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A new marker, insulinoma-associated protein 1 (INSM1), for high-grade neuroendocrine carcinoma of the uterine cervix: Analysis of 37 cases

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HIGHLIGHTS

- INSM1 was considered to be a useful new neuroendocrine marker.
- INSM1 was closely related to the development of high grade neuroendocrine carcinoma of uterine cervix (HGNCUC).
- The ratio of high risk human papillomavirus infection in HHNCUC was 72%.

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ABSTRACT

Objective. High-grade neuroendocrine carcinoma of uterine cervix (HGNCUC) has been recognized as a highly malignant tumor. Therapeutic strategy specific to neuroendocrine (NE) tumors needs to be considered, but some cases wouldn't allow simple final diagnoses. Insulinoma-associated protein 1 (INSM1), which is a zinc-finger transcription factor related to NE differentiation, is frequently expressed in NE tumors. We investigated the association between INSM1 and HGNCUC, and the possibility of INSM1 as a useful NE marker.

Methods. Thirty-seven cases of formalin-fixed and paraffin-embedded HGNCUCs were evaluated immunohistochemically for conventional NE markers and INSM1. We also surveyed polymerase chain reactions and examined the frequency and the genotype of human papillomavirus (HPV) infections.

Results. In HGNCUC, chromogranin A, synaptophysin and neural cell adhesion molecule (NCAM) were expressed in 86%, 86% and 68%, respectively. In addition, INSM1 was detected in 95%. Positivity for INSM1 was clearly evaluated histologically, because the intensity of nuclear staining on positive cells was high and nonspecific reactions were minimal. In uni- and multivariate analyses of prognostic factors on stage I and II surgical cases, the association between INSM1 expression and prognosis was insignificant. We confirmed 72% of 29 examined cases had high risk HPV infections (type 16, 14%; type 18, 86%).

Conclusions. This study has clarified that INSM1 is closely related to the development of HGNCUC, and a useful new NE marker in conducting its correct and rapid diagnosis.

1. Introduction

High-grade neuroendocrine carcinoma of the uterine cervix (HGNCUC) is a rare disease making up 1–5% of cervical cancers. Compared with other histologic types of cervical cancer, HGNCUC gives rise to hematogenous metastases from an early stage and shows a poor prognosis [1–4]. The 5-year survival rate for the International Federation of Gynecology and Obstetrics (FIGO) stage IB1 is approximately 90% for ordinary cervical cancers, but has been estimated as only 55–63% for HGNCUC [2–5]. As the biological characteristics of HGNCUC differ from those of other histologic types of cervical cancer, the therapeutic strategy must also differ. Systemic chemotherapy even in the early stages has been suggested as necessary to achieve complete control of a local HGNCUC lesion [2,6]. Small cell lung cancer is a typical classical

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example of high-grade neuroendocrine carcinoma, representing a significant percentage (14–20%) of all lung cancers, and this tumor is typically thought to be the histologic type showing the poorest prognosis. Regarding the National Comprehensive Cancer Center Network (NCCN) Clinical Practice Guidelines in Oncology (NCCN Guidelines®), the primary treatment of small cell carcinoma (SmCC) and large cell neuroendocrine carcinoma (LCNEC) of sites other than the lung is generally recommended to follow the regimen for small cell lung cancer [7,8]. Given the above, both the diagnosis of HGNCUC, which tends to metastasize even in the early stage, and the selection of a different therapeutic strategy need to be made quickly and precisely. However, diagnosis may be difficult using only staining with hematoxylin and eosin. Immunohistochemistry using conventional neuroendocrine (NE) markers is often problematic because of their low sensitivity or low specificity in some cases.

Insulinoma-associated protein 1 (INSM1), a zinc-finger transcription factor related to NE differentiation, was recently reported to be frequently expressed in NE tumors [9–11]. INSM1 was identified by Goto et al. [9] in 1992 using a human insulinoma tissue subtraction library, and is strongly and temporarily expressed during the prenatal period, but exists only locally in normal tissues in adulthood. Fujino et al. [10] and Rosenbaum et al. [11] confirmed most recently by immunostaining that INSM1 was located in the nuclei of NE tumor cells in the lung [10] and gastrointestinal tract [11]. As INSM1 appears likely to represent a superior marker to the chromogranin A (ChrA), synaptophysin (Syn) and neural cell adhesion molecule (NCAM) that have conventionally been used, verification is needed through immunohistochemical studies of more cases.

Human papillomavirus (HPV) infection is a crucial event in the path to cervical cancer [12,13]. HPV may also be associated with the development of HGNCUC [14–16]. On the other hand, smoking has been closely implicated in the development of NE carcinoma of the lung [17,18]. The cause of NE carcinoma is thought to differ between organs. To know whether the same transcription factor works in NE carcinoma of different organs in which different causes may have developed them, it seems to be important to clarify expression frequencies of the transcription factor for every primary organ. Interestingly, transcription factors related to NE differentiation such as INSM1 may become the targets of future treatments [19,20].

