

## Expression of 5 $\alpha$ -Reductases in Human Epithelial Ovarian Cancer: Its Correlation with Androgen Receptor Status

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Androgen metabolism and possible actions are considered to play some roles in human epithelial ovarian neoplasms, but the details have not been well studied. We have examined the expression of 5 $\alpha$ -reductase type 1 and type 2, which catalyze the conversion of testosterone to more active androgen, 5 $\alpha$ -dehydrotestosterone, and androgen receptor (AR), using immunohistochemistry (104 cases) and reverse transcription-polymerase chain reaction (RT-PCR) (16 cases) as a first step toward understanding the metabolism and possible actions of androgens in human common epithelial ovarian carcinoma. 5 $\alpha$ -Reductase type 1 was immunopositive in 75/104 cases (72.0%), and 5 $\alpha$ -reductase type 2 in 52/104 cases (50.0%) ( $P<0.001$ ). There was no significant correlation between patterns of immunolocalization and clinicopathological parameters examined. Median labeling index (LI) for AR was 17.8% (range 0–84.4%) which was significantly higher in serous carcinoma than other histological types ( $P<0.001$ ). There was a significant positive correlation between 5 $\alpha$ -reductase type 1 immunoreactivity and AR LI ( $P=0.0027$ ), but no significant correlation was detected in 5 $\alpha$ -reductase type 2. Results of RT-PCR analysis were also consistent with those of immunohistochemistry. The relatively wide distribution of 5 $\alpha$ -reductase type 1, and its correlation to AR status in human epithelial ovarian malignancies suggest that this isozyme plays important roles in androgen metabolism and actions in these tumors.

Key words: 5 $\alpha$ -Reductase — Androgen receptor — Ovarian cancer — RT-PCR — Immunohistochemistry

Epithelial ovarian carcinoma is the leading cause of death from gynecological malignancies in the majority of developed countries.<sup>1)</sup> Sex steroid hormones have been implicated in the etiology and/or progression of some epithelial ovarian cancers.<sup>2)</sup> Various epidemiologic findings suggest that not only estrogens, but also androgens play roles in ovarian cancer.<sup>3,4)</sup> Among these epidemiological investigations, in one case-control study, an increased risk of ovarian cancer was associated with elevated circulating levels of androgens.<sup>3)</sup> Polycystic ovary syndrome, a condition characterized by elevated circulating levels of androgens, has also been associated with an increased risk of ovarian cancer.<sup>4)</sup> Parts of these effects are considered to be due to increased intratumoral aromatization of circulating androgens and subsequent estrogenic actions that promote ovarian cancer cell growth *in vitro*.<sup>5)</sup> However, several *in vitro* studies demonstrated that androgen alone resulted in promotion of ovarian cancer cell growth and progression.<sup>6,7)</sup>

5 $\alpha$ -Reductase catalyzes the conversion of testosterone to a more bio-active androgen, 5 $\alpha$ -dehydrotestosterone (DHT).<sup>8)</sup> Therefore, this enzyme is considered an important regulator of the *in situ* actions of androgens in various

androgen-dependent tissues. Two isoforms of 5 $\alpha$ -reductase have been identified in mammals. The type 1 isozyme of 5 $\alpha$ -reductase has a neutral-basic pH optimum and is expressed primarily in the skin and liver, whereas the type 2 isozyme of 5 $\alpha$ -reductase has an acidic pH optimum and is distributed in the liver, prostate, seminal vesicle, and epididymis.<sup>8,9)</sup> The expression of 5 $\alpha$ -reductases has also been reported to be regulated by androgens in a number of tissues and species.<sup>8)</sup> However, the expression of 5 $\alpha$ -reductase isozymes has not been examined in ovarian carcinoma, and thus its biological significance remains unknown.

Androgen receptor (AR) is reported to be present in up to 90% of epithelial ovarian cancers.<sup>6,10,11)</sup> However, plasma levels of androgen have not been correlated with AR status. In addition, physiological concentrations of potent androgens have been reported to be markedly low in both normal pre- and postmenopausal women.<sup>12–14)</sup> Therefore, in this study, we studied the expression of 5 $\alpha$ -reductase type 1 and type 2, and AR, using immunohistochemistry (104 cases) and reverse transcription-polymerase chain reaction (RT-PCR) (16 cases) as a first step toward understanding the metabolism and possible actions of androgens in human common epithelial ovarian carcinoma. We also evaluated the correlation between 5 $\alpha$ -reductases and AR, in order to elucidate the possible bio-

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logical significance of intratumoral 5 $\alpha$ -reductase in ovarian malignancies.

