Expression of hypoxia-inducible 2 (HIG2) protein in uterine cancer

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Summary

For both cervical cancer (UCC) and endometrial cancer (EMC) there are no effective prognostic markers. In this study, we evaluated HIG2 protein expression in 332 uterine cancers (186 UCCs and 146 EMCs) and examined the relationship between HIG2 protein expression and clinical factors, including prognosis. Totally, HIG2 expression was detected in 58% of UCC and 66% of EMC. However, there was no significant relationship between HIG2 expression and age, clinical stage and histology in either UCC or EMC. In addition, HIG2 protein expression was not related to prognosis of UCC or EMC. The positivity rate of HIG2 protein was 56% and 61% in early-stage UCC and EMC, respectively and 67% in non-squamous cell carcinoma of UCC. The positivity rate of HIG2 protein was high even in early-stage UCC and EMC

Key words: Endometrial cancer; Cervical cancer; Adenocarcinoma; HIG2.

Introduction

Carcinoma of the uterine cervix (UCC) is a common malignant neoplasm in Japanese women and its incidence in young women is increasing [1]. Endometrial cancer (EMC) is also increasing in Japan [1]. It is very important to find prognostic markers or effective serum tumor markers, however in both UCC and EMC there are no effective prognostic or serum tumor markers. In previous reports we performed gene expression profiles in epithelial ovarian cancer (EOC) using cDNA microarrays and suggested that the HIG2 gene might be a new biomarker for EOC [2]. Furthermore, we generated a polyclonal antibody for HIG2 protein and further validated the expression of HIG2 in EOC and clear cell carcinoma of the endometrium and concluded that HIG2 might be used as a marker for ovarian and endometrial clear cell adenocarcinoma or for prediction of chemotherapy response of clear cell carcinoma of the ovary [3]. In this study we evaluated HIG2 protein expression in uterine cancer (UTC) and examined the relationship between HIG2 protein expression and clinical factors, including prognosis.

Materials and Methods

Clinical samples

A total of 332 UTCs (186 UCCs and 146 EMCs) and 14 normal endometriums (proliferative phase: 7, secretary phase: 7) and seven normal cervical epitheliums were included in the study. The median age of UCCs was 52 years old (range: 22-92). Among the UCCs, FIGO Stage was: I: 99, II: 48, III: 21, IV: 18 and histology: squamous cell carcinoma: 150, adenocarcinoma: 23, and adenosquamous carcinoma:13. The median age of EMCs was 55 years old (range: 25-83). Among the EMCs, the histologic grade was as follows: FIGO stage: I: 93, II: 5, III: 41, IV: 7 and histologic grade: G1: 66, G2: 64, G3: 16. Of 146 patients, 115 patients had estrogen receptors. All patient-derived paraffin sections were collected and archived under protocols approved by the institutional review boards (IRBs) of the parent institutions based on the Declaration of Helsinki.

Immunohistochemistry

Establishment of a polyclonal anti-HIG2 antibody was reported previously [3].

Immunolocalization of the HIG2 protein was performed using a polyclonal anti-HIG2 antibody generated by injecting the purified full-length HIG2 fusion protein into rabbits. In brief, histological sections (4 μm) were affixed to glass slides, dewaxed, and rehydrated. The sections were then incubated in 3% hydrogen peroxide for 10 min at room temperature to quench endogenous peroxidase activity. The sections were reacted with the HIG2 antibody (× 5000) at 4ºC overnight. Peroxidase activity for all proteins was visualized by applying diaminobenzidine chromogen containing 0.05% hydrogen peroxide for 2-10 min at room temperature. The sections were then counterstained with hematoxylin. The slides were observed by two independent pathologists who were blinded to the clinical background of the patients. Judgement was performed based on the cytoplasmic staining [3]. HIG2 cytoplasmic staining was divided into positive or negative. Slides of EOC known to be either positive or negative for HIG2 expression were used as positive and negative controls.

Statistical analysis

The relationship between HIG2 expression and age, clinical stage and histologic grade were analyzed the using t-test and chi-square test. Overall survival (OS) distribution was calculated by the Kaplan-Meier method. A value of $p < 0.05$ was considered statistically significant.

