mRNA expression of steroidogenic enzymes, steroid hormone receptors and their coregulators in gastric cancer

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Abstract. Epidemiological and experimental findings suggest that the development of gastric cancer (GC) is regulated by steroid hormones. In postmenopausal women and older men, the majority of steroid hormones are produced locally in peripheral tissue through the enzymatic conversion of steroid precursors. Therefore, using reverse transcription-quantitative polymerase chain reaction analysis, the mRNA expression of genes encoding steroidogenic enzymes, including steroid sulfatase (STS), hydroxy-delta-5-steroid dehydrogenase 3 beta- and steroid delta-isomerase 1 (HSD3B1), 17β-hydroxysteroid dehydrogenase type 7 and aromatase (CYP19A1), was investigated in primary tumoral and adjacent healthy gastric mucosa from 60 patients with GC. Furthermore, the mRNA levels for estrogen receptor α, estrogen receptor β (ESR2) and androgen receptor (AR), along with their coregulators, including proline, glutamate and leucine rich protein 1, CREB binding protein, nuclear receptor coactivator 1 (NCOA1), nuclear receptor corepresser 1 (NCOR1) and nuclear receptor subfamily 2 group F member 1 (NR2F1), were investigated. Additionally, the association between the mRNA expression of these genes and the clinicopathological features of patients with GC was examined. Significantly decreased levels of STS, HSD3B1, ESR2, AR, NCOA1 and NCOR1 mRNA, in addition to significantly increased levels of CYP19A1 mRNA were demonstrated in tumoral tissue samples compared with adjacent healthy gastric tissue samples. Deregulated expression of these genes in the analyzed tissue samples was associated with certain clinicopathological features of GC, such as age and localization of the tumor. The results of the current study suggest that all of the genes analyzed are expressed in tumoral and adjacent healthy gastric mucosa. In addition, the results indicate that abnormal expression of STS, ESR2, AR, NCOA1 and NCOR1 may serve a role in the development and progression of GC, and may be associated with specific clinicopathological features in patients with GC.

Introduction

The global incidence and mortality rates of gastric cancer (GC) are amongst the highest for all malignant tumor types (1). Risk factors that may increase an individual’s chance of developing GC include Helicobacter pylori infection, a diet high in salty/smoked food and low in fruit/vegetables, tobacco smoking and genetic susceptibility (2). Additionally, the incidence rate of GC is ~2 times higher in males compared with females, independently of known gender-specific variables (3). Therefore, it has been proposed that steroid hormone production influences the risk of developing GC (4,5). Furthermore, numerous studies have suggested a protective role of 17β-estradiol (E2) in gastric carcinogenesis (6-12). Although the majority of E2 is produced in the ovaries, it is also synthesized locally in peripheral tissues in males and females (13). There are two routes involved in the local synthesis of E2, the sulfatase and aromatase signaling pathways (13). The sulfatase signaling pathway involves the desulfation of dehydroepiandrosterone sulfate (DHEA-S) and estrone sulfate (E1-S) to DHEA and E1, respectively, by steroid sulfatase (STS). Subsequently, E1 is reduced to E2 by 17β-hydroxysteroid dehydrogenases (HSD17Bs; types 1, 5 and 7). In addition, DHEA is converted to androstenedione (adione) by hydroxy-delta-5-steroid dehydrogenase 3 beta- and steroid delta-isomerase 1 (HSD3B1). In the aromatase signaling pathway, adione and testosterone are converted into E1 and E2, respectively, by

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In the present study, the mRNA levels of certain genes that participate in the synthesis of E2 through the sulfatase and aromatase signaling pathways, including STS, HSD3B1, CYP19A1 and HSD17B7, were investigated in primary tumoral and healthy adjacent gastric mucosa samples from patients with GC. Furthermore, considering the fact that the cellular functions of steroid hormones are mediated through binding to their receptors and that the abnormal expression of genes encoding nuclear estrogen receptors α (ESR1) and β (ESR2), and androgen receptor (AR) have been demonstrated in GC (18,19), the mRNA expression of coactivators and corepressors of steroid hormone receptors were also determined in the tissue samples. The following coactivators and corepressors were investigated: Proline, glutamate and leucine rich protein 1 (PELP1); CREB binding protein (CREBBP); nuclear receptor coactivator 1 (NCOA1); nuclear receptor corepressor 1 (NCOR1); and nuclear receptor subfamily 2, group F, member 1 (NR2F1). Additionally, the association between the mRNA expression of the genes investigated and the clinicopathological features of patients with GC was investigated.