## MATERIALS AND METHODS

**Ovarian cancer** We studied a total of 104 cases of common epithelial ovarian carcinoma. Clinicopathological features of the patients examined are summarized in Table I. Information regarding age, performance status on admission, histology, stage, residual tumor after primary surgery, and overall survival were retrieved by review of the patients' charts. Median follow-up time of the patients in this study was 54 months (18–112 months). Surgical specimens were all fixed in 10% formalin and embedded in paraffin. Among these 104 cases, frozen specimens were available for examination by RT-PCR analysis in 16 cases. These specimens were dissected immediately into small pieces following gross dissection, quickly transferred to liquid nitrogen, and then stored at  $-80^{\circ}\text{C}$  until further use. The research protocol was approved by the ethics committee of Tohoku University School of Medicine, Sendai.

**Antibodies** 5 $\alpha$ -Reductase type 1 and type 2 antibodies were rabbit polyclonal antibodies against a synthesized peptide corresponding to amino acids 232 to 256 of 5 $\alpha$ -reductase type 1, and amino acids 227 to 251 of 5 $\alpha$ -reductase type 2, respectively.<sup>9)</sup> These antibodies were generously provided by Dr. Russell, University of Texas Southwestern Medical Center, Dallas, TX. The specificity of these two antibodies was confirmed by western blotting,<sup>9)</sup> and utilization of the antibodies for immunohistochemistry has also been reported.<sup>15)</sup> Monoclonal antibodies for AR (AR441) were purchased from DAKO (Carpinteria, CA).

**Immunohistochemistry** Immunohistochemical analysis was performed using the streptavidin-biotin amplification method using a Histofine Kit (Nichirei, Tokyo), which has been previously described in detail.<sup>16)</sup> For immunostaining of AR, the slides were heated in an autoclave at  $120^{\circ}\text{C}$  for 5 min in citric acid buffer (2 mM citric acid and 9 mM trisodium citrate dehydrate, pH 6.0) after deparaffinization. The dilutions of primary antibodies used in our study were as follows: 5 $\alpha$ -reductase type 1, 1/1000; 5 $\alpha$ -reductase type 2, 1/1000; AR, 1/100. Tissue sections of liver and prostate were used as positive controls for 5 $\alpha$ -reductase type 1 and type 2, respectively.<sup>9)</sup> As a negative control, normal rabbit or mouse IgG was used instead of the primary antibodies, and no specific immunoreactivity was detected in these sections.

**Scoring of immunostaining** For statistical analyses of 5 $\alpha$ -reductase type 1 and type 2, the carcinomas were classified into two groups: +, carcinoma with positive immunoreactivity and –, carcinoma with no immunoreactivity, by two of the authors (J.A. and T.M.) independently. For evaluation of AR immunoreactivity, labeling index (LI)

Table I. Correlations between Clinical Characteristics and Androgen Receptor Labeling Index (AR LI)

	<i>n</i>	AR LI median (range)	Level of significance
Total	104	17.8 (0–84.4)	
Age			NS
<50	47	18.0 (0–58.0)	
$\geq 50$	57	17.6 (0–84.4)	
PS			NS
0–1	69	18.8 (0–84.4)	
2–4	35	20.0 (0–70.8)	
Histology			$P < 0.001$
Serous	45	37.4 (0–84.4)	
Mucinous	18	13.6 (0–73.2)	
Endometrioid	17	12.0 (0–35.2)	
Clear cell	24	8.2 (0–68.0)	
Stage			$P = 0.029$
I–II	50	14.8 (0–70.8)	
III–IV	54	27.2 (0–84.4)	
Residual tumor			$P = 0.045$
Optimal	72	16.6 (0–73.2)	
Suboptimal	28	27.2 (0–84.4)	

PS, performance status; NS, not significant.

was determined in carcinoma cells as described by Sasano *et al.*<sup>17)</sup> In each case, more than 500 carcinoma cells were counted independently by the same two authors, and the percentage of immunoreactivity (LI) was determined.

**RT-PCR** Total RNA was extracted by homogenizing tissue specimens in guanidinium thiocyanate followed by ultracentrifugation in cesium chloride, as described previously,<sup>18)</sup> and quantified spectrophotometrically at 260 nm. An RT-PCR kit (SUPERScript Preamplification system, Gibco-BRL, Grand Island, NY) was employed in the synthesis and amplification of cDNA. cDNAs were synthesized from 5  $\mu\text{g}$  of total RNA using random hexamer and reverse transcription was carried out for 50 min at  $42^{\circ}\text{C}$  with SUPERScript II reverse transcriptase. After an initial 1-min denaturation step at  $94^{\circ}\text{C}$ , 35 cycles of PCR were carried out on a DNA thermal cycler (PTC-200 DNA Engine, MJ Research, Inc., San Francisco, CA) under the following conditions: 1-min denaturation at  $94^{\circ}\text{C}$ , 1-min annealing at  $58^{\circ}\text{C}$ , and a 2-min extension at  $72^{\circ}\text{C}$ . Primers for PCR reactions were as follows: 5 $\alpha$ -reductase type 1,<sup>19)</sup> 5' sense-TGGGAGGAGGAAAGCCTATG and 3' antisense-GCCACACCACTCCATGATTTC (347–654, 308 bp); 5 $\alpha$ -reductase type 2,<sup>20)</sup> 5' sense-CATACGGTTTAGCTTGGGTGT and 3' antisense-GCTTCCGAGATTTGGGGTAG (456–770, 315 bp); AR,<sup>21)</sup> 5' sense-GTCAAAGCGAAATGGGCCCC and 3' antisense-CTTCTGGGT-TGTCTCCTCAGT (spanning exons 2–3, 420 bp);  $\beta$ -actin,<sup>22)</sup> 5' sense-GATTCCTATGTGGGCGACGAG and 3'

