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Original contribution

The expression of sex steroid receptors and sex steroid–synthesizing/metabolizing enzymes in metastasized lymph nodes of prostate cancer☆

Yasuhiro Nakamura MD, PhD[a,b,*,1], Kazue Ise[a,b,1], Keely May McNamara PhD[b], Abdullah Azmahani PhD[c], Shun Sato MD, PhD[d,e], Fumiyoshi Fujishima MD, PhD[b], Kensuke Joh MD, PhD[b], Hiroyoshi Suzuki MD, PhD[f], Koji Mitsuzuka MD, PhD[g], Yoichi Arai MD, PhD[g,h], Hiroyuki Takahashi MD, PhD[d,e], Hironobu Sasano MD, PhD[b]

[a]*Division of Pathology, Faculty of Medicine, Tohoku Medical and Pharmaceutical University, Sendai 983-8536, Miyagi, Japan*
[b]*Department of Pathology, Tohoku University Graduate School of Medicine, Sendai 980-8575, Miyagi, Japan*
[c]*Faculty of Health Sciences, University Sultan Zainal Abidin, Kuala Nerus, Terengganu 21300, Malaysia*
[d]*Department of Pathology, The Jikei University School of Medicine, Tokyo 105-8461, Japan*
[e]*Department of Pathology, The Jikei University Hospital, Tokyo 105-8471, Japan*
[f]*Department of Pathology and Laboratory Medicine, Sendai Medical Center, Sendai 983-8520, Miyagi, Japan*
[g]*Department of Urology, Tohoku University Graduate School of Medicine, Sendai 980-8574, Miyagi, Japan*
[h]*Division of Urology, Miyagi Cancer Center, Natori, Miyagi 981-1293, Japan*

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Summary The expression statuses of sex steroid receptors and sex steroid–synthesizing/metabolizing enzymes have been reported in primary prostate cancer lesions, but that in metastatic lymph nodes has remained unknown. Therefore, in this study, we immunolocalized these proteins in primary tumors and paired metastatic lymph nodes of prostate cancer and correlated the findings with clinicopathological factors of individual patients. The expression statuses of AR and ER β was significantly increased in metastatic lymph nodes compared with primary lesions, whereas that of 17βHSD1, 17βHSD2, 17βHSD5, and STS immunoreactivity was decreased in metastatic lymph nodes. In metastatic lymph nodes, the status of 5α2 was significantly correlated with that of AR. In addition, 17βHSD5-, 5α1-, STS-, and EST-positive cases were significantly associated with Gleason score (GS) status (GS > 8 versus GS < 7) in metastatic lymph nodes. Results of our present study did demonstrate that *in situ* androgen and estrogen metabolism and

Abbreviations: AR, androgen receptor; ADT, androgen deprivation therapy; DHT, 5α-dihydrotestosterone; ERβ, estrogen receptor β; E1, estrone; E1S, estrone sulfate; E2, estradiol; H&E, hematoxylin-eosin; 17βHSD1, 17β-hydroxysteroid dehydrogenase type 1; 17βHSD2, 17β-hydroxysteroid dehydrogenase type 2; 17βHSD5, 17β-hydroxysteroid dehydrogenase type 5; 5α1, 5α-reductase type 1; 5α2, 5α-reductase type 2; STS, steroid sulfatase; EST, estrogen sulfotransferase; GS, Gleason score.

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* Corresponding author at: Division of Pathology, Faculuty of Medicine, Tohoku Medical and Pharmaceutical University, 1-15-1 Fukumuro, Miyagino-ku, Sendai, Miyagi 983-8536, Japan.

E-mail address: yasu-naka@tohoku-mpu.ac.jp (Y. Nakamura).

[1] These authors contributed equally to this work.

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action play roles in pathophysiology of prostate cancer in metastatic lymph nodes, but these steroidogenic effects could be different from those in primary lesions.

1. Introduction

Prostate cancer is known as the most common cancer in men, with blockade of androgen production and/or actions via various means (ADT) being one of the standard treatments [1]. However, the ability of the prostate tissue to produce its own androgen from circulating precursors suggests one means by which cancers may escape therapeutic effects. We previously reported that several androgen-producing enzymes 5α1, 5α2, and 17βHSD5 were expressed in prostate cancer cases, and the statuses of these enzymes were associated with more adverse clinical factors [2]. Based on these findings, it is postulated that *in situ* production of DHT from inactive androstenedione by 17βHSD5 (reduction of androstenedione to testosterone), and via 5α1 or 5α2 (metabolism of testosterone to DHT) in human prostate cancer could occur and drive prostate cancer growth [2] through its actions at the AR [3].

