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Original Article

Histological distinction of early adenocarcinoma of both stomach and colon from borderline malignancies by the immunohistochemical overexpression of Ki−67 and p53 proteins

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Early adenocarcinoma of digestive tract was immunohistochemically analized with cell cycle markers (PCNA,Ki−67), supressor gene marker (p53), and cell adhesion molecules (CD44v3,CD44v6) to establish the histological criteria of early adenocarcinoma. Tumor cells reacted strongly and diffusely with both Ki−67 and p53. Irrespective of invasion the immunohistochemical overexpression of both Ki−67 and p53 is valuable for the objective distinction of troublesome early adenocarcinomas from borderline malignancies.

Key words : histology, early adenocarcinoma, stomach, colon, p53, Ki−67

Introduction

Early adenocarcinomas included carcinoma in situ (CIS) and microinvasive carcinoma. Usual early adenocarcinomas were histologically diagnosed by atypism sharing the following features in hematoxylin−esosin (HE) staining : 1. abrupt transition betwen malignant cells and benign ones, 2. complex or atypical tubules, 3. nuclear stratification of more than half the layer, 4. loss of nuclear polarity, and 5. remarkable nuclear atypism. This tubular atypism was characterized as complex type by crowded tubules with few smooth branchings and as atypical type by serrated papillae or X− or H−shaped branchings. The latter subtype called atypical tubules was referred to as significantly diagnostic atypism for carcinoma. We coule not often distinguish CIS from corresponding borderline malignancies, including hyperplasia and adenoma with atypism or dysplasia, because borderline lesions bore a close histological resembrance to CIS.(5,6) We disclosed these worrisome histology as followings (Table 2) : i.e. (i) surface−limited carcinoma, classified as columnar cell type with several stratified oval nuclei, (ii) well differentiated type carcinoma consisted of few rows of round cells, classified as cuboidal cell type with round nuclei, and (iii) carcinoma of goblet−cell type. For avoiding this nuisance, CIS was practically referred to as an intraepithelial neoplasia (IN) of high grade, which included corresponding borderline malignancies and followed a prefix signifying organic names, e.g. cervical intraepithelial neoplasia(CIN), endometrial intraepithelial neoplasia(EIN), and prostatic intraepithelial neoplasia(PIN).(5,6) These terms as intraepithelial neoplasia, to be sure, were clinicopathologically so convenient that many pathologists were apt to use them clinically; but CIS should be scientifically distinguished from corresponding borderline malignancies.

Microinvasive adenocarcinomas were diagnosed by a finding of histological invasion. In practice it was very difficult to differentiate early invasion from budding or proliferation of tubules though the invasion of either frank invasive carcinoma or squamous cell carcinoma was easily confirmed by the surrounding desmoplastic reaction or the disruption of basal lamina, respectively.

There seemed photographical discrepancies in histological criteria among articles.(10,11,13) Because an early carcinoma however frequently lost features of both definitive invasion and obvious atypism corresponding to obvious carcinoma, it was very difficult to identify the early carcinoma

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histologically.(2, 10, 11, 13) We have tried to diagnose early carcinoma by flow cytometric DNA analyses and cytometric distinctions.(5−7) In spite of their valuable objective discrimination their time−consuming and equipment−requiring demerits prevented them from using routinely to differentiate early carcinomas from benign lesions, i.e. the more convenient method was needed is the identification of early carcinoma. (3−7) Likewise, adenocarcinomas of the goblet cell type could not be easily diagnosed histologically because goblet cells frequently lost a cellular atypism. Adenocarcinoma of goblet cell type has been confirmed only by few focally−located atypical cells, that is, because atypical cells were too rare to identify in small biopsied specimens, the identification of adenocarcinomas of goblet−cell type demanded new diagnostic procedures.

We have investigated immunohistological discri mination of carcinoma.(3,4) In this study various histological reagents were tried for early adenocarcinomas. Examined reagents consisted of cycle markers (PCNA, Ki−67), suppressor gene marker (p53), and cellular adhesion ligands (CD44v), which were reported as the most valuable reactants relating to the histological differentiation and predicting the patient's prognosis.(1,8,9,12) Ki−67 was one of the cell cycle markers and regarded as an accelerator in cellular proliferation. p53 was one of the suppressor gene products and regarded as a brake in cellular proliferation. p53−gene mutation brought loss of suppression in cell proliferation and, furthermore, aderrant p53 products remained in cytoplasm as the overexpression of p53−products. p53 overexpression was found in most tissues including normal and dysplastic cells without gene mutation and, furthermore, increased in pooly differentiated carcinoma.(1,12) Secondarily we reviewed these cases of early carcinoma positive for p53 and Ki−67 and established the histological criteria of both early adenocarcinoma and borderline malignancies in need of additional immunostaining for its distinction from carcinoma.