Table II. Correlation between 5 $\alpha$ -Reductase Type 1 Immunoreactivity and Clinicopathological Parameters in Ovarian Cancer

	5 $\alpha$ -Reductase type 1 immunoreactivity		P value
	+(n=75)	-(n=29)	
Age	53 (23-82)	46 (22-71)	NS
Histology			NS
Serous	33 (44.0%)	12 (41.4%)	
Mucinous	15 (20.0%)	3 (10.3%)	
Endometrioid	9 (12.0%)	8 (27.6%)	
Clear cell	18 (24.0%)	6 (20.7%)	
Stage			NS
I-II	34 (45.3%)	16 (55.2%)	
III-IV	41 (54.7%)	13 (44.8%)	
Residual tumor			NS
Optimal	49 (66.2%)	23 (76.7%)	
Suboptimal	23 (31.1%)	5 (16.7%)	

Data for age are presented as median (range) and others are presented as number of cases (percentage).

Table III. Correlation between 5 $\alpha$ -Reductase Type 2 Immunoreactivity and Clinicopathological Parameters in Ovarian Cancer

	5 $\alpha$ -Reductase type 2 immunoreactivity		P value
	+(n=52)	-(n=52)	
Age	51 (22-82)	49 (23-74)	NS
Histology			NS
Serous	19 (36.5%)	26 (50.0%)	
Mucinous	13 (25.0%)	5 (9.6%)	
Endometrioid	9 (17.3%)	8 (15.4%)	
Clear cell	11 (21.2%)	13 (25.0%)	
Stage			NS
I-II	28 (53.8%)	22 (42.3%)	
III-IV	24 (46.2%)	30 (57.7%)	
Residual tumor			NS
Optimal	38 (73.1%)	34 (65.4%)	
Suboptimal	12 (23.1%)	16 (30.8%)	

Data for age are presented as median (range) and others are presented as number of cases (percentage).