The importance of local estrogen production and metabolism in regulating the behavior of prostate carcinoma cells has also been previously reported. The androgen and estrogen pathway are both implicitly linked because androgens serve as the obligate precursor for the synthesis of estrogens. Therefore, an availability of localized estrogens is regulated by a number of enzymes summarized in Fig. 1. Aromatase converts circulating or localized androgens (androstenedione and testosterone) into estrogens (E1 and E2) and 17βHSD1 (the reduction of E1 to E2), whereas 17βHSD2 converts from E2 to E1, STS hydrolyzes E1S to E1, and EST sulfonates E1 to E1S [4-6]. We previously reported immunolocalization of EST and STS, as well as ERβ in prostate cancer tissues obtained from surgery [7]. Aromatase expression in carcinoma cells and cancer stromal cells in prostate cancer cases have been reported to be associated with increased time to biochemical failure and clinical failure [8], suggesting the importance of this pathway in developing therapeutic resistance.

Despite their characterization in primary lesion of prostate cancer, the statuses of these receptors and enzymes and their possible correlation with clinicopathological findings have not been examined in metastatic lymph nodes of prostate cancer cases. The status of those above in metastatic lymph nodes of prostate cancer cases could be important because the cases with therapeutic resistance often correspond to metastatic cases in which little is known about their potential for intracrine metabolism of steroids and thus sensitivity to drugs targeting this pathway. Therefore, in this study, we first immunolocalized AR, ERβ, 5α isozymes, 17βHSD isoforms, STS, EST, and aromatase in metastatic lymph nodes of prostate cancer tissue specimens. We then evaluated the possible roles of these AR/ERβ and sex steroid–synthesizing/metabolizing enzymes in metastatic lymph nodes of prostate cancer. In addition, we examined the status of these receptors and enzymes in distant metastatic lesions.

2. Materials and methods

Sixty-three pairs of specimens from both primary lesion and metastatic sites to lymph nodes in prostate cancer cases were obtained from patients who underwent surgical resections in Tohoku University Hospital (Sendai, Japan), The Jikei University Hospital (Tokyo, Japan), Sendai Medical Center (Sendai, Japan), and JCHO Sendai Hospital (Sendai, Japan), respectively. Three cases of lung metastases (resection) and 4 bone metastases

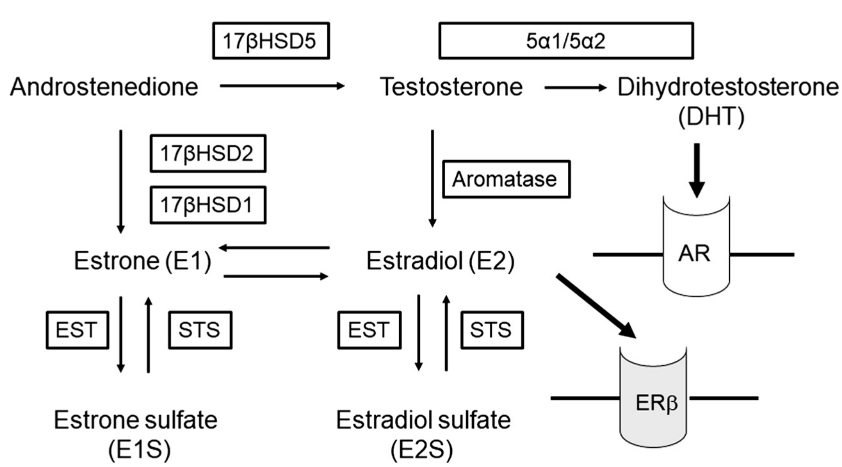

Fig. 1 The schema of local production and action of sex steroids in prostate cancer based on findings previously reported [2,7].

