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Original contribution

Molecular characteristics of colorectal neuroendocrine carcinoma; similarities with adenocarcinoma rather than neuroendocrine tumor☆

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Summary To further clarify the molecular features of colorectal neuroendocrine carcinomas (NECs), we immunohistochemically examined tumor samples from 25 NECs, including 9 small cell NECs (SCNECs) and 16 large cell NECs (LCNECs), 20 neuroendocrine tumors (NETs), and 21 poorly differentiated adenocarcinomas (PDCs) for the expression of several biomarkers (p53, β-catenin, Bcl-2, Rb, p16, p21, cyclin D1, and cyclin E) and used sequencing analysis to identify gene alterations of *TP53*, *APC*, *CTNNB1*, *KRAS*, and *BRAF*. The frequencies of aberrant p53 expression (88%), β-catenin nuclear expression (48%), and high expression of cyclin E (84%) were significantly higher in NECs than in NETs (0%, 5%, and 5%, $P < .01$, respectively). The immunohistochemical results of NECs and PDCs were similar. *TP53*, *APC*, *KRAS*, and *BRAF* gene mutations were variously detected in NECs and PDCs but not in any NETs. The frequencies of decreased expression of Rb (56%) and high expression of p16 (56%) and Bcl-2 (64%) were significantly higher in NECs than in PDCs (5%, 19%, and 5%, $P < .05$, respectively) or NETs (10%, 5%, and 5%, $P < .01$, respectively). Such immunohistochemical characteristics of NECs were more evident in SCNECs than in large cell NECs ($P < .01$). In conclusion, the molecular features of colorectal NECs are similar to those of adenocarcinomas and not to those of NETs. Decreased expression of Rb and high expression of p16 and Bcl-2 are characteristics of NECs, suggesting that Rb-p16 pathway disruption may contribute to the promotion of proliferative activity in colorectal NECs. SCNECs may be a prototype of NECs.

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1. Introduction

Colorectal neuroendocrine carcinomas (NECs) are rare but highly aggressive neoplasms [1-7]. A recent World Health Organization (WHO) classification [1] graded neuroendocrine neoplasms (NENs) into 3 groups of neuroendocrine tumors (NETs)—NET G1, NET G2, and NECs—simply based on tumor proliferative activity. Morphologically, colorectal NECs are a heterogeneous group ranging from small cell NECs (SCNECs) to large cell NECs (LCNECs). Some cases of LCNECs are difficult to distinguish from poorly differentiated adenocarcinomas (PDCs) with solid growth patterns [7].

The mechanisms of carcinogenesis and aggressiveness of colorectal NECs are still largely unknown. Although the expression of some biomarkers and the molecular features of NECs were previously analyzed, only a small number of colorectal NECs were included in most of those articles [8-13].

There is a hypothesis that they are derived from preceding adenoma/adenocarcinomas. This is supported by combined cases with conventional adenoma/adenocarcinoma and NECs and some molecular features such as the identical loss of heterozygosity [12] or identical mutation [13] of some genes in both components.

Aberrant expression of p53 was observed in approximately 80% of colorectal NECs in the previous reports [5,6]. The expression of other p53-related proteins, such as p21, cyclin E, and Bcl-2, has not been clearly described yet in colorectal NECs.

Disruption of the Rb-p16 pathway, which is another key role in the cell cycle checkpoint, was previously reported in pulmonary and gastrointestinal NECs [8,14]. Overexpression of p16 was reported in gastrointestinal NECs, including 6 cases of colorectal NECs [8]. On the other hand, low expression of p16 and the methylation of the *CDKN2A* gene were reported to be associated with poorer prognosis in some NENs, including colorectal NECs [9].

The Wnt–β-catenin pathway and the expression of cyclin D1 in NENs of various organs have been investigated [8,10,15,16], but colorectal NECs have not been the focus of attention.

In this study, we have attempted to sharpen our understanding of the molecular features of colorectal NECs systematically by analyzing a relatively large number of cases and by directly comparing the characteristics of colorectal NECs with those of NETs and PDCs.

2. Materials and methods

2.1. Patient selection

We first searched the institutional database of the Department of Anatomic Pathology of Kyushu University (Fukuoka, Japan) and related facilities to identify cases diagnosed between 1986 and 2013 as colorectal "neuroendocrine carcinoma," "endocrine cell carcinoma," "small cell carcinoma," and "carcinoma with neuroendocrine differentiation (or features)." We reviewed hematoxylin-eosin–stained sections of all the cases.

To correctly select NEC cases, we referred to the histologic criteria from the most recent WHO classification [1] and a previous report [7]. SCNECs were characterized by sheets or nests of relatively small- to medium-sized cells with high nucleus/cytoplasm ratios, hyperchromatic nuclei with finely granular chromatins, inconspicuous nucleoli, frequent nuclear moldings, and high mitotic activity and by necrosis [7]. Positive staining for neuroendocrine markers was not required for diagnosis.

LCNECs were characterized by diffuse growth patterns or a "neuroendocrine architecture" (ie, organoid or nested structures, trabeculae, peripheral palisading, or rosettes) and composed of monotonous round to oval cells with moderate amounts of cytoplasm, granular/vesicular nuclei, visible nucleoli, and high mitotic activity [7]. Necrosis was also common. To confirm diagnoses of LCNECs, we required immunohistochemical positivity for chromogranin A and/or synaptophysin in greater than 20% of the tumor area [7]. The mitotic rate was determined by counting 10 high-power fields (HPFs). The Ki-67 labeling index (LI) was calculated by manually counting Ki-67–positive nuclei among 1000 tumor cells at a hot spot.

We carefully excluded metastatic cases from other organs such as the lung.

This study was approved by the institutional review board of Kyushu University (IRB no. 25-191).

2.2. Clinical assessment

The clinical characteristics of all cases were estimated, including age, sex, tumor location, tumor size, the presence or absence of nodal/distant metastasis, and tumor stage [17,18].

2.3. Histologic assessment

We estimated histologic findings including necrosis, mitotic counts, lymphovascular invasion, tumor-infiltrating lymphocytes, focal squamous differentiation, intracytoplasmic mucin, and the presence or absence of additional adenoma/adenocarcinoma components according to the previous reports [4,5,7,19]. With regard to the 2 cases for which only biopsy specimens were available, the analyses for lymphovascular invasion, squamous differentiation, and the presence or absence of additional non-NEC components could not be performed.

2.4. Immunohistochemical assessment

Representative formalin-fixed and paraffin-embedded (FFPE) blocks were cut into 4-μm-thick slices. The antibodies used are summarized in Supplementary Table 1. For this staining, we used a polymer-based detection system