Materials and methods

Patients and tissue specimens. Primary tumoral gastric mucosa specimens were collected between December 2012 and September 2015 from 60 patients with a mean age of 67.2 years old who underwent a total gastrectomy at the First Department of Surgical Oncology and General Surgery at the Greater Poland Cancer Centre or the Department of General and Endocrine Surgery and Gastroenterological Oncology, Heliodor Święcicki Clinical Hospital at the Poznań University of Medical Sciences (Poznań, Poland). The clinicopathological characteristics of the patients are presented in Table I; however, for certain patients not all the information was available. In addition, healthy gastric mucosa tissue samples located ≥10 cm away from the tumoral lesions was obtained from each patient. Specimens were snap-frozen in liquid nitrogen and stored at -80˚C until required for RNA isolation. An experienced pathologist performed histopathological reaction in each sample was standardized by the geometric mean of B2M, β-glucuronidase and porphobilinogen deaminase, and presented as the decimal logarithm.

Statistical analysis. Normality of data distribution was assessed using the Shapiro-Wilk test, followed by Student's t-test (two-tailed) or Mann-Whitney U test to determine significant differences between mean values. P<0.01 was considered to indicate a statistically significant difference. STATISTICA software (version 10; StatSoft, Inc., Tulsa, OK, USA) was used to perform all statistical analyses.

Results

mRNA expression of STS, HSD3B1, CYP19A1, HSD17B7, ESR1, ESR2, AR, PELPI, CREBPP, NCOA1, NCOR1 and NR2F1 in primary tumoral and healthy gastric mucosa of patients with GC. RT-qPCR was used to measure the mRNA expression of genes encoding steroidogenic enzymes (STS, CYP19A1, HSD3B1, HSD17B7), steroid hormone receptors (ESR1, ESR2, AR) and coregulators of steroid hormone receptors (PELP1, CREBBP, NCOA1, NCOR1, NR2F1), in primary tumoral and adjacent healthy mucosa tissue samples from 60 patients with GC (Fig. 1). Expression of STS and HSD17B7 mRNA was detected in all tissue samples examined, whereas expression of HSD3B1 mRNA was absent in one healthy tissue sample and CYP19A1 was not detected in seven healthy specimens (Fig. 1A). Furthermore, five cancerous tissue samples demonstrated no expression of HSD3B1 mRNA. There was no difference in the mRNA level of HSD17B7 in primary tumoral and adjacent healthy mucosa (P=0.2; Fig. 1A). However,
Table I. Available clinicopathological characteristics of patients with gastric cancer.

| Clinicopathological characteristic | No. of patients |
|-----------------------------------|----------------|
| Gender (male/female)              | 36/24          |
| GC localization                   |                |
| Multisite                         | 31             |
| Cardia                            | 10             |
| Trunk                             | 6              |
| Fundus                            | 1              |
| Lesser curvature                  | 5              |
| Pylorus                           | 3              |
| Histological type                 |                |
| Diffuse                           | 19             |
| Intestinal                       | 23             |
| Undetermined                     | 12             |
| Tumor stage                       |                |
| T1                                | 2              |
| T2                                | 9              |
| T3                                | 32             |
| T4                                | 15             |
| Lymph node metastasis stage       |                |
| N0                                | 17             |
| N1                                | 8              |
| N2                                | 14             |
| N3                                | 19             |
| Metastasis stage                  |                |
| M0                                | 45             |
| M1                                | 3              |
| Histological grading              |                |
| G1                                | 1              |
| G2                                | 17             |
| G3                                | 38             |

T, tumor; N, node; M, metastasis, G, grade.