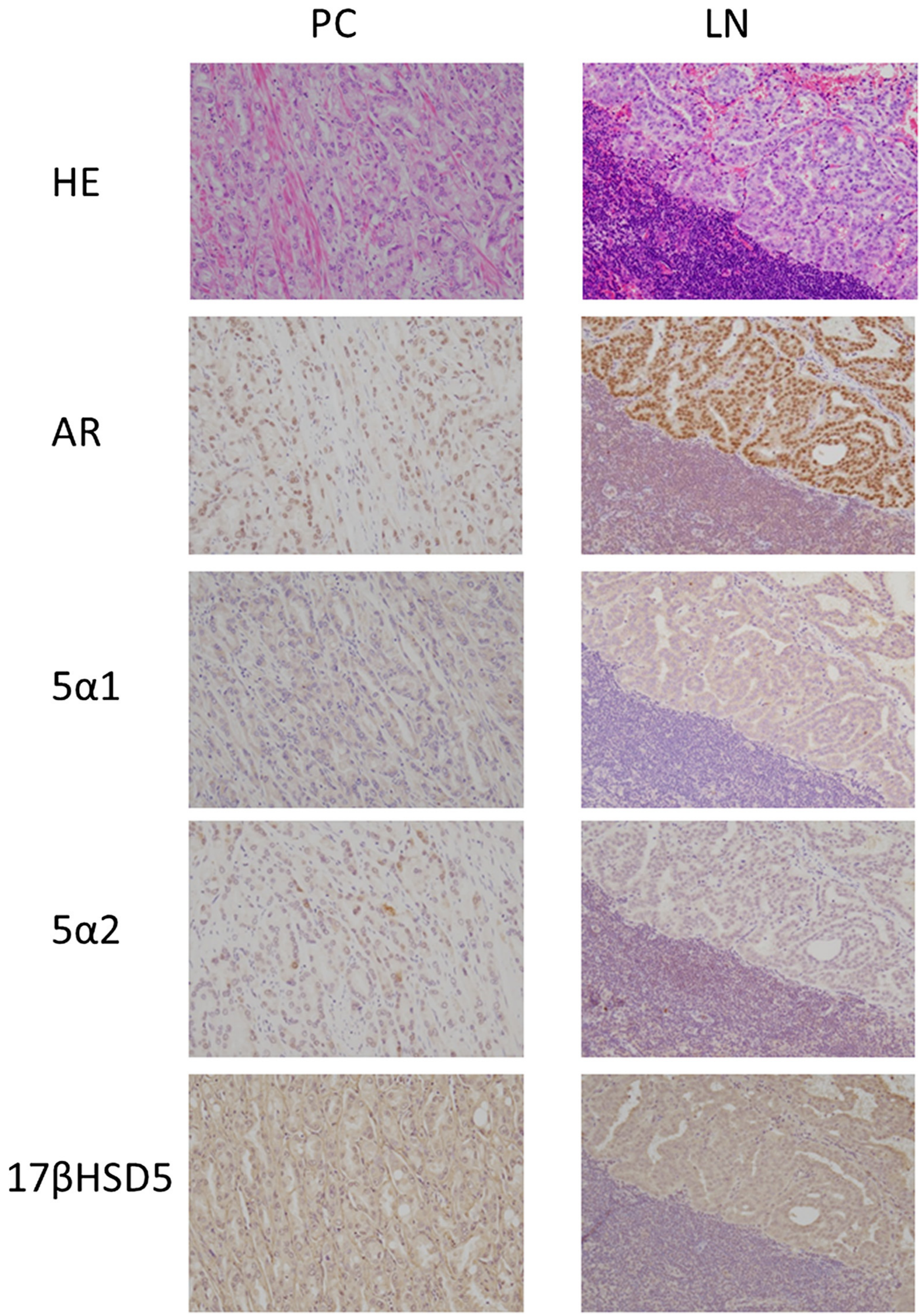

Fig. 2 Representative H&E and immunohistochemical figures (AR, 5α1, 5α2, and 17βHSD5) of primary lesion (PC) and metastatic lymph node (LN) in a prostate cancer case (original magnifications ×200).

(biopsy) were also obtained from The Jikei University Hospital and Tohoku University Hospital, respectively. These 3 cases of lung metastasis received hormone therapy as well as radiation or chemotherapy before surgery, but we microscopically confirmed distinct areas formed by viable cancer cells without degeneration or necrosis. None of other cases received radiation, chemotherapy, or hormone therapy before surgery or biopsy. The Ethics Committee at Tohoku University School of Medicine approved the research protocol for this study.

2.1. Immunohistochemistry

Tissue sections were used for H&E staining and immunohistochemistry.