Materials and Methods

Materials:

Analyzed 102 gastric cases consisted of 26 cases of normal control, 28 cases of benign lesions, and 48 cases of early adenocarcinoma. 147 colons were studied and consisted of 19 cases of normal control, 63 cases of benign lesions, and 65 cases of early adenocarcinoma. Routinely processed paraffin embedded sections were available for immunohistochemistry. Our specimens were immunostained at each first report of pathological diagnosis just after extirpations to reduce the attenuation of antigenecity.(14)

Early carcinoma were histologically differentiated by the following features : 1. abrupt transition betwen malignant cells and benign ones, 2. atypical tubules and nuclear statification of more than half the layer.(2,4−6,11,13) Benign lesion group included so−called borderline malignancies or hardly warranted specimens short of above features, hyperplasia and adenoma with atypism or dysplasia. There was no significant difference in tumor size and age among benignancies and malignancies.

Methods:

Immunohistochemical reagents:

1. Monoclonal mouse anti−proliferating cell nuclear antigen (DAKO−PCNA,PC10), provided by DAKO Co., which was the polymerase delta accesory protein and postitive in nucleus of G1−S−S2 phases, 2. monoclonal mouse anti−nuclear G1 to M phase, 3. monoclonal mouse anti−p53 protein, provided by Immunotech Co., which activated both p21 (the suppression protein in the progress through cyclin to G1−S via Rb) and apoprosis, and, furthermore, was accumulated in tumor cells as variant forms in spite of the rapid turnover and showed the disappearance of normal froms of p53, 4. polyclonal rabbit anti −CD44v3 and CDv6 (CD44 variants containing an exon 7 and exon 10, respectively), provided by Zymed Co., which were homing cell adhesion molecules and connected with extracellular hyaluronate, and, furthermore, their variants werereported to be positive in metastatic cases.

Immunohistochemical procedures:

Routinely immerced tissue sections of paraffin embedded tissues were preincubated by heat treatment with a microwave for the exposure of each antigens, and stained with peroxidase method.

Grouping of immunohistochemical positivity and its final judgement:

The immunoreaction was judged by both each intracellular staining intensity and its number of positive cells, and classified as a whole into three grades: weak, moderate, and marked degrees according to the preliminary positive control staining in twenty−five obvious carcinomatous tissues.

In regard to the classification criteria of p53−immunopositivity, firstly the "weak" grade of positivity was as following: immunopositive cells could only be identified microscopically under 200x power field and their cellular number was less than 5%. Secondly the "marked" grade of positivity consisted of two categories: (1) the immunoreactive cells were histologically found even under 40x power field and the number of positive cells was between 5 and 25%, or (2) they were confirmed under 100x power field and their number was over 25%. Thirdly the "moderate" grade occupied between above two grades, both "weak" and "marked", and we described this "moderate" grade or more as undoubtedly "positive" result in immunohistochemical analysis.

As for Ki−67, because its reactive density was generally strong, the classification of immunoreaction was based only on the number of positive cells. There were three grades: "weak", "moderate" ones, which had positive cells of 5%−25%, 25% or more in number, respectively. We also described the "moderate" grade or more as certainly "positive" result in immunohistochemical analysis.

Results

Immunostaining analyses showed that PCNA was diffusely positive in almost all the specimens irrespective of progress of lesions, and so its immunohistochemical specificity for malignancy was low. Ki−67 was also positive in most specimens but its distribution was rather restricted than that of PCNA. Ki−67 immunoreaction was convenient to identify the deep−seated normal proliferation zone strictly so that we could easily find the expansion of proliferation. The loss of normal proliferation zone, i.e. the expansion of proliferation zone, suggested the presence of a space−occupying lesion regardless of malignency. Concerning p53 immunoreactions, normal cells and most cells, irrespective of malignancy, reacted with p53 reagent, but early carcinomas showed more intense response and had higher frequency in numbers of cases than borderline lesions. (Table 1,2) There was no relationship between lesions and CD44v3 or CD44v6. Tumor cells of surface−limited carcinoma expanded beyond normal proliferation zone to luminal surface and this was well shown by Ki−67 immunostaining. This totally replaced area by Ki−67 positive cells was also diffusely stained with p53. Benign or borderline lesions were differentiated from early carciomas. (Table 1,2)

Discussion

Ki−67 and immunohistochemical analyses revealed that double immunoreaction for Ki−67 and p53 was necessary to diagnose early adenocarcinoma of well defferentiated type. The proliferation zone was fairly immunostained with Ki−67 reagent because Ki−67 positive cells were regarded as being in active cellular prolifeation. This deep−seated proliferation zone was well preserved in either normal regions or benign proliferative lesions. This proliferative zone expanded with the progress of tumorigenesis and, furthermore, the total tubular replacement by tumor cells was looked upon as carcinoma. p53 positivity of carcinoma cells was more diffuse and stronger than corresponding benign proliferative lesions. Poorly differentiated carcinoma cells showed marked atypism so that this kept further p53−immunostaining dnalysis away in the process of histological cancer diagnosis.