The majority of patients were diagnosed with an M0 metastasis stage, thus no associations between the mRNA levels of the analyzed genes and metastasis grade were investigated. Significantly lower levels of AR (P=0.0019), ESR1 (P=0.0012) and HSD17B7 mRNA, compared with adjacent healthy tissue. In addition, significant differences were observed in the expression of ESR1 mRNA between primary tumoral and adjacent healthy mucosa (P=0.0012). Amongst the steroid hormone receptors investigated, the expression levels of ESR2 and AR mRNA were the highest, with expression of ESR1 mRNA low in tumoral and adjacent healthy gastric tissue samples (data not shown). Amongst the coregulators of steroid hormone receptors examined, the expression of NCOA1 (P=0.00021) and NCOA1 (P=0.00017) mRNA was significantly reduced in tumoral mucosa compared with adjacent healthy mucosa (Fig. 1C). In addition, no significant differences were observed in the expression of NR2F1 (P=0.06), CREBBP (P=0.019) and ESR2 (P=0.11) mRNA between tumoral and healthy tissue samples (Fig. 1C).

Analysis of the clinicopathological characteristics of patients with GC and mRNA levels of STS, HSD3B1, CYP19, ESR2, AR, NCOA1 and NCOA1. Decreased expression of STS, HSD3B1, ESR2, AR, NCOA1 and NCOA1 mRNA, and increased expression of CYP19A1 mRNA in tumoral tissue samples compared with healthy controls, were associated with certain clinicopathological features of patients with GC (Table III). Expression of STS (P<0.00001), ESR2 (P=0.0001), AR (P=0.0001), NCOA1 (P=0.000028) and NCOA1 (P=0.000067) mRNA were significantly lower in tumoral tissue samples compared with the control in patients >60 years. Furthermore, males had significantly lower levels of AR (P=0.0018), NCOA1 (P=0.00051) and NCOA1 (P=0.000095) mRNA in tumoral tissue samples compared with control tissue samples, whereas in females the mRNA level of HSD3B1 was significantly lower in tumoral compared with control tissue samples (P=0.004).

The multisite localization of GC was demonstrated to be associated with a significantly lower level of HSD3B1 mRNA (P=0.0034) and tumors located in the cardia region had significantly lower STS (P=0.0012), AR (P=0.0012), NCOA1 (P=0.001) and NCOA1 (P=0.0073) mRNA, compared with healthy mucosa. However, only 10 patients with tumor localization in the cardia region were included in the analysis. Patients with the intestinal type of GC had significantly lower mRNA levels of AR (P=0.00046) and NCOA1 (P=0.0007) in tumoral tissue samples compared with the control mucosa. In addition, significantly lower levels of STS mRNA were identified in tumoral tissue compared with adjacent healthy tissue in patients with indeterminate GC (P=0.0032). Additionally, cancers tissue samples of a T3 grade expressed significantly lower levels of STS (P=0.0018) and HSD3B1 (P=0.0062) mRNA compared with healthy tissue samples. A significantly lower level of AR mRNA was also identified in tumoral tissue graded as T4 compared with the control (P=0.0086). Expression of STS mRNA was significantly decreased in the tumoral tissue samples of patients with N3 lymph node metastases compared with the controls samples (P=0.000089).
samples. However, the expression of CYP19A1 mRNA was significantly increased in tumoral tissue samples compared with the control in patients with multisite localization of GC (P=0.00001), a T3 tumor stage (P=0.00001), N0 (P=0.0024) or N3 (P=0.001) lymph node metastasis grades and G3 histological grade tumors (P<0.00001). The expression of CYP19A1 mRNA in primary tumoral tissue samples compared with healthy adjacent mucosa samples was significantly increased in all patients studied, regardless of age, gender, and histological type and localization of the tumor.