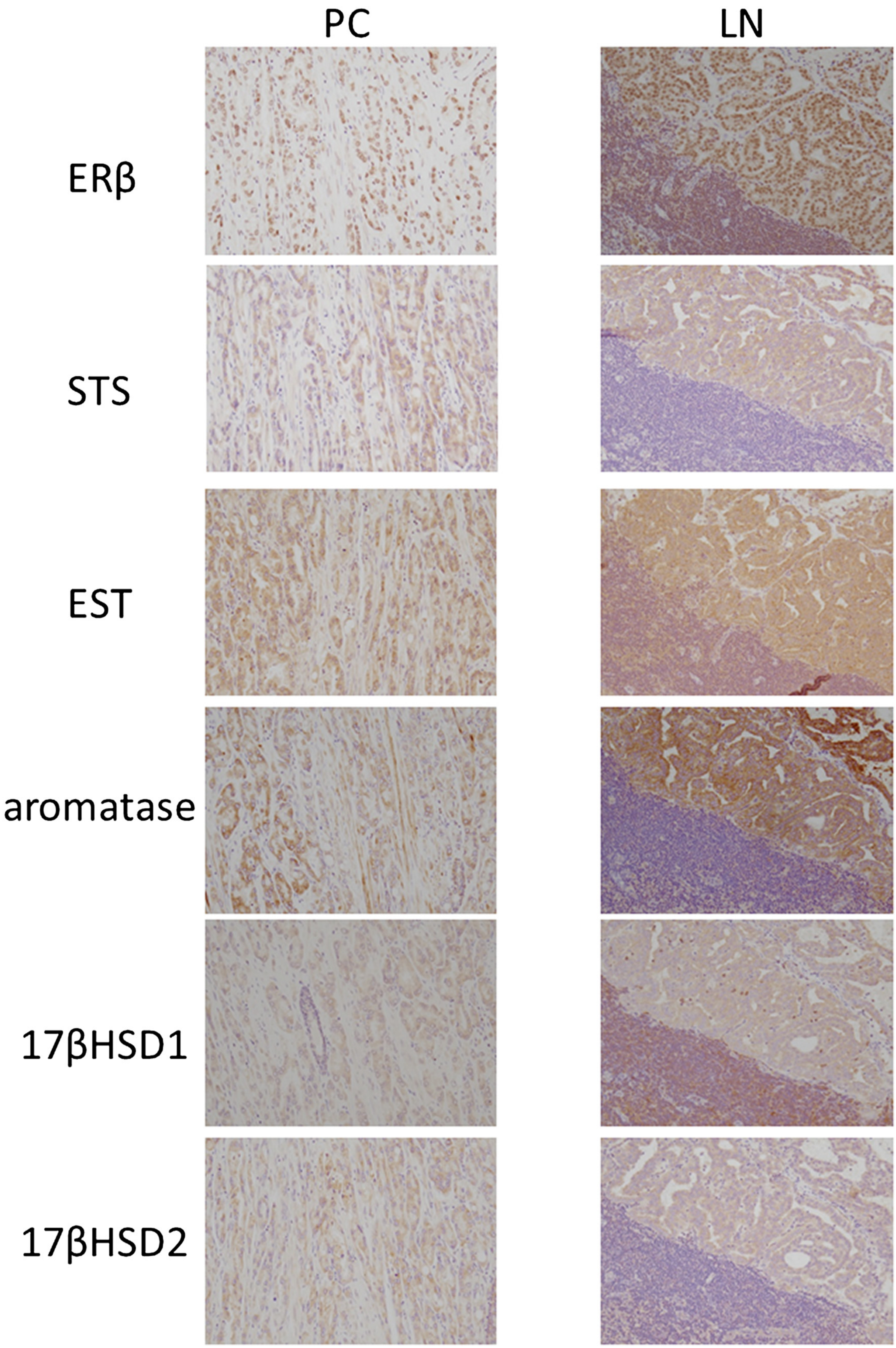

Fig. 3 Representative H&E and immunohistochemical figures (ERβ, STS, EST, aromatase, 17βHSD1, and 17βHSD2) of primary lesion (PC) and metastatic lymph node (LN) in a prostate cancer case (original magnifications ×200).

