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Immunohistochemical Characteristics of Colorectal Carcinoma with DNA Replication Errors

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It has recently been shown that nearly all cancers from hereditary nonpolyposis colorectal cancer syndrome(HNPCC), as well as a subset of sporadic colorectal cancers, have DNA replication errors(RER) at repeated sequences distributed throught their genome. These RER-positive cancers had pathological characteristics of more frequent exophytic growth, large size and poor differentiation. However, the histogenesis and immunohistochemical characteristics of these RER-positive cancers are not known. The poorly differentiated colorectal carcinomas are heterogenous group of neoplasms that differ in their histologic appearance and prognosis. We therefore examined RER from 69 sporadic colorectal carcinomas of poor differentiation and detected in 23 cases(33 %). The pathological features of RER-positive cancers differed from those without RER. The RER-positive cancers had marked preponderance of proximal location(16 / 23, 70 %, vs. 20 / 46, 43 %, $p<0.04$), no glandular differentiation with intense peritumoral immune response (12 / 23, 52 % vs, 6 / 46, 13 %, $p<0.001$). Immunohistochemically, most of the RER-positive cancers were reactive for cytokeratin(22 / 23, 96 %) and CEA(17 / 23, 74 %), and negative for NSE(2 / 23, 9 %), chromogranin(3 / 23, 13 %) and synaptophysin(0 / 23, 0 %). In comparison to 46 RER-negative tumors, RER-positive cancer had less frequent CEA expression(17 / 23, 74 % vs. 44 / 46, 96 %, $p=0.01$). We conclude that the RER-positive colorectal carcinomas have histologic characteristics of predominantly solid, poorly differentiated adenocarcinomas with intense peritumoral reaction and the tumors should be distiguished from neuroendocrine carcinomas and other more aggressive non-glandular tumors of the colon.

Key Words : *DNA replication error, Colon cancer, Immunohistochemistry*

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INTRODUCTION

Colorectal cancer results from the accumulation of several distinct genetic alterations involving the K-ras oncogene on chromosome 12(Bos et al, 1987) and tumor-suppressor genes on chromosomes 5, 17 and

18, as well as other genes(Vogelstein et al, 1988; Fearon and Vogelstein, 1990). Mutation of the APC gene on the long arm of chromosome 5 occur frequently in colorectal carcinomas(Kinzler et al, 1991), deletion of DCC gene on the long arm of chromosome 18(Fearon et al, 1990), and mutation and deletion of the p53 tumor suppressor gene located on the short arm of chromosome 17 are also frequent events in colorectal carcinomas(Baker et al, 1989).

In addition to the series of molecular genetic alterations involving both protooncogenes and tumor suppressor gene, alteration of DNA mismatch repair genes is known to occur in nearly all cancers from hereditary nonpolyposis colorectal cancer syndrome(HNPCC) and in some sporadic colorectal carcinomas(Aaltonen et al, 1993; Ionov et al, 1993; Kim et al, 1994; Thibodeau et al, 1993). The defective mismatch repair genes can be evaluated by DNA replication errors (RER) in repeated nucleotide sequences.

We have previously described the characteristics of colorectal carcinomas with RER(Kim et al, 1994). The RER-positive tumors had more frequent exophytic growth, large size and had a marked preponderance to proximal location and poor differentiation. However, the histogenesis and immunohistochemical features of the subset of sporadic colorectal carcinomas with DNA replication errors have not been studied in detail. Because poorly differentiated colorectal carcinomas are a heterogeneous group of neoplasms that differ in their histologic appearance and prognosis, an evaluation of the pathologic findings and RER in the poorly differentiated colorectal carcinomas is needed. We therefore examined DNA from 69 colorectal carcinomas with poor differentiation for the genetic alterations at microsatellites and compared them to pathological and immunohistochemical characteristics of RER-positive and RER-negative cases.

MATERIALS AND METHODS

Study Population

Sixty nine poorly differentiated sporadic colorectal carcinomas were included in this study. Cases were identified consecutively from the Yonsei University Hospital between 1986 and 1990. Patients with evdience of hereditary nonpolyposis colorectal syndrome by International Collaborative Group criteria(Vasen et al, 1991), with cancer in familial adenomatous polyposis or associated with idiopathic inflammatory bowel disease, with synchronous cancer or other primary malignancy within the previous 5 years, or with preoperative radiation or chemotherapy were not included. Of 300 eligible cases 69(23 %) were defined as poorly differentiated colorectal carcinoma and selected for this study.

