybridization of the Sdccag8 probe at various stages in 5-μm-thick sections of the seminiferous tubules of 8-week-old rat testis (A) to (C), and a sense riboprobe as a negative control (D). The tubules were expected to be (A) stages VII to VIII, (B) stages IX to XI, and (C) stages XII to XIV. Signals (purple) in the cytoplasm of the spermatocytes (arrows) can be observed. Cells were counterstained with methyl-green (green). The scale bar represents 100 μm.

Fig. 3.  Cellular Localization of Rat SDCCAG8 Protein in 8-Week-Old Rat Testis.
Immunohistochemistry using rat SDCCAG8 antisera at various stages on 5-μm-thick sections of the seminiferous tubules of 8-week-old rat testis (A) to (C), and using pre-immune serum as a negative control (D). Tubules were expected to be (A) stages VII to VIII, (B) stages IX to XI, and (C) stages XII to XIV. Signals (brown) in the cytoplasm of the spermatocytes (arrows in B and C), and spermatids (arrow in A) can be observed. The scale bar represents 100 μm.
Acknowledgments

This work was supported in part by a grant from the Education and Research Improvement and Promotion Program 2009 from the President of Tottori University. We also thank Mr. Makoto Kita, Research Center for Bioscience and Technology, Tottori University, for support with radioisotopes.

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