Table 1 Expression status of AR, ERβ, and sex steroid–synthesizing/metabolizing enzymes in PC and LN

	PC (n = 63)		LN (n = 63)		
	+	−	+	−	P
Labeling index (%)					
AR	75.9 ± 2.9		85.4 ± 2.3		.0106 *
ERβ	74.0 ± 3.1		82.3 ± 2.6		.0443 *
Expression					
17βHSD1	50	13	32	31	.0007 *
17βHSD2	40	23	18	45	<.0001 *
17βHSD5	51	12	39	24	.0171 *
5α1	20	43	17	46	.5572
5α2	20	43	11	52	.0612
STS	43	20	30	33	.0185 *
EST	56	7	50	13	.1409
Aromatase	45	18	40	23	.3413

NOTE. Statistical differences were evaluated by Student *t* test (steroid hormone receptors) or in a cross-table using the χ^2 test (synthetic/metabolizing enzymes).
Abbreviations: LN, lymph node metastasis part; PC, primary cancer part.
* $P < .05$ denotes a significant correlation.

Immunohistochemical analysis was performed using the labeled streptavidin-biotin method using Histfine SAB-PO Kit (Nichirei Biosciences, Tokyo, Japan). Detailed information on the used antibody was shown in Supplementary Table 1. The slides were deparaffinized and rehydrated for IHC. After antigen retrieval, blocking is carried out for 20 minutes at room temperature using normal serum in the kit (10% rabbit serum for mouse and goat antibody, 10% goat serum for rabbit antibody). Primary antibodies were incubated overnight at 4°C. Endogenous peroxidase inactivation treatment with methanol with hydrogen peroxide added at a concentration of 10% is performed for 10 minutes at room temperature. Secondary antibodies against each primary antibody in the kit were incubated for 30 minutes at room temperature; after washing, the avidin-labeling enzyme reagent was incubated for 30 minutes at room temperature. The antigen-antibody complex was visualized with 3,3′-diaminobenzidine solution (1 mmol/L 3,3′-diaminobenzidine, 50 mmol/L Tris-HCl buffer [pH 7.6], and 0.006% hydrogen peroxide) and then counterstained with hematoxylin.

2.2. Score of immunoreactivity

For nuclear staining, the labeling index (percentage of positive cells among 1000 tumor cells counted) was evaluated [9]. For cytoplasmic stains, those positive cells in the tumor cells with positive ratios of 10% or more were considered positive [2,7].

2.3. Statistical analysis

For the analysis of steroid hormone receptor expression with enzymes in primary cancer and LN metastasis part, Student *t* test was used. Statistical differences between immunoreactivity for synthetic/metabolizing enzymes were evaluated in a cross-table using the χ^2 test. $P < .05$ was considered significant.

3. Results

3.1. Immunohistochemical evaluation between primary lesion versus lymph node metastases

AR and ERβ immunoreactivity was both detected in the nuclei of cancer cells, whereas immunoreactivity of 17βHSD5, 5α1, 5α2, STS, EST, aromatase, 17βHSD1, and 17βHSD2 was detected in the cytoplasm of carcinoma cells in both primary lesion and metastatic lymph node (Figs. 2 and 3). The statuses of AR and ERβ expression were significantly higher in metastatic lymph node compared with primary lesion (Table 1). On the other hand, the relative expression statuses of 17βHSD1, 17βHSD2, 17βHSD5, and STS were significantly lower in metastatic lymph node compared with primary lesion (Table 1).

Table 2 Correlation between expression statuses of androgen-synthesizing/metabolizing enzymes and AR or estrogen-synthesizing/metabolizing and ERβ in lymph node metastasis part (mean ± SE)

	AR			ERβ		
	+	−	P	+	−	P
17βHSD5	89 ± 2.5	79.5 ± 4.3	.0645			
5α1	85 ± 5.3	85.5 ± 2.5	.9298			
5α2	94.4 ± 1.6	83.5 ± 2.7	.0010 *			
17βHSD1				80.3 ± 4.5	84.3 ± 2.7	.4485
17βHSD2				80.1 ± 5.3	83.2 ± 3.0	.6153
STS				78.3 ± 4.7	85.8 ± 2.6	.1642
EST				80.9 ± 3.2	87.5 ± 3.3	.1541
Aromatase				82.0 ± 3.6	82.7 ± 3.6	.8954

NOTE. Statistical differences were evaluated by Student *t* test.
* $P < .05$ denotes a significant correlation.