The histological atypism of early adenocarcinomas was reanalyzed in tumor cells. The atypism was classified in a classificatory criterion (Table 3): i.e.(a) tumor cells were morphologically

classified columnar cells with both oval nuclei and moderate cellular atypism, cuboidal cells with both round nuclei and marked cellular atypism, and goblet cells with both round nuclei and slight cellular atypism, (b) nuclear stratification meant the thickness of nuclear stratification to the full thickness of tubular epithelium, and, furthermore, the nuclear stratification was divided into two groups by their widths, (c) tubular structural atypism was classified into simple, cowplex, and atypical, that is, simple type was normal pattern, complex type was crowded pattern with smooth branching, and atypical type had saw−toothed papillae and more complicated branchings, including X− or H−shaped tubules. In this classificatory cross−table, usual early adenocarcinomas were easily diagnosed by cell atypism, unclear stratification more than half the layer, and atypical tubules ("Ca" in Table 2).

There still remained several worrisome cancerous specimens required to differentiate because of its shortness of atypism (open abbreviation "Ca" in Table 3): i.e.(1) 1. columnar cells with oval nuclei, nuclear stratification less than half the layer, and atypical tubules, or 2. columnar cells with oval nuclei, nuclear stratification more than half the layer, and complex tubules, (2) cuboidal cells with round nuclei and marked unclear atypism without atypical tubules, (3) goblet cells with atypical tubules. According to the discriminant table (Table 2), these well differentiated carcinomas were easily confirmed by diffuse and strong staining patterns for Ki−67 and p53.

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Tab. 1. p53−significance in the immunohistochemical differentiation between malignancy and benignancy in stomach

lesions/positiviy	− / ±	+ / + +	x^2−independence
normal	21	5	
benign	24	4	＊p=0.001
non−adenoma	11	3	＃p=0.07
adenoma	13	1	$p=0.03
malignant	14	34	＊＃$

＊＃&x^2−test for independence and its significant rate between two groups.

Tab. 2. p53−significance in the immunohistochemical differentiation beween malignancy and benignancy in colon

lesions/positivity	− / ±	+ / + +	x^2−independence
normal	19	0	
benign	59	4	＊p=1x10^{-10}
malignant	10	55	＊

＊x^2−test for independence and its significant rate between two gorups.

Table 3. Histological classificatory criteria of early adenocarcinoma of degesive tract

cellular atypism		stuctural atypism			
shape	grading of	nuclear	configuration of tubules		
nucleus cytoplasm	atypism	stratification	simple	complex	atypical
oval columnar	+	<1/2	−$	−	Ca#
	+	1/2≦	−	Ca	Ca*
round cuboidal	++	±	Ca	Ca	Ca
goblet cell	±	±	−	−	Ca

*: Ca: easily diagnosed only by routine sections stained with HE, which showed nuclear stratification and atypical tubules, or marked nuclear atypism, #:Ca: defined as carcinoma by the diffuse positivity for Ki−67 and p53, $: blank space in the above result table: not carcinoma but adenoma, hyperplasia, and so on.

Histological distinction of early adenocarcinoma of both stomach and colon from borderline malignancies by the immunohistochemical overexpression of Ki−67 and p53 proteins

ki-67とp53の免疫組織学的過剰発現による、胃及び大腸の早期癌とその境界病変の組織学的識別

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癌の組織学的診断において、早期癌の診断は大変難しい。実際に、臓器別の病理専門医の間でも癌診断の不一致例が多い。現在、売名的専門医による”正常と異なるものは全て癌”と自称早期癌治療臨床医による”何でも摘出”や、早期癌の過剰診断に否定的な近藤誠の”癌擬き”オデン理論が横行している現在、改めて、早期癌診断の限界を免疫組織化学的に客観的に問い直すことも意義があるのではないかと考え、細胞増殖マーカー（PCNA，Ki−67）・癌抑制遺伝子産物(p53)・細胞接着因子（CD44v3，v6)等に関して検討した。

今回、生検診断頻度の高い胃並びに大腸の早期線癌組織に関して免疫組織学的検討を加え、その客観的診断根拠を示した。即ち、早期線癌組織は、細胞周期マーカーであるKi−67による正常増殖帯消失と異所性瀰慢性陽性所見と、抑制遺伝子産物であるp53の陽性過剰発現を示すことが判明した。以上のことより、異型性の弱い分化型線癌や境界病変の癌診断に客観的根拠を与えることが可能となった。

キーワード；組織、早期腺癌、胃、大腸、Ki-67、p53

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