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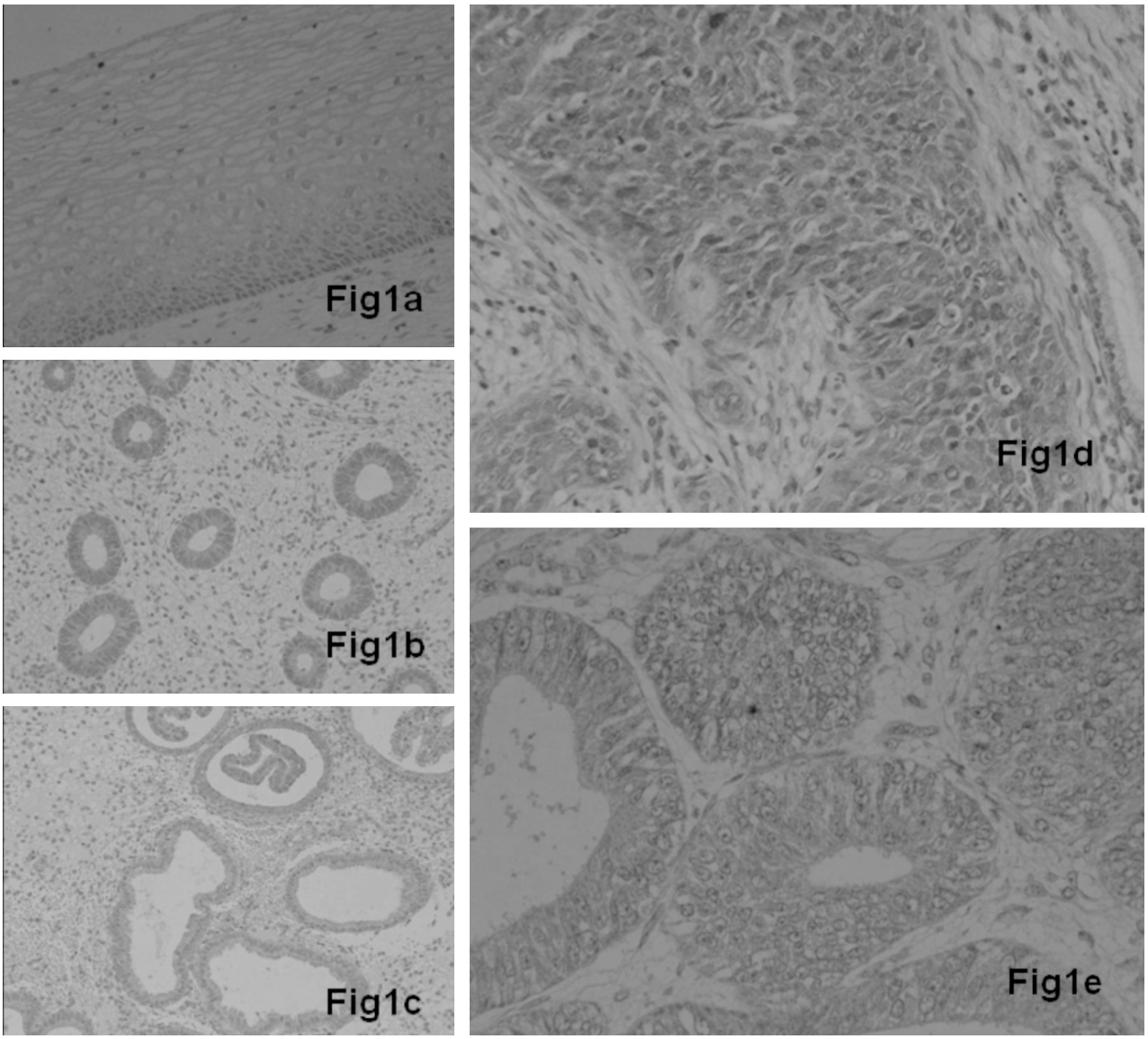

Figure 1. — Typical images of HIG2 immunohistochemical staining. (a) cervical intraepithelium, (b) proliferative phase endometrium, (c) secretory phase endometrium, (d) cervical cancer, (e) endometrial cancer. Weak nuclear staining and cytoplasmic staining were observed.

