

Immunohistochemical Study Of Steroidogenic Acute Regulatory (StAR) Protein In Ovarian Tumors

T Sugawara, N Hoshi, N Sakuragi

Citation

T Sugawara, N Hoshi, N Sakuragi. *Immunohistochemical Study Of Steroidogenic Acute Regulatory (StAR) Protein In Ovarian Tumors*. The Internet Journal of Gynecology and Obstetrics. 2003 Volume 3 Number 2.

Abstract

Objectives: Steroidogenic acute regulatory (StAR) protein is a 30-kDa mitochondrial protein that promotes steroid hormone production in the ovary, testis and adrenal gland. In this study, we examined the expression of StAR protein using immunohistochemistry.

Methods: We studied four cases of functional tumors, including one case of theca cell tumor, one case of granulosa cell tumor, one case of sclerosing stromal tumor and one case of mucinous cystadenocarcinoma

Results: All of the tumors examined had StAR expression in the tumor tissues. Our immunohistochemical studies of the steroid hormone-producing tumors revealed that StAR protein expression is present in stromal cells.

Conclusions: Immunostaining with a StAR protein antibody enables speculation on not only the production of steroid hormones but also the origin of tumor cells.

INTRODUCTION

Ovarian tumors are common neoplasms in the female reproductive tract. The frequency of functioning tumors has been reported to be 0.8% to 1.2% in ovarian tumors and to be about 8% in all solid ovarian tumors (1,2,3). Specific signs are caused by internal secretion of produced steroid hormones. Signs in cases of estrogen-producing tumors are precocious maturity, irregular genital bleeding, marked estrogenic vaginal smears and endometrial hyperplasia, and the sign in cases of androgen-producing tumors is masculinization (4, 5). When clinical characteristics due to the effects of steroid hormones are seen, speculation regarding the histology of ovarian tumors can sometimes be made. However, the possibility of the existence of a functioning tumor cannot be excluded simply by the amount of steroid hormones produced because there are no specific signs if only a small amount of steroid hormones is produced. Theca cell tumors and granulosa cell tumors produce estrogen in tumor cells, and Sertoli cell tumors and Leydig cell tumors produce androgen. It has also been reported that some epithelial ovarian tumors, which are considered to be tumors that do not produce steroid hormones, are steroid hormone-producing tumors.

The first reaction in the biosynthesis of steroid hormones is catalyzed by the cytochrome P450 cholesterol side-chain

cleavage enzyme (P450_{scc}), which resides on inner mitochondria membranes. Steroidogenic acute regulatory (StAR) protein promotes pregnenolone production from cholesterol by regulating the movement from the outer to the inner mitochondrial membranes (6). StAR mRNA is expressed in hormone-producing tissues, which are in the adrenal gland, ovary and testis (7). StAR is a candidate gene of lipoid congenital adrenal hyperplasia, which is a severe impairment of steroid biosynthesis (8). StAR protein is associated with steroid hormone production (7). In this study, we examined the expression of StAR protein in steroid hormone-producing cord-stromal ovarian tumors and in a mucinous cystadenocarcinoma using immunohistochemistry.

MATERIALS AND METHODS

We studied four cases of functional tumors, including one case of theca cell tumor, one case of granulosa cell tumor, one case of sclerosing stromal tumor and one case of mucinous cystadenocarcinoma. In all cases, diagnosis had been made on the basis of clinical findings, including results of hormone assays, and surgery had been performed. Pathological diagnosis was made after surgery. Ovary tissues that had StAR protein expression before menopause were used as positive controls. Phosphate buffer was used instead of StAR protein antibody as a negative control. A polyclonal rabbit antibody IgG fraction against StAR protein provided

by Dr. Jerome F. Strauss III of the University of Pennsylvania, Philadelphia, USA was used (9).