**Discussion**

In the present study, the mRNA levels of specific genes involved in the synthesis of E2, in addition to genes encoding steroid hormone receptors and their coregulators, were investigated in primary tumoral and adjacent healthy gastric mucosa samples obtained from patients with GC. The presence of mRNA was detected for all genes analyzed in the majority of gastric specimens examined. Furthermore, it was identified that the expression of STS, HSD3B1, ESR2, AR, NCOA1 and
NCOR1 mRNA was significantly decreased, whereas, the level of CYP19A1 mRNA was significantly increased, in cancerous gastric tissue compared with the control samples. To the best of our knowledge, no previous studies have investigated the expression of STS, HSD3B1 and NCOA1 mRNA in patients with GC, although their association with other tumor types has been examined.

Previous studies have identified lower levels of STS mRNA in primary colorectal adenocarcinoma compared with adjacent healthy colon mucosa (23). This is consistent with the results of the present study, which identified significantly decreased levels of STS mRNA in tumoral compared with healthy gastric tissue. As E1 and DHEA are typically found in their inactive sulfated form in the blood and have to undergo STS-mediated activation prior to entering the steroidogenesis pathway (24,25), downregulation of STS in a particular tissue may suppress the synthesis of estrogens and androgens (26). By contrast, higher levels of E2 have been identified in cancerous tissue compared with healthy tissue in patients with breast cancer (26,27). In addition, numerous studies have reported

Figure 1. Relative expression of STS, HSD3B1, CYP19A1, HSD17B7, ESR1, ESR2, AR, PELP1, CREBBP, NCOA1, NCOR1 and NR2F1 mRNA in healthy and primary tumoral gastric tissue samples. (A) Expression of genes encoding steroidogenic enzymes. (B) Expression levels of genes encoding steroid hormone receptors. (C) Expression of genes encoding coregulators of steroid hormone receptors. White dots represent healthy tissue and black dots represent primary tumoral tissue from patients with gastric cancer, which were analyzed through reverse transcription-quantitative polymerase chain reaction analysis and normalized to expression levels of β2-microglobulin, β-glucuronidase and porphobilinogen deaminase. The quantity of analyzed genes is presented as the decimal logarithm that represents the ratio between the amount of target gene in a sample and the target gene in the calibrator. STS, steroid sulfatase; HSD3B1, hydroxy-delta-5-steroid dehydrogenase 3 beta- and steroid delta-isomerase 1; CYP19A1, aromatase; HSD17B7, 17β-hydroxysteroid dehydrogenase type 7; ESR1, estrogen receptor α; ESR2, estrogen receptor β; AR, androgen receptor; PELP1, proline, glutamate and leucine rich protein 1; CREBBP, CREB binding protein; NCOA1, nuclear receptor coactivator 1; NCOR1, nuclear receptor corepressor 1; NR2F1, nuclear receptor subfamily 2, group F, member 1.
an increase in the activity and expression of STS in breast cancer (28-33). Therefore, it has been suggested that upregulation of STS may be associated with higher concentrations of intratumoral E2 (28,30,32).

Desulfated DHEA that is converted into adione, typically by HSD3B1, may serve as a substrate of E2 synthesis through the aromatase signaling pathway (25). However, low expression of HSD3B1 mRNA was observed in tumoral and healthy gastric tissue samples, with expression lower in the tumoral mucosa compared with the adjacent healthy tissue. Conversely to the results of the present study, increased expression of HSD3B1 mRNA has been identified in benign prostatic...

Table III. Association between the expression of STS, HSD3B1, CYP19, ESR2, AR, NCOA1 and NCOR1 mRNA in tumoral and healthy gastric tissue samples and the clinicopathological characteristics of patients with GC.