Table 3 Correlation between expression statuses of sex steroid–synthesizing/metabolizing enzymes in lymph node metastasis part of prostate cancer

	P						
	17βHSD2	17βHSD5	5α1	5α2	STS	EST	Aromatase
17βHSD1	.0056 *	.0964	.1767	.1035	.0033 *	.0029 *	.3780
17βHSD2		.6206	.4783	.0444 *	.0541	.2157	.1267
17βHSD5			.3826	.4070	.0006 *	<.0001 *	.2297
5α1				.1443	.6072	.2690	.1833
5α2					.0008 *	.2615	.1465
STS						.0415 *	.1196
EST							.8699
Aromatase							

NOTE. Statistical differences were evaluated in a cross-table using the χ^2 test.
* $P < .05$ denotes a significant correlation.

3.2. Correlation between the expression level of steroid hormone receptors and its corresponding synthesizing/metabolizing enzymes in lymph node metastases

To assess the impact of receptor-enzyme pathways, we compared the labeling index of the steroid receptors between positive and negative enzymes cases (ie, AR, 17βHSD5, 5α1 and 5α2, ERβ, 17βHSD1, 17βHSD2, STS, EST, and aromatase) in lymph node metastasis. Significantly lower levels of AR were detected in 5α2-negative cases, whereas there was no significant difference in 5α1 or 17βHSD5 (Table 2). For ERβ, no significant correlation was detected with statuses of any estrogen-synthesizing/metabolizing enzyme expression (Table 2).

3.3. Correlation between synthetic/metabolizing enzymes in lymph node metastases

To try and discover functional pathways or networks, we examined the association of expression of the enzymes in lymph node metastasis. Statistically significant positive correlations were detected between 17βHSD1 and 17βHSD2, 17βHSD1 and STS, 17βHSD1 and EST, 17βHSD2 and 5α2, 17βHSD5 and STS, 17βHSD5 and EST, 5α2 and STS, and STS and EST in metastatic lymph node lesion, respectively (Table 3).

3.4. Correlation between GS and expression status of sex steroid–synthesizing/metabolizing enzymes in lymph node metastases

In lymph node metastasis lesion, the GS was significantly lower when 17βHSD5, 5α1, STS, or EST were negative, respectively (Table 4).

3.5. Expression status of sex steroid–synthesizing/metabolizing enzymes and sex steroid receptors in distant metastatic lesions

AR and ERβ immunoreactivity was both detected in the nuclei of cancer cells, whereas immunoreactivity of

Table 4 Correlation between expression statuses of sex steroid–synthesizing/metabolizing enzymes and groups divided by GS status (≤7 or ≥8) in lymph node metastasis part of prostate cancer

GS	≤7	≤8	*P*
AR	83.7 ± 4.8	86.2 ± 2.5	.6404
ERβ	88.0 ± 2.7	79.4 ± 3.6	.0631
17βHSD1			
+	8	24	.1526
−	13	18	
17βHSD2			
+	5	13	.5503
−	16	29	
17βHSD5			
+	7	32	.0010 *
−	14	10	
5α1			
+	2	15	.0189 *
−	19	27	
5α2			
+	3	8	.6432
−	18	34	
STS			
+	6	24	.0301 *
−	15	18	
EST			
+	13	37	.0183 *
−	8	5	
Aromatase			
+	12	28	.4614
−	9	14	

NOTE. Statistical differences were evaluated by Student *t* test (steroid hormone receptors) or in a cross-table using the χ^2 test (synthetic/metabolizing enzymes).
* $P < .05$ denotes a significant correlation.

