**In Situ Androgen Production in Human Gastric Carcinoma – Androgen Synthesizing and Metabolizing Enzymes**

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**Abstract.** Background: It is well known that the incidence of gastric carcinoma is lower in females than in males. Therefore, androgens have been proposed to play an important role in modifying the development of gastric carcinoma. 5Alpha-reductase (5α-reductase) types 1 and 2 and 17β-hydroxysteroid dehydrogenase type 5 (17β-HSD type 5) are considered important local regulators of androgen production in human androgen-responsive tissues and cancer. Materials and Methods: The immunoreactivities of these steroidogenic enzymes, as well as of the androgen receptor (AR), were evaluated in human gastric carcinoma obtained from endoscopic mucosal resection (EMR) (n=117). Results: 17β-HSD type 5 immunoreactivity was detected in 99 cases (85%), 5α-reductase type 1 in 69 cases (59%), 5α-reductase type 2 in 57 cases (49%) and AR in 46 cases (39%). Conclusion: These androgen-producing enzymes are expressed in human gastric carcinomas and are involved in the in situ production and possible regulation of androgenic activity in human gastric carcinoma.

The incidence of gastric carcinoma, especially of the intestinal type, is well known to be lower in females than in males (1-4). In addition, several types of sex steroid hormone receptors have been reported to be expressed in gastric carcinoma (5-7), data which suggests that sex steroid hormones may be involved in the development of gastric carcinoma. An association between gastric cancer development and sex steroid hormones, especially estrogens, was previously reported (8-11). Kominea et al., however, recently demonstrated that androgen receptor (AR) expression was an independent unfavorable prognostic factor in patients with gastric carcinoma (12). In addition, the in situ androgen production and expression of the AR have also been considered important in the development of tumor, possibly as in the cases of prostate adenocarcinoma, a well-established androgen-dependent neoplasm (13). Therefore, an examination of the status of androgen metabolizing and/or producing enzymes in gastric carcinoma patients is of interest.

5Alpha-reductase (5α-reductase) types 1 and 2, and 17beta-hydroxysteroid dehydrogenase type 5 (17β-HSD type 5) are considered to play important roles as local regulators of androgens in several human tissues and neoplasms arising from these tissues as an intracrine mechanism (13). Testosterone, the major circulating androgen, is converted by 5α-reductases into the bioactive and potent androgen, 5α-dihydro-testosterone (DHT) (14). In addition, 17β-HSD type 5, which specifically catalyzes the reduction of androstenedione to testosterone, was recently isolated and cloned (15). The type 1 isozyme of 5α-reductase is located on the distal short arm of chromosome 5, and is mainly expressed in the liver and skin (14, 16). The type 2 isozyme of 5α-reductase is located in band q23 of chromosome 5, and is also markedly expressed in the prostate, seminal vesicle and epididymis in addition to the liver (16, 17). 17β-HSD type 5 is expressed in various peripheral human tissues (18). However, the status of expression for these enzymes and their possible relationship to clinicopathological findings have never been examined in human gastric carcinoma. Therefore, the immunolocalization of 5α-reductase isozymes and 17β-HSD type 5 in human gastric carcinoma tissue specimens obtained from endoscopic mucosal resection (EMR) were examined. These findings were then correlated with various clinicopathological parameters of the patients, including the AR status, in order to evaluate the possible roles of these androgen metabolizing/synthesizing enzymes as well as of androgen activity in human gastric carcinoma, especially regarding their possible involvement in the early stages of carcinoma development.

**Materials and Methods**

**Tissue collection and preparation.** Formalin (10%)-fixed and paraffin-embedded blocks of gastric carcinoma from 117 patients.
(87 males, 30 females; mean, 70.3 ± 0.8 years of age), who received EMR from 1999 to 2001 at the Department of Gastroenterology, Tohoku University Hospital, Japan, were retrieved for immunohistochemical studies. In addition, the histological types of gastric carcinoma (tub1; 96 cases, tub2; 21 cases) were based on the Japanese Classification of Gastric Carcinoma (13th edition), by the Japanese Gastric Cancer Association. This study protocol was approved by the Ethics Committee of Tohoku University School of Medicine (Sendai, Japan) (2000-147).

Immunohistochemistry. The immunohistochemical analysis was performed by employing the streptavidin-biotin amplification method using a Histofine Kit (Nichirei, Tokyo, Japan). The characteristics of the primary antibodies used in this study have previously been described in detail (13). The antigen-antibody complex was visualized with 3,3'-diaminobenzidine (DAB) solution [1 mM DAB, 50 mM Tris-HCl buffer (pH 7.6) and 0.006% H2O2], and was counterstained with hematoxylin. Human adrenal gland, prostate and liver were used as positive controls for immunostaining. For negative controls, normal goat IgG instead of the primary antibody was used and no specific immunoreactivity was detected in these tissue sections.