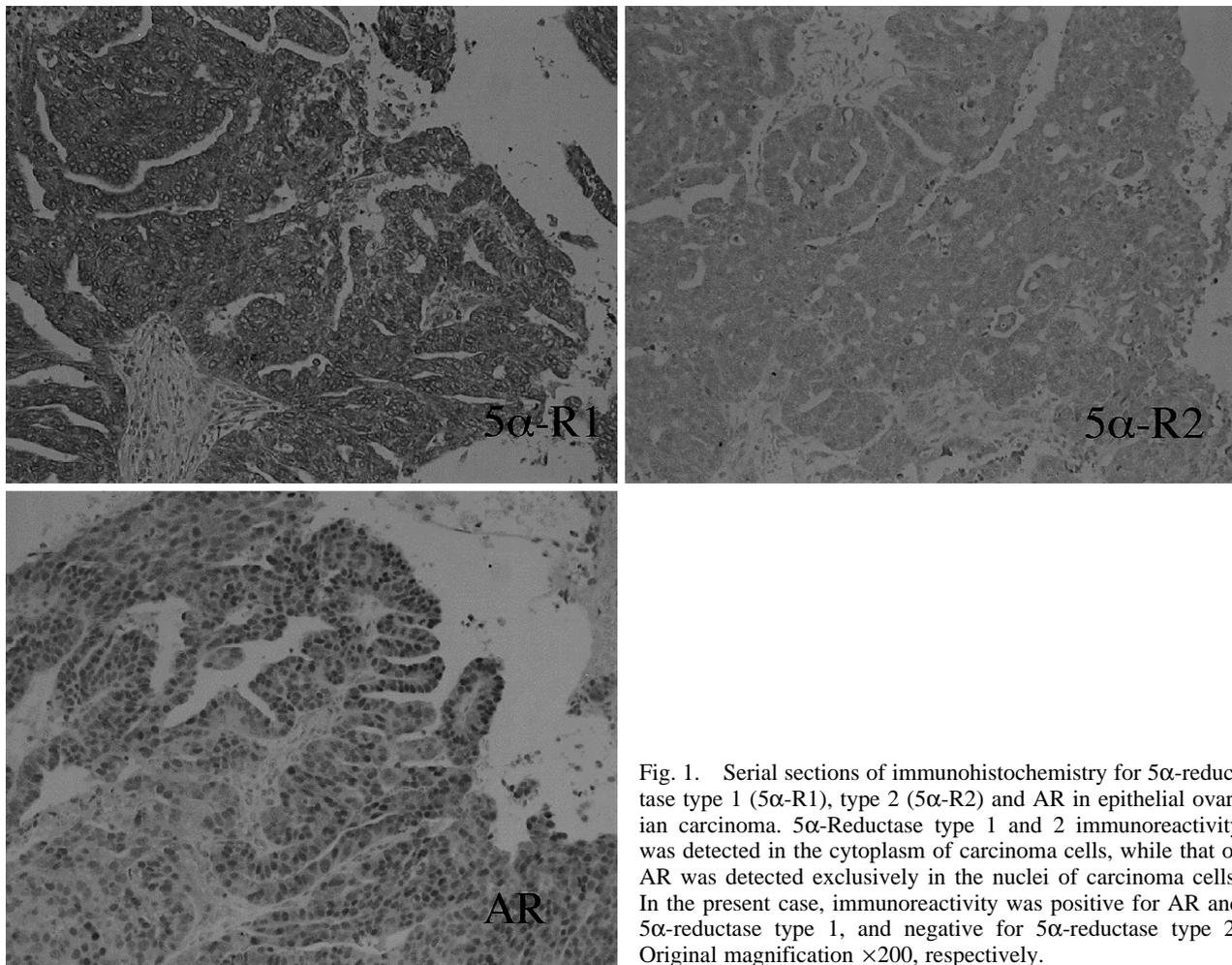


Fig. 1. Serial sections of immunohistochemistry for 5 $\alpha$ -reductase type 1 (5 $\alpha$ -R1), type 2 (5 $\alpha$ -R2) and AR in epithelial ovarian carcinoma. 5 $\alpha$ -Reductase type 1 and 2 immunoreactivity was detected in the cytoplasm of carcinoma cells, while that of AR was detected exclusively in the nuclei of carcinoma cells. In the present case, immunoreactivity was positive for AR and 5 $\alpha$ -reductase type 1, and negative for 5 $\alpha$ -reductase type 2. Original magnification  $\times$ 200, respectively.

antisense-CCATCTCTTGCTCGAAGTCC (192–723, 532 bp).  $\beta$ -Actin primers were utilized as positive controls. Negative controls without RNA and without reverse transcriptase were also performed.

**Statistical analysis** The statistical significance of association between AR status and characteristics of the patients was evaluated using the Mann-Whitney *U* test, Kruskal-Wallis, and Scheffe analysis. Associations between AR status and immunoreactivity of 5 $\alpha$ -reductases were evaluated using the Mann-Whitney *U* test. Associations between clinical parameters and immunoreactivity of 5 $\alpha$ -reductases were evaluated in a cross-table using the  $\chi^2$  test. Univariate analysis of prognostic significance for AR status was performed using a log-rank test, after each survival curve was obtained by the Kaplan-Meier method. Results were considered significant when the *P* value was less than 0.05.

## RESULTS

**5 $\alpha$ -Reductase** Results of immunohistochemistry for 5 $\alpha$ -reductase type 1 and type 2 and their correlation with clinicopathological parameters are summarized in Table II, Table III. Positive immunoreactivity for both 5 $\alpha$ -reductase type 1 and type 2 was detected in the cytoplasm of carcinoma cells (Fig. 1). No immunoreactivity was detected in stromal cells. The number of positive cases was 75 out of 104 cases examined (72.0%) for 5 $\alpha$ -reductase type 1, and 52 out of 104 cases examined (50.0%) for 5 $\alpha$ -reductase type 2 (the difference was significant,  $P < 0.001$ ). There was no significant association between immunoreactivity and clinicopathological parameters for both 5 $\alpha$ -reductase

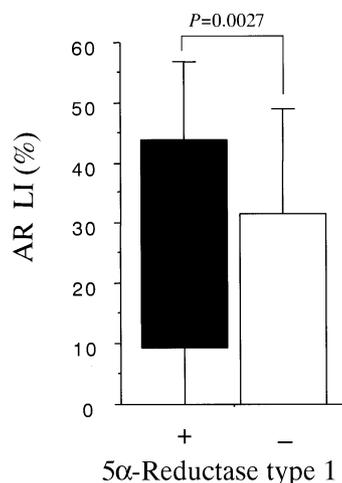


Fig. 2. Relationships between immunohistochemistry for 5 $\alpha$ -reductase type 1 and LI of AR. A significantly positive correlation was detected among them.

Table IV. Correlation between 5 $\alpha$ -Reductase Type 1 and Type 2 Immunoreactivity in Ovarian Cancer

	5 $\alpha$ -Reductase type 1 immunoreactivity		<i>P</i> value
	+	-	
5 $\alpha$ -Reductase type 2 immunoreactivity			
+	46 (61.3%)	6 (20.7%)	$P < 0.001$
-	29 (38.7%)	23 (79.3%)	

5 $\alpha$ -Reductase immunoreactivity is presented as number of cases (percentage).

subtypes. No significant correlation was detected between 5 $\alpha$ -reductase type 1 or type 2 immunoreactivity and overall survival.