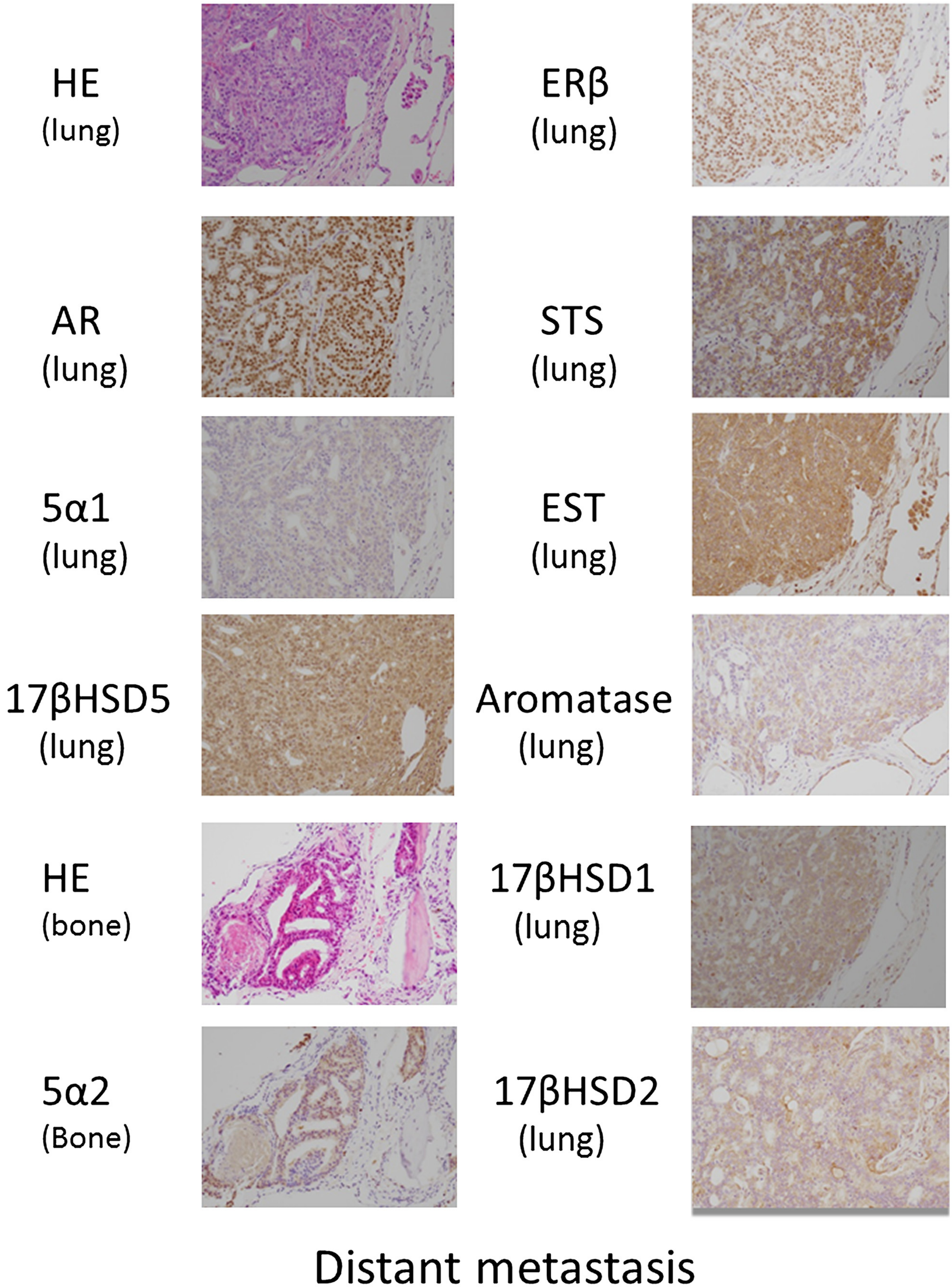

Fig. 4 Representative H&E and immunohistochemical figures (AR, 5α1, 5α2, 17βHSD5, ERβ, STS, EST, aromatase, 17βHSD1, and 17βHSD2) of distant metastatic lesions of prostate cancer (lung or bone; original magnifications ×200).

17βHSD5, 5α1, 5α2, STS, EST, aromatase, 17βHSD1, and 17βHSD2 was detected in the cytoplasm of carcinoma cells in distant metastatic lesion (Fig. 4). Except for 5α1 and 5α2, the statuses of sex steroid receptors and sex steroid–synthesizing/metabolizing enzymes were positive in all the cases examined in this study (Supplementary Table 2).