Evaluation of immunostaining intensity. The scoring of immunoreactivity was performed based on the results of our previous report (13). For statistical analysis of 5α-reductase type 1 and type 2 and 17β-HSD type 5 immunoreactivity, the carcinoma cases were classified into the following two groups: +, positive, more than 10% positive cells; and −, no immunoreactivity, less than 10% positive cells. The evaluation was performed by two of the authors (N.S. and T.S.), independently. Discordant results between the investigators were re-evaluated together using multi-headed light microscopy. The AR scoring in carcinoma cells was performed on high-power fields (×400) using standard light microscopy. In each case, more than 500 carcinoma cells were counted independently by the two authors above and the percentage of immunoreactivity, i.e., labelling index (LI), was determined. Cases with more than 10% LI were regarded as positive and cases with less than 10% LI as negative. Interobserver differences were less than 5%, and the mean of these two values was obtained.

Statistical analysis. Statistical differences between the two factors were evaluated in a cross-table using the χ2-test. P < 0.05 was considered significant.

Results

The immunoreactivity of 5α-reductase type 1 and type 2, and 17β-HSD type 5 was detected in the cytoplasm of carcinoma cells, whereas AR immunoreactivity was detected in the nuclei of carcinoma cells (Figure 1 A-D). 17β-HSD type 5 immunoreactivity was detected in 99 cases (85%), 5α-reductase type 1 in 69 cases (59%), 5α-reductase type 2 in 57 cases (49%) and AR in 46 cases (39%) (Table I). 17β-HSD type 5 immunoreactivity was significantly correlated with that of AR (p = 0.0325) (Table III). However, 5α-reductase type 1 immunoreactivity was significantly inversely correlated with that of AR (p = 0.0184) (Table I). These immunoreactivities, however, were not significantly correlated with other clinicopathological parameters, including histological types (between tub1 and tub2) (Table I). However, in adjacent non-neoplastic gastric epithelium, immunoreactivity for 17β-HSD type 5 and AR was detected in foveolar gland cells, but 5α-reductase type 1 and type 2 were not (Figure 1 E-H).

Discussion

In the present study, 17β-HSD type 5 immunoreactivity was detected in 85% of gastric carcinoma tissues. In addition, 17β-HSD type 5 immunoreactivity was significantly correlated with that of AR in these cases. Saito et al. previously demonstrated the immunoreactivity for testosterone in gastric cancer tissues (8). These findings all suggest that 17β-HSD type 5 is involved in the process of in situ production of testosterone, which exerts some effects on carcinoma cells in the human stomach. However, the immunoreactivities for 17β-HSD type 5 and AR were detected in the non-neoplastic adjacent gastric mucosa and, thus, these proteins may not be involved in the pathogenesis or development of stomach cancer.

The results of our study also demonstrated the immunoreactivities of 5α-reductase type 1 in 69 cases (59%) and 5α-reductase type 2 in 57 cases (49%). Both types of 5α-reductase have been reported to be expressed in several normal human tissues and cancer and were considered very important in the process of in situ production of DHT in these tissues (13). In addition, the immunoreactivities of both 5α-reductase type 1 and type 2 were negative in the non-neoplastic gastric epithelium. Therefore, both type 1 and type 2 5α-reductases are important in the local production and activity of DHT in human gastric carcinoma and may be involved in the pathogenesis or development of stomach cancer. The expression of 5α-reductase type 1 was more frequent than that of 5α-reductase type 2 and, thus, the different expression levels of the 5α-reductase subtypes in gastric cancer may be important with regard to androgenic activity through in situ androgen production, as seen in other normal tissues and/or prostate cancer (13).
Our results also demonstrated that 5α-reductase type 1 immunoreactivity had a significant inverse correlation with that of AR. Oduwole and colleagues reported negative correlations between 17β-HSD type 1, an estrogen-producing enzyme, and the expression and abundance of estrogen receptor in human breast carcinoma, suggesting the involvement of the enzyme in the regulation of estrogen receptor expression and its role as an independent prognostic factor in breast cancer (19). In addition, Kominea et al. demonstrated that AR expression was an independent factor of unfavorable prognosis in gastric cancer (12). Therefore, the accumulated DHT via 5α-reductases, especially type 1, possibly regulates the expression of AR in gastric carcinoma and modifies the characterization of gastric carcinoma cells. Kominea et al. also demonstrated that there were no differences in AR expression in tumors of male and female subjects and suggested that both androgen-dependent and -independent activation of AR may occur in gastric cancer (12). It has also been postulated that the androgen-independent activation of AR may occur through interactions with multiple cytokines and peptide growth factors, including insulin-like growth factor 1, interleukin 6 and cyclin E (12, 20, 21). Therefore, investigations are required to clarify the significance of 17β-HSD type 5 and the 5α-reductases regarding androgenic effects on gastric cancer.

In summary, it was demonstrated that 5α-reductase types 1 and 2 and 17β-HSD type 5 were all detected in a relatively large proportion of the cases of gastric cancer. These findings also indicated that these androgen metabolizing/synthesizing enzymes are involved in the local production of androgens, which may exert some biological effects on human gastric carcinoma.

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