The characteristics of the study population are summerized in Table 1. The informations about demographics, tumor site and patient were obtained from the Tumor Registry of Yonsei University and chart review. The population contained 30 females and 39 males ranging in age from 25 to 91 years (mean, 54±16 years). Of the patients, 31 were stage II, 23 stage III and 15 were stage IV. Thirty six tumors were located in the right side and 33 were in the left colon.

Immunohistochemistry for Epithelial and Neuroendocrine Markers

Sections were immunostained with anti-cytokeratin monoclonal antibody(Biogenex, San Ramon, CA) anti-CEA monoclonal antibody(Zymed, San Francisco, CA), anti-neuron specific enolase(Dako, Carpenteria, CA), anti-chromogranin(Dako, Carpenteria, CA), anti synaptoshysin(Dako, Carpenteria, CA). Avidin-biotin complex methodology (Vectastain, Vector Laboratories, Burlingame, CA) was employed. The chromogen was 3-amino-9-ethylcarbazole. Counterstaining of the nuclei was done with hematoxylin. As a negative control, irrelevant antibody UPC10 (Cappell, Organon Teknika, West Chester, PA) was used in each immunohistochemistry run.

Analysis of DNA replication errors (RER)

The analysis of RER was performed as previously described(Goelz et al, 1985; Jen et al, 1994). Briefly, the DNA was extracted from routine formalin-fixed paraffin-embedded tissue sections. The area of the tumor and area of non-neoplastic tissue for comparison were chosen by microscopic examination. In each tumor, the most homogeneous area with tumor cells was selected to reduce contamination by non-neoplastic inflammatory and stromal cells. The desired areas of the tissue were scraped from the slide, deparaffinized in xylene, and digested overnight at 58℃ with 500 μg/ml proteinase K and 1 % sodium dodecyl sulfate. After digestion, the DNA was extracted with equal volume of phenol/chloroform. The

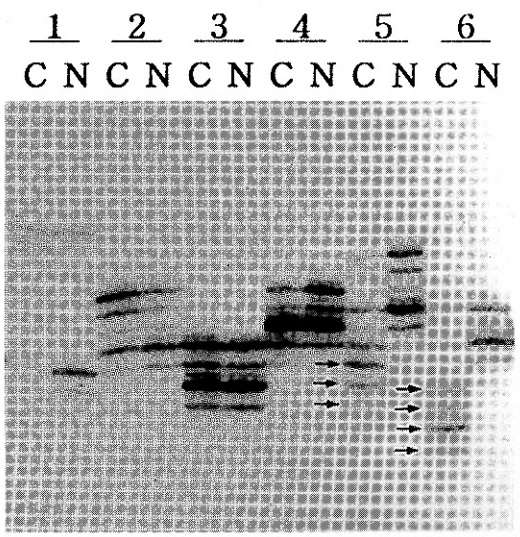

Fig. 1. The DNA replication errors(RER) in colorectal carcinomas. The patient numbers are shown above the lane. The PCR products shown were from normal(N) and Cancer(C) tissue. In RER-positive colorectal carcinomas (case 5 and 6), many additional bands(arrows) are present.

DNA pellet was obtained by ethanol precipitation and dissolved in 10 l of 3 mM Tris buffer(pH 7.5) and 0.3 mM ethylenedinitrilo-tetraacetic acid per slide.

Three microsatellite markers(D18S58, D18S61, D18S64) on the long arm of chromosome 18 were chosen for the determination of RER status by PCR. RER was determined by mobility shift of PCR products using denaturating gel electrophoresis(Fig. 1). In colorectal carcinomas with RER abnormality, numerous bands are found in the normal allele region. We defined a tumor as RER-positive when at least one band was present in the tumor specimen that was not found in the corresponding non-neoplastic tissue, and when these mobility shifts were found with at least two of the three microsatellite markers.

Pathological Analysis

Tumor size was obtained from the surgical pathology records. Conventional histopathologic features(Jass et al., 1987) were examined without knowledge of the patient outcome. Grading of tumor was based on the formation of glands. Poorly differentiated tumors were categorized when the proportion of gland-forming tumor cells was less than 30 %. Histopathologically, the poorly differentiated colorectal carcinomas can be categorized as medullary ; non-glandular, solid, poorly differentiated adenocarcinoma of the colon with intense peritumoral(Gram and Appelman, 1990) and intratumoral lymphocytic infiltration(Fig. 2a), mucinous ; non-glandular mucin producing poorly differentiated