**Androgen receptor** Results for AR immunohistochemistry are summarized in Table I. AR immunoreactivity was confined exclusively to the nuclei of tumor cells. No immunoreactivity was detected in stromal cells (Fig. 1). Median LI for AR was 17.8% (range 0–84.4%) and was significantly higher in carcinoma showing serous differentiation than other histological types ( $P < 0.001$ ). Median LIs for AR were also significantly higher in advanced stages (stage III or IV) than early stages (stage I or II) and suboptimal cytoreduced patients ( $P = 0.029$  and  $0.045$ , respectively). Univariate analysis of prognostic significance for AR revealed that there was no survival difference between AR-positive and negative groups. In this analysis, we determined the positive cases as those with an LI of more than 10%.

**Correlation between 5 $\alpha$ -reductase and AR** The relationships between immunoreactivity for 5 $\alpha$ -reductase type 1 and LI of AR are shown in Fig. 2. Median AR LI values of cases for positive and negative 5 $\alpha$ -reductase type 1 were 27.0 (range 0–84.4) and 15.4 (0–53.6), respectively, and the difference was significant ( $P = 0.0027$ ). Significant correlations were also detected between immunoreactivity for 5 $\alpha$ -reductase type 1 and type 2 ( $P < 0.001$ , Table IV). There was no significant correlation between 5 $\alpha$ -reductase type 2 and AR status.

**RT-PCR analysis** Results of RT-PCR analysis of 9 cases that were positive for AR immunoreactivity are demonstrated in Fig. 3. mRNA expression for 5 $\alpha$ -reductase type 1, type 2, and AR, was detected in 9 out of 9 (100%), 6 out of 9 (66.7%), and 9 out of 9 (100%), respectively. Results of RT-PCR analysis were consistent with those of immunohistochemistry (data not shown).

## DISCUSSION

In androgen-dependent tissues, DHT is one of the most potent androgens. Its actions are different from that of tes-

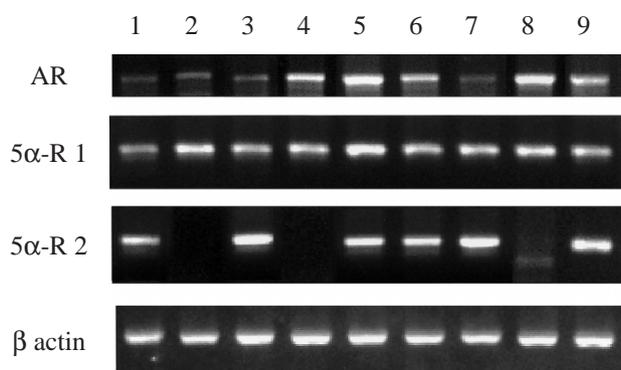


Fig. 3. RT-PCR analysis of total RNA extracted from epithelial ovarian carcinoma tissues. All of the cases shown here were immunohistochemically positive for AR. Bands of the correct size for AR (420 bp), 5 $\alpha$ -reductase type 1 (308 bp), 5 $\alpha$ -reductase type 2 (315 bp), and  $\beta$ -actin (450 bp) were detected in each histological subtype of ovarian cancer (1–4, serous; 5,6, mucinous; 7,8, endometrioid; 9, clear cell).  $\beta$ -Actin was used as a positive control. Note that all of the cases are positive for both AR mRNA and 5 $\alpha$ -reductase type 1 mRNA.

tosterone.<sup>8)</sup> 5 $\alpha$ -Reductase is well-known to convert testosterone to DHT. Intratumoral expression of this enzyme has also been reported to play very important roles in androgen actions in human prostate malignancies.<sup>19)</sup> Therefore, it is very important to study the correlation between 5 $\alpha$ -reductases and AR expression in androgen-dependent tissues, in order to clarify androgen actions. In our present study, 5 $\alpha$ -reductase type 1 immunoreactivity was widely detected in parenchymal cells of common epithelial ovarian carcinoma and was significantly correlated with AR. On the other hand, 5 $\alpha$ -reductase type 2 immunoreactivity was detected only in half of the cases examined, and there was no correlation with AR status in individual patients diagnosed with ovarian malignancies. These results suggest that 5 $\alpha$ -reductase type 1 may play a more important role than type 2 in the regulation of androgenic actions in human common epithelial ovarian carcinoma.