In this study, we examined the following points: 1) whether differences exist in the expression of INSM1 among HGNCUC and other histologic types or organs; 2) whether INSM1 can be a useful NE marker in the histological diagnosis of HGNCUC; 3) whether immunohistochemical expression of INSM1 can be a prognostic factor; and 4) frequency and genotype of HPV infections of HGNCUC.

2. Material & methods

2.1. Case selection

This study was approved by the ethics committees at Shizuoka Cancer Center Hospital, Shizuoka, Japan, and Keio University Hospital, Tokyo, Japan. The present study covered patients diagnosed with FIGO stage IB1 to IVB cervical cancer with SmCC, LCNEC and NE carcinoma that could be classified as HGNCUC between 1985 and 2013 at Shizuoka Cancer Center and Keio University Hospital. Thirty-seven cases were retrieved, comprising 19 from Shizuoka Cancer Center (biopsy only, 9 cases; radical hysterectomy, 10 cases) and 18 from Keio University Hospital (all radical hysterectomy). All the specimens for histological analysis did not experience any preceding chemo- or radiotherapy. In prognostic analysis, radical hysterectomy cases comprising stage I and II cases, excluding cases treated with NAC, were evaluated.

Non-HGNCUC cases evaluated as FIGO stage IB1 to IIB with squamous cell carcinoma (SqCC)/adenocarcinoma (AD)/adenosquamous carcinoma (ADSq), which were all radical hysterectomy cases, were retrieved from the database of Shizuoka Cancer Center (SqCC, 11 cases; AD, 6 cases; ADSq, 3 cases). For comparison, cases diagnosed as lung cancer with surgical treatment were selected (small cell lung cancer, 5 cases and lung SqCC, 5 cases; all from Shizuoka Cancer Center). In prognostic analysis, clinical information was obtained from follow-up records.

2.2. Cell lines

N231 cell lines, derived from a small cell lung cancer, were purchased from the American Type Culture Collection (Rockville, MD). The LCN1 cell line, derived from a LCNEC of the lung, was established in Kitasato University [21]. The RERF cell line, derived from lung squamous cell carcinoma, was purchased from the RIKEN BioResource Center. A549 cell lines, derived from lung adenocarcinoma, were purchased from the Japanese Cancer Research Resources Bank (Tokyo, Japan). These cell lines were maintained and fixed in the Department of Applied Tumor Pathology at the Graduate School of Medical Sciences of Kitasato University [22].

2.3. Preparation of hematoxylin and eosin-stained sections and immunohistochemically stained sections

Three-micrometer-thick formalin-fixed paraffin embedded (FFPE) sections were deparaffinized in xylene and rehydrated in a descending ethanol series. For the histologic reevaluation of all cases, slides stained with hematoxylin and eosin (H&E) were used.

For immunohistochemistry, thin tissue sections were prepared in the same methods and simultaneously as those for H&E stain, and rehydrated.

The sources of the antibodies to five NE markers are shown in Table 1. In addition, lung cancer cell lines were used as NE (N231 and LCN1) and non-NE (RERF and A549) controls.

Antibody against INSM1 was newly purchased from Santa Cruz (Table 1), and the optimal dilution of the antibody was ×250.

Sections were treated with 3% hydrogen peroxide for 10 min. Antigens were retrieved by autoclaving in 0.01-M citrate buffer (pH 6.0) with a final concentration of 0.1% Tween 20 at 121 °C for 10 min. After blocking with 2% normal swine serum for 10 min, sections were reacted with diluted primary antibodies overnight at room temperature. After rinsing in TBS (0.01 M Tris-HCl, pH 7.5, 150 mM NaCl) 3 times for 5 min each, sections were reacted with ChemMate EnVision (Dako) for 30 min at room temperature. Finally, sections were visualized using a Stable DAB solution (Invitrogen, Carlsbad, CA) and then counterstained with Mayer's hematoxylin (Fig. 2).

ChrA, Syn and NCAM have been traditionally and routinely used as NE markers at our institution (Table 1, Fig. 1-E–G).

Immunohistochemical staining was performed at the same time for each antibody.

2.4. Histologic analysis

As the 2014 WHO histopathologic diagnostic criteria for NE tumors of the uterine cervix are only vaguely described [23,24], pathologic criteria in the present study were defined in accordance with the 2015 WHO criteria for NE tumors of the lung [25] (Fig. 1-A to D). In the 2014 WHO classification of the uterine cervix, two categories are listed for NE tumors of the uterine cervix [24]: low-grade and high-grade NE tumors. Low-grade tumors comprise carcinoid tumors and atypical carcinoid tumors, while high-grade tumors comprise SmCC and LCNEC. In our study, low-grade tumors were not included. Combined-type HGNCUC was either a SmCC or LCNEC with components of adenocarcinoma or squamous cell carcinoma.

The pathologic criteria for non-HGNCUC and lung SqCC are available in the 2014 WHO classification of tumors of the uterine cervix [24] and 2015 WHO classification of tumors of the lung [25], respectively.

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