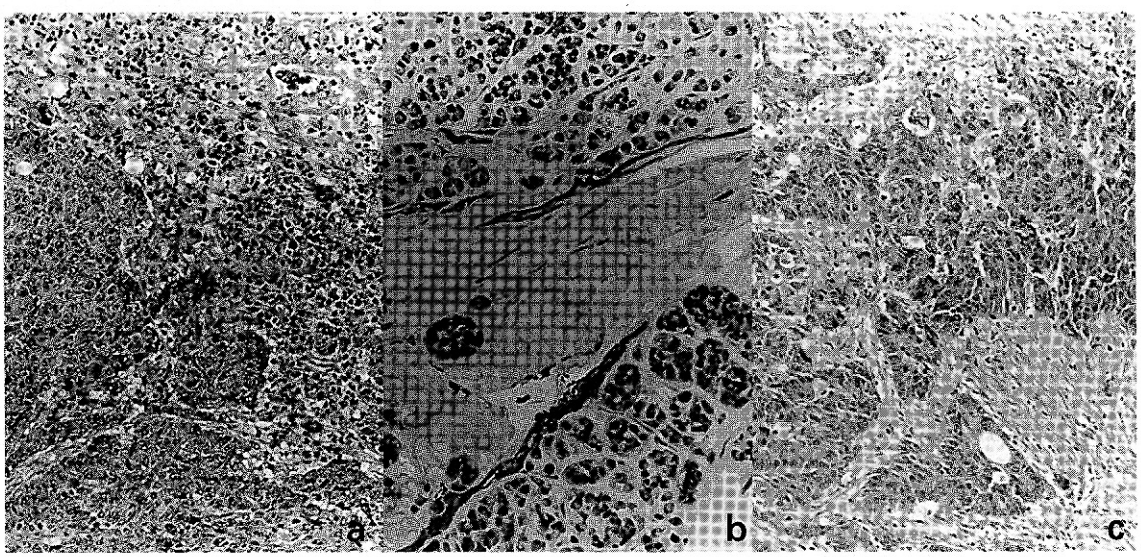

Fig. 2. Histologic subtypes of poorly differentiated colorectal carcinoma. a) Medullary type : solid pattern of tumor cells with many inflammatory cell infiltration is evident. b) Mucinous type : mucin producing carcinomas with many signet ring cells are shown. c) Non-glandular type : poorly differentiated tumor cells with minimal tubule formation and intense desmoplastic reaction is evident.

adenocarcinoma mostly composed of signet ring cells(Fig. 2b), and non-glandular ; minimal gland forming adenocarcinoma(Fig. 2c) without mucin production or peritumoral lymphocytic infiltration.

Statistical Analysis

The clinical and pathological variables were cross tabulated with source(colorectal carcinoma patients with DNA replication errors), and significance of association was determined using a chi-square test.

RESULTS

Clinicopathologic features of poorly differentiated colorectal carcinomas with RER abnormality

RERs were found in 23 cases(33 %) among 69 evaluable poorly differentiated colorectal carcinomas. A slight male preponderance (11 female and 12 males) was noted, but no gender preponderance was found when these cases were compared to RER-negative patients (19 females and 27 males, p=0.8). There was marked preponderance of right-sided cancers (16 right-sided and 7 left sided) comparing to the RER-negative patients (20 right-sided and 26 left sided, $p<0.04$). When the poorly differentiated colorectal carcinomas are subdivided as medullary, mucinous and non-glandular, many of the RER-positive tumors were medullary type ; 12(52 %) RER-positive cases were classified as medullary, 7 cases(30 %) were mucinous and 4 cases(17 %) were non-glandular type(Table 1). In comparison to the 46 RER-negative cancers, RER-positive tumors had strong tendency towards the medullary type(12/23, 52 % vs. 6/46, 13 %, $p<0.001$).

Immunohistochemical features of poorly differentiated colorectal carcinomas with RER abnormality

The cytokeratin was expressed in the cytoplasm and detected in all of the RER-positive colorectal tumors and the intensity did not different from RER-negative tumors. The CEA was also expressed in the cytoplasm of the non-gland forming tumor cells (Fig. 3a). In the area of tumor cells forming glands, strong staining was noted in the apical portion of the cytoplasm(Fig. 3b). The CEA was detected in 17 cases(74 %), and the expression was lower than the RER-negative tumors(12/17, 74 % vs. 44/46, 96 %, p=0.01). The neuroendocrine markers were expressed as scattered(Fig. 3c) and diffuse(Fig. 3d) pattern. Among the neuroendocrine markers the NSE was present in 2 cases(9 %), chromogranin was in 3 cases(13 %). Synaptophysin was not present in any case of RER-positive tumors. There was no significant differences between RER-positive and RER-negative tumors(Table 2).