Tissue distribution surveys of 5 $\alpha$ -reductases have been extensively studied in many human tissues, including androgen target tissues such as the prostate, epididymis, and others.<sup>19, 23–25)</sup> In male reproductive organs, 5 $\alpha$ -reductase type 2 is predominantly expressed, compared to type 1 subtype. Males with congenital 5 $\alpha$ -reductase type 2 deficiency have a biochemical defect in the synthesis of DHT in the embryo, which in turn leads to a developmental defect in the formation of external genitalia and prostate.<sup>26, 27)</sup> On the other hand, in female mice, mutation of the 5 $\alpha$ -reductase type 1 gene affects reproduction by decreasing fertility and blocking parturition, whereas mutation of the type 2 gene does not affect development and/or reproductive functions.<sup>28, 29)</sup> Mahendroo and Russell

therefore support the notion that the type 1 gene is a female-specific 5 $\alpha$ -reductase isoenzyme, whereas the type 2 is a male-specific isoenzyme.<sup>30)</sup> In our study of epithelial ovarian malignancies, 5 $\alpha$ -reductase type 1 was more widely distributed than type 2. This finding is also consistent with the gender differences of distribution of these two subtypes of 5 $\alpha$ -reductase described above.

Increasing evidence is becoming available to support the hypothesis that steroid hormones play an important role in ovarian cancer. However, information on the prognostic importance of androgen metabolism and the response to endocrine treatment remains controversial. In general, progesterone receptor (PR) immunoreactivity is associated with better prognosis, while estrogen receptor (ER) and AR are not found in epithelial ovarian cancer.<sup>31–35)</sup> Results from our present study have demonstrated that AR was expressed much more frequently in ovarian carcinoma with serous differentiation. Quinn *et al.* also reported that serous tumors were more frequently ER-positive than other types of cancers.<sup>36)</sup> Therefore, serous differentiation of ovarian tumors may be more hormone-dependent than other types of differentiation, but this hypothesis awaits further investigation.

5 $\alpha$ -Reductase expression is regulated by androgen, especially DHT, in a number of tissues and species.<sup>8, 37)</sup> These findings indicated that the product of 5 $\alpha$ -reductases is involved in regulating the expression of the gene that encodes the enzyme, i.e., feed-forward regulation.<sup>8)</sup> This may explain the significantly positive correlation between AR and 5 $\alpha$ -reductase type 1 expression detected in our study. In breast carcinoma, Recchione *et al.* reported that DHT concentration was significantly higher in cancer tissue than in plasma, and suggested the possible local production of DHT.<sup>38)</sup> The simultaneous expression of AR and 5 $\alpha$ -reductase type 1 in ovarian carcinoma indicated that *in situ* accumulation of DHT also occurs in carcinoma tissues, as in breast carcinoma. Various *in vitro* studies demonstrated that DHT may promote ovarian cancer progression by decreasing the expression of transforming growth factor  $\beta$  (TGF- $\beta$ ) receptor.<sup>7)</sup> Slotman and Rao also reported that anti-androgens inhibit the growth of ovarian cancer cells.<sup>6)</sup> These results suggest that reduction of *in situ* production and/or accumulation of DHT can be a treatment option for ovarian cancer patients as an endocrine therapy. It is true that the clinical usefulness of sex steroid hormone manipulation, such as administration of agonists and/or antagonists, in the treatment of ovarian cancer has yet to be determined.<sup>39)</sup> However such an approach may lead to a better understanding of the role of steroid hormones and a rational basis for the selection of a more effective treatment in patients with common epithelial ovarian carcinoma.

In conclusion, the wide distribution of 5 $\alpha$ -reductase type 1 and its relation to hormone receptor status suggest

that this isozyme may play important roles in epithelial ovarian cancer in regulation of hormones, especially androgens, while the significance of the type 2 isozyme remains unclear. Further investigations are required to clarify the precise functions of 5 $\alpha$ -reductase isozymes in human ovarian epithelial carcinoma.

