Establishment and Analysis of Biological Characteristics of a Human Duodenal Carcinoma Cell Line, WDC-1

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Background: Duodenal carcinoma is very rare and its culture cell lines have rarely been established. Methods: Tumor cells separated from a surgically resected primary tumor of duodenal carcinoma were put into culture. The patient was an 81-year-old female and had metastatic lymph nodes. We investigated the biological characteristics of the culture cells including in vitro cell kinetics, karyotype, expression of tumor markers and integrins and tumorigenicity and histology in nude mice.

Results: A new cell line, designated WDC-1, was established. This duodenal carcinoma cell line proliferated in a monolayered sheet with a doubling time of 50 h. The histological findings of the xenograft in nude mice were similar to those of the primary tumor. WDC-1 cells produced carcinoembryonic antigen and expressed 1 integrin and very late antigen (VLA)-4d in vitro.

Conclusions: A duodenal carcinoma cell line was established, which is rare and may contribute to progress in understanding the biological features of duodenal cancer.

Key words: duodenal carcinoma — tumor cell line — carcinoembryonic antigen — adhesion molecule — neoadjuvant chemotherapy

INTRODUCTION

The incidence of primary adenocarcinoma of the duodenum is very low (1,2). The tumor may infiltrate the duodenal wall, causing obstruction, and frequently metastasize to regional lymph nodes and the liver. It is crucial to investigate the biological characteristics of tumor cell lines, including the sensitivity against anticancer agents, for the improvement of the prognosis of patients with duodenal carcinoma. However, there are very few cell lines of human duodenal carcinoma and it has been insufficient to analyze their biological characteristics (3). In this work, we established a human duodenal carcinoma cell line from an 81-year-old woman with aggressive metastases to regional lymph nodes.

MATERIALS AND METHODS

CASE FROM WHICH THE CELL LINE WAS ESTABLISHED

An 81-year-old Japanese woman was diagnosed as having primary duodenal carcinoma which originated from the epithelium of the second portion of the duodenum and simultaneously metastatic lymph nodes existed. The levels of serum tumor markers on admission were carcinoembryonic antigen (CEA) 0.4 ng/ml, carbohydrate antigen 19-9 (CA19-9) 7 U/ml, a-fetoprotein (AFP) <1 mg/l. The neoadjuvant chemotherapy [cisplatin (CDDP) 75 mg day 1; 5-fluorouracil (5-FU), 500 mg days 1-5] was performed, because it was difficult to remove the metastatic lymph nodes completely. The metastatic lymph nodes were reduced in size by the neoadjuvant chemotherapy and we could execute the pylorus preserving pancreaticoduodenectomy with lymph node dissection (Fig. 1). The tumor, which formed a large ulcer in the duodenal mucosa, invaded the entire duodenal wall, but the pancreas was not affected by the tumor. The tumor was histologically diagnosed as a poorly differentiated adenocarcinoma of the duodenum.

CELL CULTURE

The tumor cells were separated from the primary tumor tissues by enzymatic digestion as described previously (4–7). The separated tumor cells were suspended in RPMI-1640 medium (Nissui, Tokyo, Japan) supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 mg/ml streptomycin, 50 mM 2-mercaptoethanol and 10% heat-inactivated fetal bovine serum (FBS) (Gibco, Grand Island, NY, USA) (complete medium) and cultured at 37°C in a humidified 5% CO₂ atmosphere. Cell cultures were subcultured with brief trypsin treatment until detachment of cell islands. The established cell line was desig-
nated WDC-1. The levels of CEA, CA19-9 and sialyl Le\(^x\) (SLX) were determined by ELISA in the culture medium of 1 \(\times 10^6/\text{ml}\) WDC-1 cells cultured for 7 days.

**Transplantation of WDC-1 Cells into Nude Mice**

WDC-1 cells at 1 \(\times 10^7\) in 0.5 ml of physiological saline were inoculated subcutaneously on the back of BALB/c nude mice and observed for 4 weeks. The xenograft was examined microscopically with hematoxylin–eosin staining.

**Chromosomal Analysis**

Chromosomes were analyzed by the G-banding technique (8). Briefly, WDC-1 cells were treated with 0.1 g/ml of colcemid, resuspended in 0.075 M KCl hypotonic solution and fixed. The cells were dropped on glass slides, stained with Giemsa solution and photographed for counting.