DISCUSSION

In this study we evaluated the pathological characteristics of poorly differentiated colorectal carcinomas with RER abnormality. The RER-positve cancers differed from RER-negative colorectal carcinomas in their right side predominance, medullary histologic

Table 1. Clinicopathologic features of colon cancer with RER abnormality

Variables	Category	Case Number	Patients Evaluated		P value
			RER+	RER−	
Sex	male	39	12	27	0.78
	female	30	11	19	
Age			53±18	54±15	
TNM stage	2	31	13	18	0.27
	3	23	7	16	
	4	15	3	12	
Tumor side	right	36	16	20	0.04
	left	33	7	26	
Histologic subtype	medullary	18	12	6	0.0002
	mucinous	16	7	9	
	non-glandular	35	4	31	
Growth pattern	expanding	12	6	6	0.13
	mixed	10	5	5	
	infiltrative	47	12	35	

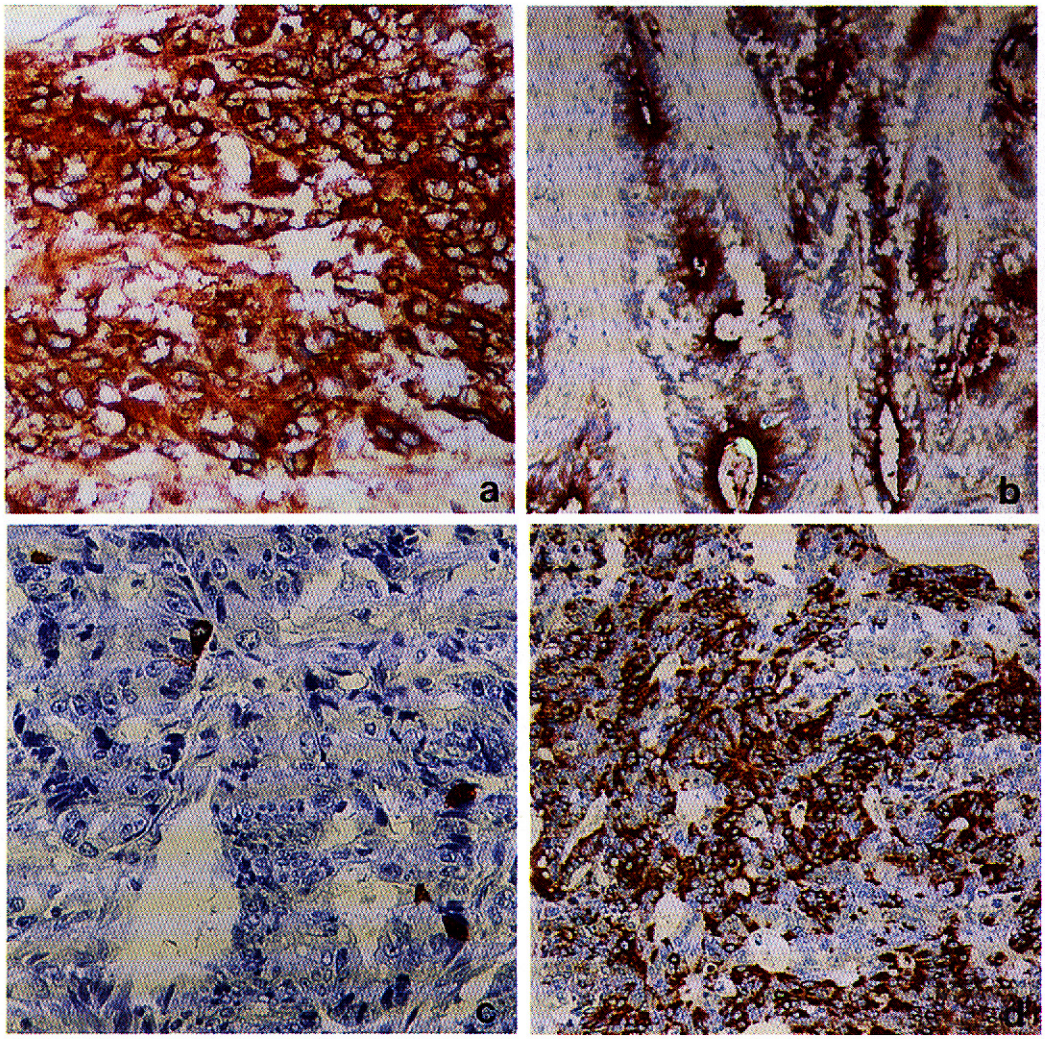

Fig. 3. Immunohistochemical features of RER-positive colorectal carcinomas. a) CEA expression in poorly differentiated carcinomas. The diffuse expression in the cytoplasm is noted. b) CEA expression in tubule forming colorectal carcinomas. The expression of CEA is confined to the apical portion of the cytoplasm. c) Chromogranin expression, scattered pattern. Some of the tumor cells showed chromogranin expression in their cytoplasm. d) Chromogranin expression, diffuse pattern. Most of the tumor cells are positive for chromogranin.

patterns, and decreased CEA expression.

The RER-positive cancers are genetically unstable (Parsons et al., 1993 ; Shibata et al., 1993). The RER has been shown in nearly all cancers from HNPCC patient, as well as 12-18 % of sporadic colorectal carcinomas(Aaltonen et al., 1993 ; Ionov et al., 1993 ; Thibodeau et al., 1993 ; Kim et al., 1994) and variable fractions of other tumor types(Han et al., 1993 ; Risinger et al., 1993 ; Burks et al., 1994 ; Merio et al., 1994). On the basis of the biochemical and genetic analysis, it has been suggested that most of the HNPCC patients have hereditary defects in DNA mismatch repair genes and sporadic colorectal carcinoma with RER have somatic mutations of DNA mismatch repair genes(Leach et al., 1993 ; Liu et al., 1994). The product of DNA mismatch repair genes participate in the recognition and correction of mismatch base pairs resulting from DNA replication errors(Modrich, 1991 ; Kunkel, 1993 ; Strand et al., 1993), and five human genes homologous to the

Table 2. Immunohistochemical features of colon cancer with RER abnormality

Variables	Category	Case Number	Patients Evaluated		P value
			RER+	RER−	
Cytokeratin	no expression	2	1	1	0.56
	expression	67	22	45	
Carcinoembryonic Antigen	no expression	8	6	2	0.01
	expression	61	17	44	
NSE	no expression	64	21	43	0.8
	expression	5	2	3	
Chromogranin A	no expression	57	20	37	0.17