IMMUNOHISTOCHEMISTRY

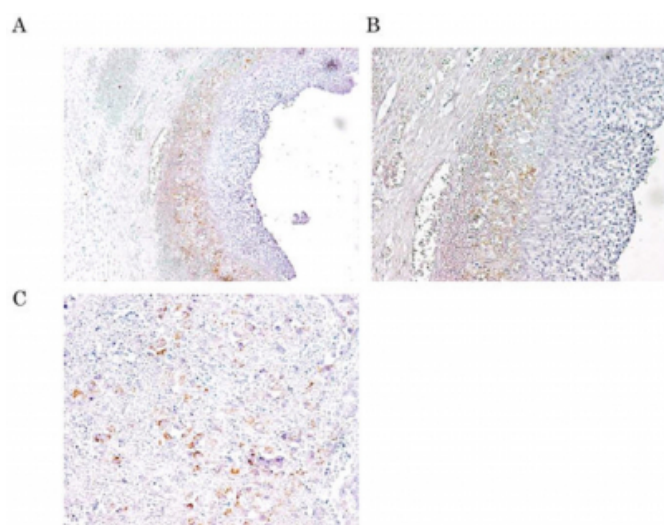
Immunohistochemistry was performed on 2.5- μ m-thick sections on slides using the biotin-streptavidin amplified technique with a histofine immunostaining kit (Nichirei, Tokyo, Japan). Briefly, the procedure was as follows. After deparaffinization, endogenous peroxides were inactivated with 3% H₂O₂ in methanol for 15 minutes at room temperature. This was followed by incubation with 10% goat serum for 10 min at room temperature. The slide was then incubated with a primary antibody to StAR protein (rabbit IgG fraction) at 4 °C for 7 hr. The StAR protein antibody dilution was 1 to 1,000. The sample was further incubated with a biotinylated goat anti-rabbit antibody for 1 hr and then with peroxidase-conjugated streptavidin for one hour at room temperature. Finally, a colorimetric reaction was carried out with a solution containing 50 mM Tris HCl, pH 7.6, 0.66 mol/l of 3,3'-diaminobenzidine, and 0.06% of H₂O₂, and then counterstaining with hematoxylin stain was performed.

RESULTS AND DISCUSSION

Five different ovarian specimens before menopause were analyzed, and similar results were obtained. StAR proteins were limited to theca cells in the phase of preovulation. Preovulatory granulosa cells and ovarian stromal cells did not contain StAR proteins. StAR protein-stained portions were seen in the cytoplasm of cells (Fig. 1).

Figure 1

Figure 1: Detection of StAR protein expression in the human ovary by immunohistochemistry.

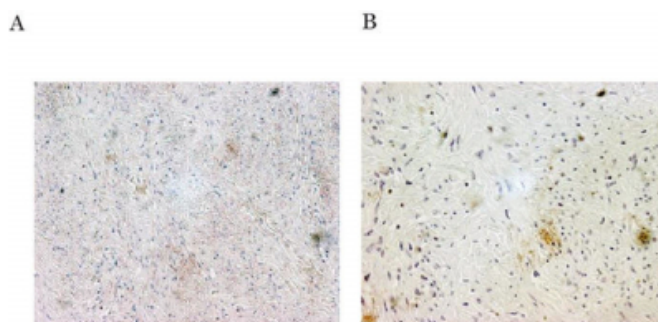


StAR protein expression was detected in theca cells in the preovulatory follicle. Magnification $\times 100$. (B) Magnification $\times 200$. (C) StAR protein staining is positive only in theca cells in the follicle at higher magnification ($\times 400$).

The theca cell tumor cells were epithelioid in appearance, and bundles of tumor cells were separated by fibrous tissues. StAR protein expression was detected in the cytoplasm of theca cell tumor cells (Fig. 2).

Figure 2

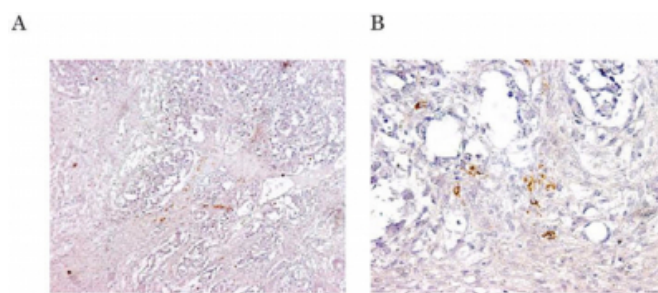
Figure 2: Detection of StAR protein in a theca cell tumor. (A) Cells are epithelioid in appearance, and bundles of connective tissues are distributed in the tumor. StAR protein staining with patches of weak intensity can be seen. Magnification $\times 200$. (B) StAR protein staining in the epithelioid cells is shown (arrow). Magnification $\times 400$.



Adenomatoid granulosa cell tumors gave no signal for StAR protein expression, and StAR protein was stained predominantly in thecomatoid stromal cells that had a large cytoplasm (Fig. 3).

Figure 3

Figure 3: Detection of StAR protein in a granulosa cell tumor.



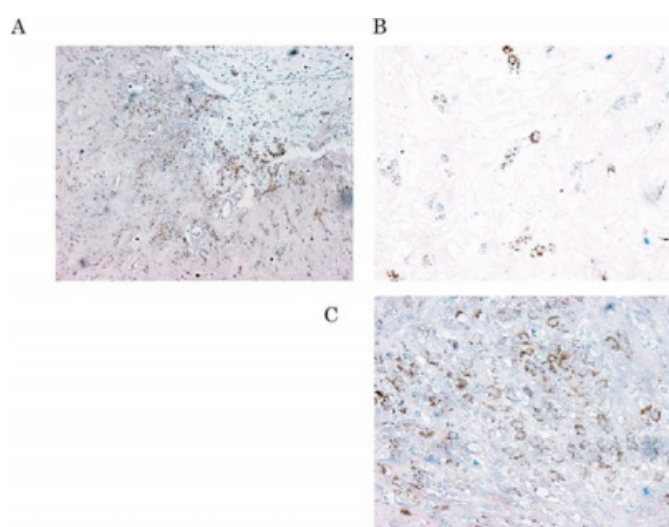
(A) StAR protein staining was not seen in the granulosa cells but was observed in connective tissues. Magnification $\times 100$. (B) StAR protein is present in theca cell-like cells in connective tissues. Magnification $\times 400$.