| Clinicopathological characteristic | Gene analyzed (P-value, GC vs. control tissue) |
|------------------------------------|-----------------------------------------------|
| All patients                        | ↓STS<sup>a</sup> | <0.00001 | 0.0051 | <0.00001 | 0.0097 | 0.00029 | 0.00021 | 0.00017 |
| Age (years old)                     |                                | 0.9 | 0.2 | 0.00074 | 0.89 | 0.35 | 0.27 | 0.22 |
| ≤60                                |                                | <0.00001 | 0.02 | 0.00012 | 0.0024 | 0.0001 | 0.00028 | 0.00067 |
| >60                                |                                | 0.0014 | 0.28 | 0.00072 | 0.08 | 0.018 | 0.00051 | 0.00095 |
| Gender                             |                                | 0.000069 | 0.004 | 0.0023 | 0.056 | 0.04 | 0.11 | 0.48 |
| Male                               |                                | 0.013 | 0.034 | 0.00001 | 0.04 | 0.026 | 0.12 | 0.11 |
| Female                             |                                | 0.0012 | 0.57 | 0.054 | 0.55 | 0.0012 | 0.001 | 0.0073 |
| Cardia                             |                                | 0.034 | 0.034 | 0.0018 | 0.73 | 0.69 | 0.36 | 0.038 |
| Body                               |                                | 0.037 | 0.022 | 0.0074 | 0.022 | 0.000046 | 0.0007 | 0.019 |
| Fundus                             |                                | 0.0032 | 0.14 | 0.0099 | 0.047 | 0.47 | 0.71 | 0.98 |
| Lesser curvature                   |                                | 0.013 | 0.034 | 0.00001 | 0.04 | 0.026 | 0.12 | 0.11 |
| Pylorus                            |                                | 0.0012 | 0.57 | 0.054 | 0.55 | 0.0012 | 0.001 | 0.0073 |
| Diffuse                            |                                | 0.034 | 0.034 | 0.0018 | 0.73 | 0.69 | 0.36 | 0.038 |
| Intestinal                         |                                | 0.037 | 0.022 | 0.0074 | 0.022 | 0.000046 | 0.0007 | 0.019 |
| Indeterminate                      |                                | 0.0032 | 0.14 | 0.0099 | 0.047 | 0.47 | 0.71 | 0.98 |
| T1                                 |                                | 0.57 | 0.96 | 0.11 | 0.79 | 0.19 | 0.093 | 0.077 |
| T2                                 |                                | 0.00018 | 0.0062 | 0.00001 | 0.022 | 0.027 | 0.016 | 0.038 |
| T3                                 |                                | 0.014 | 0.11 | 0.089 | 0.14 | 0.0086 | 0.046 | 0.074 |
| N0                                 |                                | 0.17 | 0.11 | 0.0024 | 0.29 | 0.16 | 0.039 | 0.027 |
| N1                                 |                                | 0.18 | 0.27 | 0.052 | 0.76 | 0.035 | 0.014 | 0.024 |
| N2                                 |                                | 0.019 | 0.49 | 0.041 | 0.22 | 0.38 | 0.48 | 0.53 |
| N3                                 |                                | 0.000089 | 0.06 | 0.001 | 0.045 | 0.048 | 0.062 | 0.21 |
| M0                                 |                                | 0.0021 | 0.003 | <0.00001 | 0.014 | 0.00064 | 0.0028 | 0.0018 |
| M1                                 |                                | - | - | - | - | - | - | - |
| G1                                 |                                | 0.07 | 0.07 | 0.036 | 0.24 | 0.0062 | 0.0027 | 0.042 |
| G2                                 |                                | 0.000054 | 0.0012 | <0.00001 | 0.027 | 0.044 | 0.021 | 0.0049 |

*Two-tailed Student’s t-test, bMann-Whitney U test. GC, gastric cancer; STS, steroid sulfatase; HSD3B1, hydroxy-delta-5-steroid dehydrogenase 3 beta- and steroid delta-isomerase 1; CYP19A1, aromatase; ESR2, estrogen receptor β; AR, androgen receptor; NCOA1, nuclear receptor coactivator 1; NCOR1, nuclear receptor corepressor 1; T, tumor; N, node; M, metastasis; G, grade; ↓/↑, down/up-regulation in gastric cancer in compare to healthy controls.
hyperplasia compared with healthy adjacent prostate tissue samples (34), in addition to castration-resistant metastases compared with primary prostate tumors (35). HSD3B1 activity is essential for the production of adione, which is subsequently used as a substrate for the synthesis of testosterone, an important hormone that regulates the proliferation of prostate cells (36). Furthermore, adione and testosterone can be directly converted to E1 and E2, respectively, by CYP19A1 (25). In the current study, expression of CYP19A1 mRNA was demonstrated in the majority of healthy gastric tissue samples and all tumoral gastric tissue samples. A previous study identified the presence of CYP19A1 in 23/30 GC tissue samples; however, all healthy gastric mucosa specimens tested negative for this enzyme (37). The presence of CYP19A1 mRNA and protein has been revealed in healthy and tumoral gastric tissue samples, although no significant difference in the level of CYP19A1 mRNA was demonstrated between healthy and tumoral gastric tissue samples (38). These findings oppose the results of the present study; however, this may have been due to the fact that the previous study analyzed a total of five cases (38).