	expression	12	3	9	
Synaptophysin	no expression	62	23	39	0.14
	expression	7	0	7	

responsible for mismatch repair have been discovered(Fishel et al., 1993; Leach et al., 1993; Papadopoulos et al., 1994; Nicholaides et al., 1994; Palombo et al., 1995).

Not only the genetic alterations but the clinicopathological features of RER positive sporadic colorectal carcinomas are similar in many ways to the features of colorectal carcinomas in the HNPCC syndrome. The histopathologic characteristics of colorectal carcinomas of HNPCC are of poorly differentiated carcinoma with marked peritumoral inflammatory infiltrates and mucinous carcinoma with many signet ring cells(Mecklin et al., 1986; Smyrk et al., 1990; Lynch et al., 1993). We have previously demonstrated these histopathologic features were also found in sporadic RER-positive carcinomas. Based on our previous experiments, we selected poorly differentiated sporadic carcinomas for this study and found a high incidence of RER-positive patient in this subgroup. Our RER-positive cases differ from the RER-negative cases in their marked right-sided predominance and strong tendency for little tubule formation. These characteristic histologic appearances mimic endocrine or composite carcinomas. However, most of the tumors were reactive for cytokeratin, CEA and negative for chromogranin, NSE and synaptophysin. These findings indicate that RER-positive colorectal carcinomas are different from the neuroendocrine carcinomas. The altered expression of epithelial and neuroendocrine markers may be due to the numerous mutations that develop in RER-positive tumors. The pathologic features of poor differentiation and marked peritumoral inflammatory responses are also thought to be due to the numerous genetic alterations in RER-positive tumors. Among the characteristic pathologic features of RER-positive cancers, the peritumoral inflammatory response is of particular interest. Previous studies suggested that RER-positive sporadic carcinomas have a better prognosis than those with RER-negative cancers(Thibodeau et al., 1993; Lothe et al., 1993). The discrepance between the aggressive histological features and the more favorable prognosis may be related to the marked peritumoral inflammatory responses of the RER-positive cancers.

In conclusion, the RER-positive colorectal carcinomas have histologic characteristics of predominantly solid, poorly differentiated adenocarcinomas with many lymphocytic infiltration or mucin production. These tumors should be distinguished from neuroendocrine carcinomas and other more aggressive, non-glandular tumors of the colon.

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