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#### REFERENCES

- 1) Nakashima, N., Nagasaka, T., Fukata, S., Oiwa, N., Nara, Y., Fukatsu, T. and Takeuchi, J. Study of ovarian tumors treated at Nagoya University Hospital, 1965–1988. *Gynecol. Oncol.*, **37**, 103–111 (1990).
- 2) Risch, H. A. Hormonal etiology of epithelial ovarian cancer, with a hypothesis concerning the role of androgens and progesterone. *J. Natl. Cancer Inst.*, **90**, 1774–1786 (1998).
- 3) Helzsofer, K. J., Alberg, A. J., Gordon, G. B., Longcope, C., Bush, T. L., Hoffman, S. C. and Comstock, G. W. Serum gonadotropins and steroid hormones and the development of ovarian cancer. *JAMA*, **274**, 1926–1930 (1995).
- 4) Schildkraut, J. M., Schwingl, P. J., Bastos, E., Evanoff, A. and Hughes, C. Epithelial ovarian cancer risk among women with polycystic ovary syndrome. *Obstet. Gynecol.*, **88**, 554–559 (1996).
- 5) Kaga, K., Sasano, H., Harada, N., Ozaki, M., Sato, S. and Yajima, A. Aromatase in human common epithelial ovarian neoplasms. *Am. J. Pathol.*, **149**, 45–51 (1996).
- 6) Slotman, B. J. and Rao, B. R. Response to inhibition of androgen action of human ovarian cancer cells *in vitro*. *Cancer Lett.*, **45**, 213–220 (1989).
- 7) Evangelou, A., Jindal, S. K., Brown, T. J. and Letarte, M. Down-regulation of transforming growth factor  $\beta$  receptors by androgen in ovarian cancer cells. *Cancer Res.*, **60**, 929–935 (2000).
- 8) Russell, D. W. and Wilson, J. D. Steroid 5 $\alpha$ -reductase: two genes/two enzymes. *Annu. Rev. Biochem.*, **63**, 25–61 (1994).
- 9) Thigpen, A. E., Silver, R. I., Guileyardo, J. M., Casey, M. L., McConnell, J. D. and Russell, D. W. Tissue distribution and ontogeny of steroid 5 alpha-reductase isozyme expression. *J. Clin. Invest.*, **92**, 903–910 (1993).
- 10) Hamilton, T. C., Davies, P. and Griffiths, K. Androgen and oestrogen binding in cytosols of human ovarian tumours. *J. Endocrinol.*, **90**, 421–431 (1981).
- 11) Chadha, S., Rao, B. R., Slotman, B. J., Van, Vroonhoven, C. J. and van der Kwast, H. An immunohistochemical evaluation of androgen and progesterone receptors in ovarian tumors. *Hum. Pathol.*, **24**, 90–95 (1993).
- 12) Cheng, Y. and Prusoff, W. H. Relationship between the inhibition constant (K<sub>1</sub>) and the concentration of inhibitor which causes 50 percent inhibition (I<sub>50</sub>) of an enzymatic reaction. *Biochem. Pharmacol.*, **22**, 3099–3108 (1973).
- 13) Abraham, G. E. Ovarian and adrenal contribution to peripheral androgens during the menstrual cycle. *J. Clin. Endocrinol. Metab.*, **39**, 340–346 (1974).
- 14) Vermeulen, A. and Verdonck, L. Factors affecting sex hormone levels in postmenopausal women. *J. Steroid Biochem.*, **11**, 899–904 (1979).
- 15) Silver, R. I., Wiley, E. L., Thigpen, A. E., Guileyardo, J. M., McConnell, J. D. and Russell, D. W. Cell type specific expression of steroid 5 alpha-reductase 2. *J. Urol.*, **152**, 438–442 (1994).
- 16) Suzuki, T., Sasano, H., Kimura, N., Tamura, M., Fukaya, T., Yajima, A. and Nagura, H. Immunohistochemical distribution of progesterone, androgen and oestrogen receptors in the human ovary during the menstrual cycle: relationship to expression of steroidogenic enzymes. *Hum. Reprod.*, **9**, 1589–1595 (1994).
- 17) Sasano, H., Frost, A. R., Saitoh, R., Harada, N., Poutanen, M., Vihko, R., Bulin, S. E., Silverberg, S. G. and Nagura, H. Aromatase and 17 $\beta$ -hydroxysteroid dehydrogenase type 1 in human breast carcinoma. *J. Clin. Endocrinol. Metab.*, **81**, 4042–4046 (1996).
- 18) Sambrook, J., Fritsch, E. F. and Maniatis, T. Extraction, purification and analysis of messenger RNA from eukaryotic cells. In “Molecular Cloning, A Laboratory Manual,” 2nd Ed., Vol 1, pp. 7.1–7.87 (1989). Cold Spring Harbor Laboratory Press, Plainview, NY.
- 19) Delos, S., Carsol, J. L., Fina, F., Raynaud, J. P. and Martin, P. M. 5 alpha-Reductase and 17 beta-hydroxysteroid dehydrogenase expression in epithelial cells from hyperplastic and malignant human prostate. *Int. J. Cancer*, **75**, 840–846 (1998).
- 20) Andersson, S., Berman, D. M., Jenkins, E. P. and Russell, D. W. Deletion of steroid 5 alpha-reductase 2 gene in male pseudohermaphroditism. *Nature*, **354**, 159–161 (1991).
- 21) Zhu, X., Daffada, A. A. I., Chan, C. M. W. and Dowsett,

