Abstracts of the Alumni Association Medical Research Fund Prize Memorial Lecture (3)

Pathological and Biomolecular Analyses of Colorectal Endocrine Carcinoma

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Introduction

Colorectal endocrine carcinomas are a heterogeneous group of tumors that display aggressive clinical behavior and have a poorer prognosis compared with common colorectal adenocarcinomas. On the other hand, poorly differentiated (PD) colorectal carcinomas (CRCs) can rapidly metastasize to distant organs and have a poorer prognosis than do well-differentiated or moderately differentiated CRCs. In 2001, Grabowski *et al.* reported that neuroendocrine (NE) cell differentiation can be used as an independent prognostic factor in stage III and stage IV CRCs. PDCRCs often show NE cell differentiation in some parts of the tumor and are found more frequently than well-differentiated or moderately differentiated CRCs. Recent research shows that many types of cancer contain their own stem cells, that is cancer stem cells have self-renewing capacity and differentiation ability. It is thought that gastrointestinal cancer stem cells can differentiate into epithelial cells or endocrine cells. However, the mechanism underlying the aggressiveness of endocrine carcinomas and the role of NE cell differentiation in CRC remain unclear. The aims of this study were to investigate the expression of NE cell differentiation in PDCRC tissue and to examine the clinicopathological significance of the expression and its biological metastatic mechanisms. Furthermore, we focused on the anticancer activity of STI571 (i.e., the c-kit tyrosine kinase inhibitor that exhibits substantial therapeutic activity in patients with chronic myeloid leukemia and gastrointestinal stromal tumors associated with the constitutive activation of the BCR-ABL and c-kit tyrosine kinases, respectively) in human colorectal endocrine carcinoma cells in vitro.

Materials and Methods

From January 1990 through December 2003, 2,204 patients with CRC underwent surgery at the Nippon Medical School Hospital (Bunkyo-ku, Tokyo) or the Nippon Medical School Chiba Hokusoh Hospital (Inba, Chiba). Forty-eight patients (2.2%) with PDCRC were examined. NE cell differentiation was analyzed immunohistochemically with anti-chromogranin A and anti-synaptophysin antibodies. When the immunoreactivity of either of the markers was more than 2% in the tumor cells, a diagnosis of PDCRC with NE cell differentiation was made in accordance with a previous report. Immunostaining results for NE

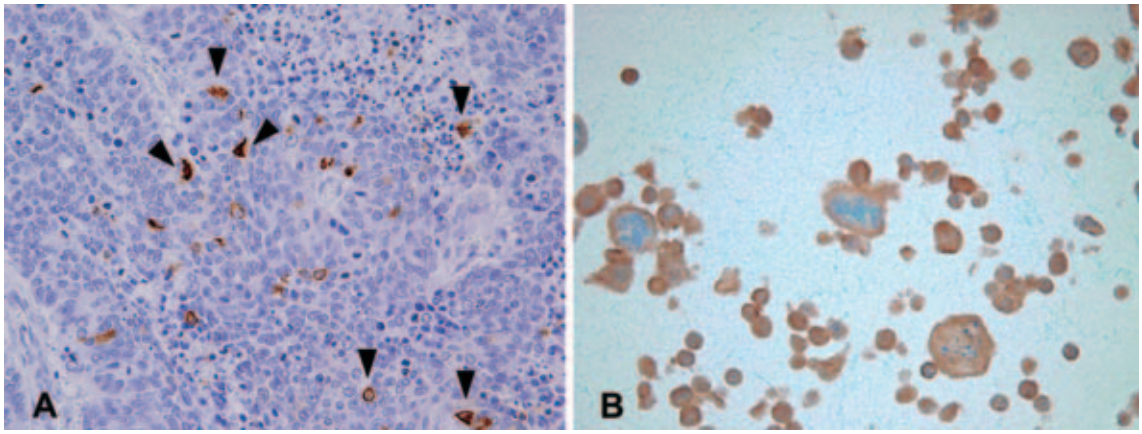

Fig. 1 Immunohistochemical staining for chromogranin **A (arrowheads)** showing cytoplasmic localization in poorly differentiated colorectal carcinoma cells with NE cell differentiation (A). Immunoreactivity for c-Kit was observed in the cytoplasm (B).

differentiation were compared with clinicopathological factors. Microvessel density (MVD) was determined with slides immunostained with an anti-CD34 antibody. After scanning at 100 × to identify areas with the highest number of microvessels, each image was captured with a digital camera system (DP12, Olympus Optical, Tokyo) attached to a microscope (AX80, Olympus Optical) at 200 × (0.64 mm^2). Vascular endothelial growth factor (VEGF) protein and mRNA expression levels were examined with immunohistochemistry and in situ hybridization. With our established MELS-1 cell line derived from human rectal endocrine carcinoma, c-Kit localization and mRNA expression levels were examined with immunohistochemical staining and real-time polymerase chain reaction (PCR) analysis, respectively. Furthermore, the antitumor effect of STI571 was analyzed.

Results

With immunohistochemical staining for chromogranin A **(Fig. 1A, arrowheads)** and synaptophysin, NE cell differentiation was observed in eight patients (16.7%). NE cell differentiation correlated with liver metastasis (P= 0.03). In patients with PDCRC the microvessel density and VEGF protein expression level were higher in those with NE cell differentiation than in those without NE cell differentiation (P=0.13 and 0.068, respectively). The disease-free survival and overall survival rates were slightly but not significantly lower for patients with NE cell differentiation. Serial tissue sections from the immunohistochemical staining and in situ hybridization analysis showed VEGF protein and mRNA expression in the cancer cells (data not shown). In vitro examination of MELS-1 showed immunoreactivity for c-Kit in the cytoplasm **(Fig. 1B)** and c-kit mRNA expression. Furthermore, STI571 inhibited MELS-1growth.

Discussion and Conclusions

NE cell differentiation in PDCRCs may correlate with liver metastasis through microvessel formation in tumors induced by VEGF. In PDCRC, immunohistochemical analysis of NE markers is important for confirming the presence of NE cell differentiation. Further study is necessary to evaluate the effectiveness of anti-angiogenic drugs for PDCRC with NE cell differentiation. These results indicate the importance of further preclinical investigations and clinical trials of the use of the c-kit inhibitor STI571 as a chemotherapeutic agent in colorectal endocrine carcinoma.