Results

Immunolocalization of HIG2 protein

Previously we reported that immunohistochemical analysis demonstrated strong cytoplasmic HIG2 staining as well as weak nuclear staining, and that the anti-HIG2 antibody is specific to the HIG2 protein, which is predominantly located in the cytoplasm of the cells. Typical images of HIG2 immunohistochemical staining are shown in Figure 1.

HIG2 expression in normal cervical intraepithelum, normal endometrium, UCC and EMC

HIG2 protein was weakly expressed in two of seven cervical intraepitheliums. In all cases, HIG2 protein expression was detected in 108/186 (58%) UCCs. There was no significant relationship between age, clinical stage, histologic type and HIG2 expression. HIG2 protein was weakly expressed in two of seven proliferative endometriums and four of seven secretory phase endometriums. In all cases, HIG2 protein expression was detected in 96 of 146 (66%) EMCs. There was no significant relationship between age, clinical stage, histologic grade, estrogen receptor status and HIG2 expression (Table 1). There was no significant difference in overall survival between HIG2 positive and negative cases in either UCC or EMC.

Table 1. — *HIG2 expression and clinical factors*.

Parameter	HIG2 expression
Cervical cancer	
Age	
< median	51% (47/93)
> median	66% (61/93)
FIGO stage	
I+II	56% (83/147)
III+IV	64% (25/39)
Histology	
squamous cell	56% (84/150)
non-squamous cell	67% (24/36)
Endometrial cancer	
Age	
< median	64% (47/73)
> median	67% (49/73)
FIGO stage	
I+II	61% (61/100)
III+IV	76% (35/46)
Histologic grade	
G1+G2	66% (85/129)
G3	65% (11/17)

Discussion

We previously reported that HIG2 might be used as a marker for ovarian and endometrial clear cell adenocarcinoma and for prediction of chemotherapy response of clear cell carcinoma of the ovary [3]. Togashi *et al*. reported that HIG2 plays an essential role in proliferation of renal CCC cells in an autocrine manner and HIG2 protein is highly expressed in renal clear cell carcinoma [4]. In this study we evaluated HIG2 protein expression in UCC and EMC and examined the relationship between HIG2 expression and clinical factors including prognosis. HIG2 protein expression was detected in cervical intraepithelium, (2/7) proliferative endometrium (2/7) and in secretary phase endometrium (4/7). Totally, HIG2 expression was detected in 58% of UCC and 66% of EMC. However, there was no significant relationship between HIG2 expression and age, clinical stage and histology in either UCC or EMC. In addition, HIG2 protein expression was not related with prognosis of UCC and EMC. However, the positivity rate of HIG2 protein was 56% and 61% in early stage of UCC and EMC and 67% in non-squamous cell carcinoma of UCC.

In both UCC and EMC, there are no effective tumor markers. For example, elevated serum levels of SCC are found in 57% and 65% of women with primary squamous cell carcinoma of the UCC [5, 6]. Especially the incidence of elevated serum levels of SCC is low in poorly differentiated squamous cell tumor or early stage tumor [7]. In addition, the incidence of elevated serum levels of CA125, CA19-9 and CEA are low in adenocarcinoma of the UCC [8]. A low incidence of patients with early-stage EMC also have elevated serum CA125 or CEA levels [9-11]. A previous immunohistochemical study reported that CEA was detected in 64% of adenocarcinoma of UCC [12] and, the positivity rate of CA125, CEA and CA19-9 was 65%, 58% and 60%, respectively, in EMC [13-15] and the positivity rate of CA125 was 88% in benign endometrium [16]. In our study, the positivity rate of HIG2 was similar with previous reports, however, positivity rates of HIG2 were slightly high in early-stage UCC and EMC, and low in benign cervical intraepithelium and endometrium, compared with previous reports [12-16]. HIG2 is a secretory molecule and an ELISA system using polyclonal antibody for HIG2 protein has been developed [4]. In the future, HIG2 may be used as a serum marker.

Conclusions

The positivity rate of HIG2 protein was high even in early stages of UCC and EMC. We are planning to measure serum HIG2 protein by using the new ELISA system by monoclonal antibody for HIG2 protein in gynecologic malignancies in future studies.

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