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Original contribution

Global histone modification of histone H3 in colorectal cancer and its precursor lesions☆,☆☆

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Summary Chromatin remodeling through histone modification is an important mechanism of epigenetic gene dysregulation in human cancers. However, little is known about global alteration of histone status during tumorigenesis and cancer progression. Histone H3 status was examined in benign and malignant colorectal tumors by immunohistochemistry and Western blotting. For immunohistochemical evaluation, 4 anti-histone H3 antibodies, specific to dimethylation at lysine 4 (H3K4me2), acetylation at lysine 9 (H3K9ac), dimethylation at lysine 9 (H3K9me2), and trimethylation at lysine 27 (H3K27me3), were used. On immunohistochemistry, H3K4me2, H3K9ac, and H3K27me3 showed no significant changes between normal and colorectal tumors. On the other hand, the global level of H3K9me2 was distinctly higher in neoplastic cells (adenoma and adenocarcinoma) than in normal glandular cells. In addition, it was significantly higher in adenocarcinoma than in adenoma. Correspondingly, Western blotting confirmed that H3K9me2 expression was significantly higher in adenocarcinomas than in normal colorectal mucosa. No alteration of H3K9me2 was observed with tumor differentiation and with the histological subtypes of colorectal cancers. These results suggest that aberration of the global H3K9me2 level is an important epigenetic event in colorectal tumorigenesis and carcinogenesis involved with gene regulation in neoplastic cells through chromatin remodeling.

1. Introduction

Colorectal cancer is one of the most common malignancies in the world and is the major cause of cancer-related death [1]. In addition to environmental and alimentary factors, colorectal cancers are thought to occur via a multistep process, resulting in the accumulation of numerous genetic

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Table 1 Immunohistochemical results of H3K9 in normal and neoplastic colorectal tissues

Histology	n	IHC score (H3K9me2)				IHC score (H3K9ac)			
		Negative	Low	Moderate	High	Negative	Low	Moderate	High
Normal colon mocusa	85	0	57 (67.1%)	21 (24.7%)	7 (8.2%) **, ***	0	0	23 (27.1%)	62 (72.9%)
Tubular adenoma	25	0	9 (36%)	10 (40%)	6 (24%) *	0	0	9 (36%)	16 (64%)
Adenocarcinoma	60	0	0	11 (18.3%)	49 (81.7%)	0	0	17 (28.3%)	43 (71.7%)

* $P < .001$ (adenoma vs adenocarcinoma).
** $P = .055$ (normal vs adenoma).
*** $P < .001$ (normal vs adenocarcinoma).

and epigenetic alterations, including activation of oncogenes and the inactivation of tumor suppressor genes [2-4].

Epigenetic events include DNA methylation and histone modifications. Histone protein, around which DNA is wrapped, can be chemically modified in residues by the addition of acetyl, methyl, phospholyl, or other groups. Histone modification is mainly accounted for by acetylation and methylation of histone core tails, which mostly occurs at lysine or arginine residues of NH_2 termini. Histone modification plays an important role in reversible regulation of chromatin dynamics [5]. In addition, it is known that histone modification acts as an activator or suppressor of gene transcription through chromatin remodeling [6]. Generally, condensed chromatin (heterochromatin) is associated with gene inactivation, whereas sparse chromatin (euchromatin) promotes gene transcription. This depends on the site of modified residues and the type of modification (ie, acetylation, methylation, phosphorylation, and sumoylation), that acts on gene transcription [7].

So far, interest in epigenetic involvement in human epithelial cancers has focused on DNA hypermethylation of specific genes, resulting in carcinogenesis by silencing tumor suppressor genes [8]. It has also been shown that histone modification and DNA methylation are closely related to each other [9]. The correlation between the methylation status of CpG islands in the specific genes and histone modification has been investigated mainly using the chromatin immunoprecipitation (ChIP) assay [10,11]. Recently, global histone modification levels have been intensively studied in cancers of various organs using immunohistochemistry [12-21]. In these reports, global alteration of histone modification in certain cancers has been closely associated with patient prognosis and tumor aggressiveness. With regard to colorectal cancers, there has been only one report investigating global histone status and its implications in tumorigenesis and/or carcinogenesis of the neoplasms [21].

In the current study, to clarify the epigenetic environments in colorectal cancers and its precursor lesions, the global histone H3 status was studied in individual cell nuclei of 60 adenocarcinomas, 25 adenomas, 4 adenocarcinomas with adenoma component, and the adjacent nonneoplastic mucosa by immunohistochemistry with 4 specific histone markers (H3K4me2, H3K9ac, H3K9me2, and H3K27me3), along with Western blotting with one histone marker (H3K9me2).

2. Material and methods

2.1. Human colonic tissues

A total of 56 surgically and 33 endoscopically resected specimens of colorectal tumors from 89 Japanese patients was studied. Specimens were collected from the pathological files of Yamanashi University Hospital. Informed consent had been obtained before resection in all patients. The materials consisted of 25 tubular adenomas and 60 adenocarcinomas, including 14 well-differentiated adenocarcinomas, 18 moderately differentiated adenocarcinomas, 14 poorly differentiated adenocarcinomas, 14 mucinous adenocarcinomas, and 4 adenocarcinomas accompanied by an adjacent tubular adenoma component. Adenocarcinomas limited to the mucosa were excluded, and the adenocarcinomas showing invasion to layers deeper than the lamina propria were selected. For these specimens, pathological diagnoses were

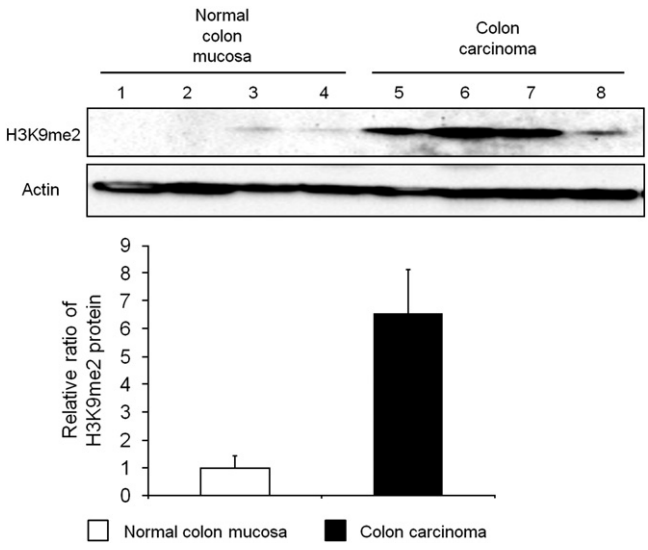

Fig. 1 The results of Western blotting for H3K9me2. Pairs of lanes (1, 5), (2, 6), (3, 7), and (4,8) are the samples obtained from the same patients with moderately differentiated adenocarcinoma, respectively. H3K9me2 expression is up-regulated in moderately differentiated adenocarcinomas (lanes 1-4) compared with individual-matched normal tissues (lanes 5-8), as shown by the densitometric assessments below ($P = .0005$). Columns, mean of densitometric values of normal colon mucosa (n = 4) or colon carcinomas (n = 4); bars, SE.