**Flow Cytometry**

WDC-1 cells were examined by flow cytometry, using phycoerythrin (PE)-labeled anti-\(\beta\)1 integrin monoclonal antibody (MoAb) (Serotec, Oxford, UK) and anti-very late antigen (VLA)-4d MoAb (specific for the complex of integrin \(\alpha_4\) and \(\beta_1\) chains; Immunotech, Marseille, France), as described previously (6). Briefly, WDC-1 cells (1 \(\times 10^7/\text{ml}\)) were suspended in phosphate-buffered saline (PBS; Nissui, Tokyo, Japan) containing 0.1% bovine serum albumin. The cell suspension was mixed with 100 \(\mu\)l of MoAb/ml, then incubated for 30 min at 4°C, washed twice with the PBS. WDC-1 cells were resuspended in PBS–0.1% NaN\(_3\) for flow cytometric examination (FACScan, Becton Dickinson).

**Results**

**Establishment of WDC-1 Cells**

A few days after starting the primary culture, some epithelial cell-like colonies were observed on the bottom of the plastic flask. The cells, trypsinized and washed, were seeded into a plastic flask. WDC-1 cells grew rapidly and the doubling time for the logarithmic growth phase was 50 h (Fig. 2). This cell line has been maintained for over 60 passages since the primary culture.

Phase-contrast microscopy of WDC-1 cells revealed a monolayer cobblestone-like pattern, with clear cytoplasm and oval nuclei (A). On light-microscopic examination (H–E stain), both primary lesion (B) and WDC-1 xenograft (C) showed irregularity of nuclei and tubule formation.

**Xenograft into BALB/c Nude Mice**

WDC-1 cells were successfully transplanted and grown in logarithmic mode in BALB/c nude mice. It was shown pathologically that irregular large cells with a large nucleus and nucleolus patterned the ductal formation. The depth of invasion was the layer of serosa, hence the tumor did not invade the pancreas. The pathological examination revealed that the
Table 1. Secretion of tumor markers in the supernatant of WDC-1 cells, measured by ELISA

<table>
<thead>
<tr>
<th>Marker</th>
<th>Concentration</th>
</tr>
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<tbody>
<tr>
<td>CEA</td>
<td>36 ng/ml</td>
</tr>
<tr>
<td>CA19-9</td>
<td>&lt;6 U/ml</td>
</tr>
<tr>
<td>SLX</td>
<td>6 U/ml</td>
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Hence the established human duodenal carcinoma cell line is rare (10, 11). The WDC-1 cell line was established from human primary duodenal carcinoma.

WDC-1 cells showed a monolayer cobblestone-like pattern and the doubling time was 50 h. WDC-1 cells were successfully transplanted and grown in BALB/c nude mice. The histological finding of xenograft was similar to that of the original primary duodenal carcinoma and was classified as a poorly differentiated adenocarcinoma. In addition, WDC-1 cells indicated structural abnormalities in G-banded karyotype. However, the mice did not have lymph node metastases. We intend to examine the metastatic potential using the orthotopic transplantation model in the future.

The level of CEA was elevated in the culture medium of WDC-1 cells, indicating that WDC-1 cells produced CEA. The serum CEA level of the patient, however, was within the normal range. The serum level of CEA is not always correlated with tumor aggressiveness and progression, because CEA is inactivated in the liver and its serum level is usually normal in gastric carcinoma patients unless they have liver or lymph node metastases (12). Further, serum levels of CEA do not always correlate with the expression of CEA in tumor tissue (13).

The expression of integrins is an important factor for cancer metastasis, although it cannot be judged only from expression of integrins on the tumor cells whether the tumor cells have highly metastatic capacities. Most integrins are expressed on a wide variety of cells and most cells express several integrins (14). Integrins are heterodimers, composed of an α subunit and a β subunit, and play an important role in the attachment of a cell to a cell and to an extracellular matrix. VLA-4 is the complex of α4 and β1 subunits and is the receptor to fibronectin. The number of adherent cells to a VCAM-1-coated well is significantly decreased by vitamin D3, which suppresses the expression of α4 integrin mRNA (15). The adhesion of renal cell carcinoma cells was also inhibited by...
anti-α4 antibody (16). These facts might suggest the involvement of α4 integrin in hematogenous metastasis.

The processes of migration and invasion are likely to involve specific cell surface receptors, such as receptors for the extracellular matrix molecules (17). WDC-1 cells highly expressed β1 integrin. It is necessary to evaluate whether high expression of the β1 integrin makes the tumor cells metastasize to lymph nodes or not because anti-β1 integrin monoclonal antibody inhibits the migration and invasion of tumor cells (18,19). We are now proceeding to investigate the mechanism for the metastasis of WDC-1 cells and these findings may improve the understanding of the biological features of duodenal carcinoma.

References