Numerous animal studies have demonstrated that parietal cells are capable of converting circulating androgens into estrogens, whilst simultaneously expressing CYP19A1 (39-44). Furthermore, the synthesis of E2 through the aromatization of exogenous testosterone has been demonstrated in various GC cell lines (38). Considering these findings and the numerous evidence for the protective role of E2 against GC (6-12), the increased level of CYP19A1 mRNA in tumoral tissue samples compared with the control group observed in the present study is difficult to explain. However, it was observed that the expression of CYP19A1 mRNA in cancerous and healthy tissues was maintained at a low level, indicating that the role CYP19A1 serves in estrogen synthesis in gastric tissue may be limited. Additionally, it has been demonstrated that E2 may inhibit CYP19A1 and STS activity in breast cancer cells (45,46), suggesting that the increased mRNA expression of CYP19A1 in GC could be due to lower intracellular concentrations of E2.

Cellular responses to steroid hormones are facilitated by steroid hormones binding to their receptors, such as ESR1, ESR2 and AR (47). Studies that investigated the association between these receptors and GC have produced inconsistent results (18,19,48). ESR1 has been suggested to mediate the cancer-promoting effects of E2 in breast (49,50), colon (51), prostate (52) and gastric (53-57) cells, whereas binding of E2 to ESR2 could inhibit cell proliferation in tumors of these tissues (51,53,55,57-61). In the current study, no significant difference in the expression of ESR1 mRNA was identified between healthy and tumoral gastric tissue samples; however, the expression of ESR2 mRNA was significantly lower in cancerous mucosa compared with the control. Thus, the results of the present study support the hypothesis that reduced ESR2 expression is associated with the development of GC. Conversely, Matsuyama et al (62) suggested that the role served by ESR2 in GC may differ depending on the subtype. Furthermore, Guo et al (63) demonstrated that certain splicing variants of ESR2 mRNA (ESR2-1, -2 and -5) are differentially expressed in GC and healthy tissue samples. Additionally, higher levels of ESR2-5 mRNA were detected in GC compared with healthy tissue samples, and were associated with tumor-node-metastasis (TNM) staging, while decreased mRNA levels of ESR2-1 in GC did not correlate with any clinicopathological characteristics. In the current study, patients who were >60 years old had significantly lower levels of ESR2 mRNA in tumoral compared with healthy gastric tissue samples; however, all ESR2 splicing variants were analyzed simultaneously. A previous study suggested that ESR1 may exhibit antiproliferative activity, and reduce the motility and invasion of GC cells (64). Furthermore, ESR1 has been associated with an early TNM stage in GC (18). Thus, further studies investigating the role of ESRs in the etiology of GC are warranted.

In addition to investigating ESRs, the expression of AR mRNA was investigated in the current study. Previous studies have proposed that AR expression is an unfavorable factor in GC (19,65,66). In the present study, it was demonstrated that expression of AR mRNA was significantly decreased in GC tissue compared with controls. Similarly, decreased mRNA expression of AR in GC tissue samples has been reported by Gan et al (18). Low expression of AR and ESR1 mRNA was observed in cancerous and wild-type gastric mucosa, whereas the ESR2 mRNA was predominantly expressed in the both of these tissues. However, the expression of AR and ESR2 mRNA in GC tissue samples and matched controls was the highest amongst the analyzed steroid hormone receptors in the present study. The STS substrates E1-S and DHEA-S have been demonstrated to induce transactivation of ESRs and AR in a concentration-dependent manner in the MVLN invasive ductal carcinoma and CHO-K1 ovarian cell lines, respectively (67). The results of the current study suggest that DHEA-S is hydrolyzed by STS prior to AR activation, whereas E1-S may be active prior to STS-mediated hydrolysis. Thus, the decreased expression of AR mRNA in GC may have been due to reduced androgen synthesis caused by STS down-regulation. Notably, decreased expression of HSD3B1 mRNA, which is essential for androgen synthesis from DHEA, was detected in the present study.

