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Original contribution

Androgen and androgen-metabolizing enzymes in metastasized lymph nodes of breast cancer☆

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Summary Androgen receptor and androgen metabolizing enzymes, 17β-hydroxysteroid dehydrogenase type 5 (17βHSD5) and 5α-reductase1 (5α1), are frequently detected in primary tumor of breast cancer, but their status in metastatic lymph nodes has not been examined. The biological role of androgen in breast cancer and its metastatic process also remain unknown. In this study, we used immunohistochemistry to localize the expression of androgen receptor, 17βHSD5, and 5α1 in primary tumors and paired metastatic lymph nodes and correlated the findings with clinicopathologic factors of individual patients. Approximately 70% of primary tumors and paired metastatic lymph nodes expressed androgen receptor, with significant correlation between both lesions. However, 17βHSD5 and 5α1 immunoreactivity was decreased in metastatic lymph nodes. Alone or in tandem with androgen receptor, 5α1 was associated with significantly lower Ki-67 index, lower pathologic grade, and higher estrogen receptor positivity, but androgen receptor/5α1 double positivity in lymph nodes was associated with larger lymph node metastasis and higher TNM stage. In conclusion, androgen receptor immunoreactivity remained stable during the process of metastasis, whereas androgen-metabolizing enzymes decreased. Although results of our study and previous reports imply additional roles of androgen metabolism in the metastasis process, especially conversion by 5α1, there may be divergence between its effects on primary tumor and those in metastatic lymph nodes.

1. Introduction

The androgen receptor (AR) is positive in 80% of breast cancer patients [1]. Androgen is generally recognized as a tumor suppressor, but its role has remained controversial [2]. This controversy is especially centered around estrogen

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receptor (ER)–negative cancers, including the triple-negative breast cancer subtype (ER−/progesterone receptor [PR]−/human epidermal growth factor receptor type2 [HER2]−) that is difficult to treat. Although the results of in vitro experiments have suggested androgens as a growth-promoting factor, studies of histologic samples indicate that the presence of AR is associated with better prognosis [3,4]. Resolving this issue is important as triple-negative breast cancer subtype represents approximately 25% of all breast cancers, and androgens are a potentially promising therapeutic target.

Although much of the current research has focused on the role of androgens in the primary tumor (PT), research into any potential role of androgens in metastasis has been unexplored. It is becoming apparent that steroid metabolism plays an important role in metastatic cancer as various reports have suggested biological roles for estrogenic pathways. Studies examining ER expression have reported decreased immunoreactivity in the metastatic lymph nodes, which may explain the development of resistance to endocrine therapy during tumor progression [5]. In contrast to this, we recently reported that aromatase expression in metastatic lymph nodes of ER-positive breast cancer is maintained [6], suggesting that cells retain their ability to synthesize estrogens following metastasis. In contrast, although the expression of AR in distant metastasis has been examined recently [7], the role of androgens in metastatic lymph nodes is unexplored.

The intracrine mechanisms of androgens in human breast cancer tissues can be summarized as follows: androstenedione is converted into testosterone by 17β-hydroxysteroid dehydrogenase type 5 (17βHSD5); subsequently, testosterone is metabolized into dihydrotestosterone (DHT) by the actions of 5α-reductase, type1 and type 2 (5α1 and 5α2). We have previously shown that 5α1 is the dominant isoform in breast cancer tissues between these 2 isoforms, 5α1 and 5α2 [8]. In an alternate pathway, testosterone is metabolized into estradiol by the aromatase enzyme. As estradiol promotes tumor growth, the role of androgen depends on the relative expression levels of these enzymes. Therefore, it is essential to examine the combined expression of 17βHSD5 and 5α1 in addition to AR, and to the best of our knowledge, this is the first study to do so in metastatic lymph nodes using immunohistochemistry.

2. Materials and methods

2.1. Patients

Surgical pathology specimens of breast cancer were obtained from 57 patients who underwent breast and lymph node excision between 2003 and 2011 at the Department of Surgery, Tohoku University Hospital (Sendai, Japan). The Ethics Committee at Tohoku University School of Medicine approved the research protocol for this study. Only the cases positive for lymph node metastasis but negative for clinical distant metastasis were included. Exclusion criteria based on study design were cases treated with neoadjuvant therapy and cases with small lymph node metastasis, in which multiple tissue sectioning was not possible technically.

The mean age of the patients was 57.5 years (range, 28-82). Clinical data were retrieved from the patients' charts, and the pathologic types and histologic grades of individual tumors (Table 1) were evaluated by 3 of the authors (Y. S., C. S., and Y. M.). All specimens were fixed with 10% formalin and embedded in paraffin. Of the 57 cases, 44 ER-positive cases were used in our previous article for immunohistochemistry [6], and clinicopathologic data of those cases were used in this study.

2.2. Antibodies

Methodologies for AR (AR441; DakoCytomation, Kyoto, Japan), 17βHSD5 (Sigma-Aldrich, St Louis, MO), and 5α1

Table 1 Patient characteristics

Patient characteristics	n	%
Age (y)		
Median	56	
Mean (range)	58	(28-82)
Age class		
≤40	5	8.8
41-50	11	19.3
51-60	21	36.8
61-70	8	14.0
≥71	12	21.1
pTNM stage [a]		
I	37	64.9
II	20	35.1
III	0	0.0
Primary tumor size (cm)		
≤2	21	36.8
2-5	33	57.9
>5	3	5.3
Tumor grade [b]		
1	12	21.1
2	22	38.6
3	23	40.4
Pathologic type		
Scirrhous	36	63.2
Papillotubular	7	12.3
Solid-tubular	12	21.1
Mucinous	1	1.8
Metaplastic	1	1.8
IMPCa	3	5.3
No. of metaLN		
1-3 (pN1)	38	66.7
4-9 (pN2)	15	26.3
≥10 (pN3)	4	7.0

Abbreviations: IMPCa, invasive micropapillary carcinoma; metaLN, metastatic lymph nodes.

[a] pTNM (pathological TNM) stage was determined according to UICC, Seventh Edition, 2009 [21].

[b] Tumor grade was evaluated according to the Nottingham Histological Grading Method [22].