The sclerosing stromal tumor had two compartments, one in which many tumor cells were tightly packed and a stromal

portion, which was edematous. StAR protein immunoreactivity was found in both areas but was predominant in the area of tightly packed tumor cells. In both areas, the cytoplasm portions of cells were stained (Fig. 4).

Figure 4

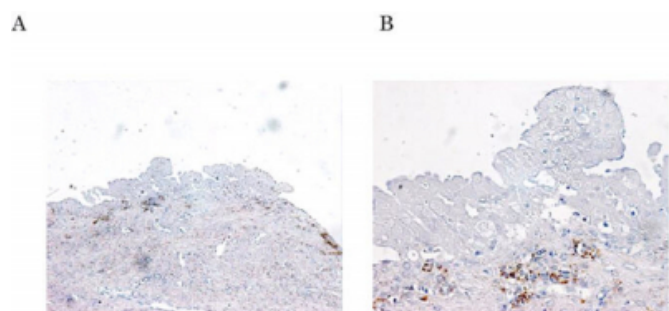
Figure 4: Detection of StAR protein in a sclerosing stromal tumor. (A) The tumor consists of two compartments: an edematous area and a solid cellular area. The solid cellular area is mainly stained with a StAR protein antibody. Magnification $\times 100$. (B) Large round tumor cells were stained in the cytosolic portions of the cells. Magnification $\times 400$. (C) There is a small number of round cells in the edematous area. StAR protein staining can be seen in the tumor cells. Magnification $\times 400$.



The origin of a sclerosing stromal cell tumor is thought to be stromal cells before the development of theca cells and granulosa cells (10, 11). In the mucinous cystadenocarcinoma, StAR protein expression was absent in tumor cells but was detected in stromal cells. StAR protein-stained cells were present beneath the cystadenocarcinoma cells (Fig. 5).

Figure 5

Figure 5: Detection of StAR protein in mucinous adenocarcinoma.



(A) StAR protein staining was seen in the stromal tissues.

Magnification $\times 100$. (B) StAR protein is present in the stromal cells beneath the carcinoma cells. Magnification $\times 400$.

Our immunohistochemical studies of the steroid hormone-producing tumors revealed that StAR protein expression is present in stromal cells. It is difficult to stain steroid hormones in tumor tissues because steroid hormones are unstable on paraffin block slides. There are sometimes discrepancies in results of immunostaining and clinical studies (12). Enzymes associated with synthesis of steroid hormones include P450scc, P45017 β and P450arom. StAR protein seems to be a critical protein for production of steroid hormones. Detection of StAR protein expression in tumor tissues is useful for speculation on the presence of steroid hormone-producing cells in ovarian tumors. In normal cells, StAR protein expression is limited to cells in the testis, ovary and adrenal gland, which produce steroid hormones (7). It is generally difficult to determine the origin of malignant cells, especially totipotent tumor cells. Immunostaining with a StAR protein antibody enables speculation on not only the production of steroid hormones but also the origin of tumor cells.

It has been reported that plasma levels of steroid hormones are elevated in patients with ovarian cancer and that cells derived from ovarian cancer produce steroid hormones (13,14,15). Steroid hormones produced by tumor cells bind to receptors and activate the transcription of specific genes. Estrogen and progesterone receptors are expressed in almost half of ovarian cancers (16). Because steroid hormones synthesized in local tissues are thought to be biologically active with an autocrine or paracrine action even if their amounts are small (17,18,19), steroidogenesis may have an effect on the survival of ovarian cancer patients. Further investigation is needed to elucidate the effect of steroidogenesis on prognosis of ovarian cancer.

CORRESPONDENCE TO

Teruo Sugawara, Department of Biochemistry, Hokkaido University School of Medicine Kita15, Nishi 7, Sapporo 060-8638, Japan Tel: 81-11-706-5047 FAX: 81-11-727-6006 Email: terusuga@med.hokudai.ac.jp

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Author Information

Teruo Sugawara

Department of Biochemistry, Hokkaido University Graduate School of Medicine

Nobuhiko Hoshi

Laboratory of Experimental Animal Science, Kitasato University School of Veterinary Medicine and Animal Sciences

Noriaki Sakuragi

Department of Obstetrics and Gynecology, Hokkaido University Graduate School of Medicine