- M. Identification of an exon 3 deletion splice variant androgen receptor mRNA in human breast cancer. *Int. J. Cancer*, **72**, 574–580 (1997).
- 22) Willey, J. C., Crawford, E. L. and Jackson, C. M. Expression measurement of many genes simultaneously by quantitative RT-PCR using standardized mixtures of competitive templates. *Am. J. Respir. Cell Mol. Biol.*, **19**, 6–17 (1998).
- 23) Bonkhoff, H., Stein, U., Aumuller, G. and Remberger, K. Differential expression of 5 alpha-reductase isoenzymes in the human prostate and prostatic carcinomas. *Prostate*, **29**, 261–267 (1996).
- 24) Bjelfman, C., Soderstrom, T. G., Brekkan, E., Norlen, B. J., Egevad, L., Unge, T., Andersson, S. and Rane, A. Differential gene expression of steroid 5 $\alpha$ -reductase 2 in core needle biopsies from malignant and benign prostatic tissue. *J. Clin. Endocrinol. Metab.*, **82**, 2210–2214 (1997).
- 25) Bruchofsky, N., Sadar, M. D., Akakura, K., Goldenberg, S. L., Matsuoka, K. and Rennie, P. S. Characterization of 5 $\alpha$ -reductase gene expression in stroma and epithelium of human prostate. *J. Steroid Biochem. Mol. Biol.*, **59**, 397–404 (1996).
- 26) Walsh, P. C., Madden, J. D., Harrod, M. J., Goldstein, J. L., MacDonald, P. C. and Wilson, J. D. Familial incomplete male pseudohermaphroditism, type 2. Decreased dihydrotestosterone formation in pseudovaginal perineoscrotal hypospadias. *N. Engl. J. Med.*, **291**, 944–949 (1974).
- 27) Imperato-McGinley, J., Guerrero, L., Gautier, T. and Peterson, R. E. Steroid 5 $\alpha$ -reductase deficiency in man: an inherited form of male pseudohermaphroditism. *Science*, **186**, 1213–1215 (1974).
- 28) Mahendroo, M. S., Cala, K. M. and Russell, D. W. 5 $\alpha$ -Reduced androgens play a key role in murine parturition. *Mol. Endocrinol.*, **10**, 380–392 (1996).
- 29) Mahendroo, M. S., Porter, A., Russell, D. W. and Word, R. A. The parturition defect in steroid 5 $\alpha$ -reductase type 1 knockout mice is due to impaired cervical ripening. *Mol. Endocrinol.*, **13**, 981–992 (1999).
- 30) Mahendroo, M. S. and Russell, D. W. Male and female isoenzymes of steroid 5 $\alpha$ -reductase. *Rev. Reprod.*, **4**, 179–183 (1999).
- 31) Rao, B. R. and Slotman, B. J. Action and counter-action of hormones in human ovarian cancer. *Anticancer Res.*, **9**, 1005–1008 (1989).
- 32) Langdon, S. P., Gabra, H., Bartlett, J. M. S., Rabiaz, G. J., Hawkins, R. A., Tesdale, A. L., Ritchie, A. A., Miller, W. R. and Smyth, J. F. Functionality of the progesterone receptor in ovarian cancer and its regulation by estrogen. *Clin. Cancer Res.*, **4**, 2245–2251 (1998).
- 33) Slotman, B. J., Kuhnel, R., Rao, B. R., Dijkhuizen, G. H., deGraaf, J. and Stolk, J. G. Importance of steroid receptors and aromatase activity in the prognosis of ovarian cancer: high tumor progesterone receptor levels correlate with longer survival. *Gynecol. Oncol.*, **33**, 76–78 (1989).
- 34) Akahira, J., Inoue, T., Suzuki, T., Ito, K., Konno, R., Sato, S., Yajima, A., Moriya, T. and Sasano, H. Progesterone receptor isoforms A and B in human epithelial ovarian carcinoma: immunohistochemical and RT-PCR studies. *Br. J. Cancer*, **83**, 1488–1494 (2000).
- 35) Kuhnel, R., De Graaff, J., Rao, B. R. and Stolk, J. G. Androgen receptor predominance in human ovarian carcinoma. *J. Steroid Biochem.*, **26**, 393–397 (1987).
- 36) Quinn, M. A., Pearce, P., Rome, R., Funder, J. W., Fortune, D. and Pepperell, R. J. Cytoplasmic steroid receptors in ovarian tumours. *Br. J. Obstet. Gynecol.*, **89**, 754–759 (1982).
- 37) Normington, K. and Russell, D. W. Tissue distribution and kinetic characteristics of rat steroid 5 alpha-reductase isozymes: evidence for distinct physiological functions. *J. Biol. Chem.*, **267**, 19548–19554 (1992).
- 38) Recchione, C., Venturelli, E., Manzari, A., Cavalleri, A., Martinetti, A. and Secreto, G. Testosterone, dihydrotestosterone and oestradiol levels in postmenopausal breast cancer tissues. *J. Steroid Biochem. Mol. Biol.*, **52**, 541–546 (1995).
- 39) Ahlgren, J. D., Ellison, N. M., Gottlieb, R. J., Laluna, F., Lokich, J. J., Sinclair, P. R., Ueno, W., Wampler, G. L., Yeung, K. Y., Alt, D. and Fryer, J. G. Hormonal palliation of chemoresistant ovarian cancer: three consecutive Phase II trials of the Mid-Atlantic Oncology Program. *J. Clin. Oncol.*, **11**, 1957–1968 (1993).