The activity of nuclear steroid hormone receptors is regulated by various coactivators and corepressors (68). In the current study, NCOA1 and NCOA1 were demonstrated to be downregulated in GC tissue samples compared with healthy controls. Furthermore, mRNA and protein expression of NCOA1 has been identified to be downregulated in gastrointestinal stromal tumors, where it has been proposed to serve as a tumor suppressor through the SMAD signaling pathway (69). In contrast to these findings, another study identified increased mRNA expression of NCOA1 in malignant endometrial tissue samples compared with healthy tissue samples (70). Furthermore, high mRNA expression of NCOA1 has been associated with the improved prognosis of patients with breast cancer (71), whereas a loss of nuclear NCOA1 has been revealed to cause increased expression of cancer-associated genes and be significantly associated with the progression of invasive malignant melanoma (72). Upregulation of NCOA1 mRNA and protein expression, induced by progestins, has been associated with the suppression of estrogen-induced growth in T47D breast cancer cells (73).

In the present study, it was demonstrated that expression of NCOA1 mRNA was reduced in tumoral compared with healthy gastric tissue samples. Similarly, downregulation
of NCOA1 mRNA has been identified in cancerous bladder urothelium samples (74). Additionally, high mRNA levels of NCOA1 have been observed in healthy breast tissue samples, intermediate levels in tumoral tissue samples and low levels in breast cancer cell lines (75). However, numerous studies have identified an association between increased expression of NCOA1 and enhanced angiogenesis, cell proliferation and survival, disease recurrence, higher tumor grade and poor prognosis in breast cancer (76−81). Notably, upregulation of NCOA1 has been observed in patients with breast cancer treated with aromatase inhibitors (82). Thus, the decreased levels of NCOA1 mRNA in GC tissue identified in the present study may have been due to upregulation of CYP19A1 mRNA in tumoral gastric mucosa. Tai et al (83) demonstrated that overexpression of NCOA1 enhanced the E2-induced growth of MCF-7 breast cancer cells. Increased expression of NCOA1 has also been associated with the DHEA-mediated activation of AR in prostate cancer (84). Therefore, the decreased expression of AR and NCOA1 mRNA that were observed in cancerous gastric tissue samples in the present study may have been the result of limited desulfitation of DHEA-S due to the downregulation of STS.

In conclusion, the presence of STS, HSD3B1, CYP19A1, HSD17B7, ESR1, ESR2, AR, PELP1, CREBBP, NCOA1, NCOR1 and NR2F1 mRNA was demonstrated in the majority of tumoral and adjacent healthy gastric mucosa tissue samples. Furthermore, significantly decreased mRNA levels of STS and HSD3B1, in addition to significantly increased expression levels of CYP19A1 mRNA, in tumoral gastric tissue samples compared with matched controls were identified. However, compared with STS, the expression of HSD3B1 and CYP19A1 mRNA was low in the majority of the examined tissue samples, indicating that their role in gastric carcinogenesis is limited. Additionally, tumoral gastric tissue samples exhibited decreased levels of NCOA1 and NCOR1 mRNA compared with adjacent healthy controls, suggesting that deregulated expression of these coregulators may serve a role in gastric carcinogenesis. A limitation of the current study was the use of homogenized tissue samples, and thus the possibility that the samples included non-cancerous cells, such as fibroblasts, endothelial cells, smooth muscle cells or blood cells, could not be excluded. Furthermore, mRNA abundance does not always correlate with protein expression. Therefore, further studies on the expression levels of the genes involved in the steroidogenesis pathway and their role in gastric carcinogenesis are warranted.

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