4. Discussion

Sex steroid hormones, including androgens and estrogens, are known to play important roles in various target tissue including normal human prostate, prostate cancer, and benign prostatic hyperplasia [2,7,10,11]. Therefore, in this study, we reported the presence of the AR and ERβ in primary prostate cancer, metastatic lymph nodes, and distant metastatic lesion with relevant enzymes (17βHSD1, 17βHSD2, 17βHSD5, 5α1, 5α2, STS, EST, and aromatase), indicating that *in situ* androgen and estrogen metabolism and action could also play pivotal roles in microenvironment and development of prostate cancer in metastatic lymph nodes and distant metastatic lesions as well as the primary lesions. Of particular importance, the statuses of these markers in metastatic samples can differ from those in primary lesions, suggesting that the most effective steroid-dependent therapies could also differ depending on clinical progression. Although the issue of steroid receptor discordance and its implication for therapy is often talked about for breast cancers, this is one of the first studies to raise the possibility that it may also be relevant to prostate cancer. However, further studies are required to clarify this aspect because only a few cases of distant metastasis were available in this study and some of them received hormone therapy as well as radiation or chemotherapy before surgery.

As mentioned in Introduction, one of the marked drivers of prostate cancer and thus one of its most important therapeutic targets is AR signaling [12]. Intratumoral production of DHT may play important roles in biological behavior of prostate carcinoma cells [12]. In our previous study, we reported a statistically significant positive correlation between AR and 5α2 expression levels in primary prostate cancer, but not between AR and 5α1 expression status [2]. In our present study, we demonstrated that AR was significantly higher in metastatic lymph node than in primary lesion, and a statistically significant positive correlation between AR and 5α2 expression levels in metastatic lymph node. However, 17βHSD5 expression was significantly lower in metastatic lymph node compared with primary lesions. Results of our present study regarding AR are in line with those in metastatic castration-resistant prostate cancer previously reported [13]. This suggests that the changes in AR expression may be an early step in metastatic progression. In addition, results of our present study suggest that the roles of 5α2 could be more important for local androgen production and action in prostate cancer cells. We also suggest that some aspect of metastasis results in decreased expression level of 17βHSD5 in prostate cancer cases, which seems to be consistent with previous findings [14,15]. However, further investigations including clarification in this mechanistic aspects and the analysis of detecting DNA amplification of AR are required to study the significance of the findings of our present study.

Less classically considered but no less important is the putative role of estrogen signaling in prostate cancers [16,17]. By extension, localized metabolism of estrogens is therefore also important in understanding prostate cancer progression. Results from our previous study revealed that STS, EST, aromatase, and 17βHSD1 proteins were detected in primary prostate cancer lesions [7]. EST immunoreactivity was significantly associated with clinical stage (TNM stage pT3 versus pT2), whereas the status of 17βHSD1 was significantly and inversely correlated with ERβ status in primary lesions [7]. Results of our present study also demonstrated that the relative expression level of ERβ was significantly higher in metastatic lymph node compared with primary lesion, whereas those of 17βHSD1, 17βHSD2, 17βHSD5, and STS were significantly lower in metastatic lymph node than in primary lesions. On the other hand, we also demonstrated that statistically significant positive correlations between the expression statuses of estrogen synthesizing/metabolizing enzymes: 17βHSD1 and 17βHSD2, 17βHSD1 and STS, 17βHSD1 and EST, and STS and EST in metastatic lymph node lesion. Therefore, these findings indicated that estrogen-synthesizing/metabolizing enzymes played pivotal roles in pathophysiology in metastasized lymph nodes of prostate cancer as well as in primary lesions, but the status and significance of *in situ* estrogen metabolism and action could be different from those in primary lesion. In addition, a previous study reported that expression of ERβ was significantly decreased in cases with lymph node metastasis [18]. Therefore, further studies are required to clarify the details.

Beyond the discreet actions of the androgen- and estrogen-synthesizing/metabolizing pathways, this study did not substantiate the cross-talk between these pathways and others. We have previously demonstrated an interconnectivity between enzymes expression in primary prostate cancers [2]; however, whether these relationships remain in metastatic cancers was less certain. The correlations found between the androgen and estrogen pathways with conjugation enzymes in metastatic lymph nodes suggest a complex intracrine environment that may prove interesting to probe in future studies. Of particular importance, in the metastatic lesions, we also demonstrated positive correlations between the GSs and the status of 17βHSD5, 5α1, STS, and EST. This also indicated that in contrast to primary tumors, steroid metabolism could play a larger role in determining the tumor biology (grade) in a metastatic setting. This in turns has implications for therapeutic choice in the metastatic setting.

In summary, this study demonstrated the importance of expression of various sex steroid–synthesizing/metabolizing enzymes in local production and action of in situ androgen and estrogen synthesis and metabolism involved in metastasized lymph nodes of prostate cancer.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humpath.2018.09.011.

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