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# Expression of Keratin 7 in the Boundary Epithelial Cells of Colorectal Neoplasm

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**Background:** Cytokeratin 7 (CK7) is an effective immunohistochemical diagnostic tool for the study of the origins of tumors in various organs. However, the specific phenomenon of CK7 expression in the boundary epithelial cells of colorectal neoplasms has not been adequately studied. We evaluated this specific expression pattern of CK7 in colorectal tumors and tried to explain its possible relationship to tumorigenesis. **Methods:** The study comprised 130 cases of colon adenocarcinoma, 105 colon adenoma, 15 benign nonadenomatous tumors of the colorectum, 12 inflammatory bowel disease and 17 secondary metastatic carcinomas of the colorectum. Immunohistochemical staining for CK7 was performed for all cases. **Results:** One hundred and seven cases (82.3%) in the primary adenocarcinoma group revealed positive staining at the tumor boundary with sharp transition from CK7-negative carcinomatous tumor to CK7-positive adenomatous epithelium (boundary epithelium) then to CK7-negative normal-appearing mucosa. Eighty-eight cases (83.8%) in the primary adenoma group showed similar boundary patterns. However, other lesions, including benign non-adenomatous tumors, inflammatory bowel disease and secondary metastatic carcinomas of the colorectum, did not show this pattern. **Conclusion:** Because of the characteristic location of these CK7-positive cells, they may be related to dedifferentiation and redifferentiation of cells in colorectal tumorigenesis. In this article, we present a hypothetical "ripple model" to interpret the role of the specific CK7 boundary features in the possible pattern of clonal expansion in the development of colon tumors.

Key words: keratin 7, colorectum, carcinoma, boundary epithelium, tumorigenesis

## INTRODUCTION

Cancer presumably arises from a dedifferentiation of mature cells that return to a fetal phenotype with the potential for proliferation and renewal, or from stem cells preserved in an undifferentiated state since fetal development[1-3]. Dedifferentiation in this context could represent a transitional phase that cells pass through before they switch to redifferentiation, metaplasia or neoplasia[4]. Keratins are intermediate filaments that are critical in cytoskeletal organization. Their roles in cellular processes are underscored by inherited human diseases in which germline mutations of keratins are found, as well as by transgenic and knockout mouse models that recapitulate those diseases[5,6]. According to previous reports, CK7 is present in fetal, largely absent in normal adult, and transiently neoexpressed in metaplastic and neoplastic epithelial cells of the stomach[2]. Hence, CK7 neoexpression in the stomach could define a fetal-like, dedifferentiated, cellular phenotype during the development of metaplasia and neoplasia[2]. In contrast, although adenocarcinoma of the colorectal region is typically positive for cytokeratin 20 (CK20) and negative for CK7[7,8], the role of CK7 neoexpression in the colorectum remains unclear. In the present study, we found that CK7 is uniquely expressed in the boundary epithelial cells of colon neoplasia. This suggests that CK7 expression could correlate with important cell fate decisions in colon epithelia. We tried to explain the relationship between this CK7-positive boundary phenomenon and tumorigenesis of colorectal adenocarcinoma by developing a "ripple model". The critical roles of CK7-associated proteins or molecules within this hypothetical model remain to be determined.

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## MATERIALS AND METHODS

### Human Materials

Formalin-fixed and paraffin-embedded colon tissue was derived from the diagnostic files of the pathology department of the Tri-Service General Hospital and National Defense Medical Center, Taipei, Taiwan, between 2001

and 2004. One hundred and fifty-six primary colon adenocarcinomas (Ca), 124 adenomatous polyps (Ap) of the colon, 15 benign non-adenomatous tumors of the colorectum (five hyperplastic polyps; four carcinoid tumors; two lipomas; four gastrointestinal stromal tumors (GISTs)) and 12 cases of inflammatory bowel disease (six ulcerative colitis; six Crohn's disease) were included. Seventeen cases of secondary carcinoma metastatic to the colorectum, including four cases of ovarian adenocarcinoma, four cases of endometrial adenocarcinoma, four cases of hepatocellular carcinoma, two cases of urinary bladder transitional cell carcinoma, two cases of cervical squamous cell carcinoma and one case of esophageal squamous cell carcinoma were also evaluated. Specimens were included irrespective of an immunohistochemically detectable sublemmal or cytoplasmic CK7 expression.

**Immunohistochemistry**

Expression of CK7 was investigated by a mouse anti-keratin 7 monoclonal antibody (1:100; clone OV-TL 12/30; Dako, Glostrup, Denmark). Single staining in paraffin sections was performed as previously described[9]. One paraffin-embedded pulmonary tissue specimen from an adult cadaver donor was used as an internal control for CK7 immunostaining. A negative control was performed by omitting the primary antibody.

**Evaluation of immunostaining**

CK7 immunostaining was evaluated semiquantitatively. The categories for adenocarcinoma of colon (including ascending, descending, sigmoid and rectal colon) were defined arbitrarily as *negative*: less than ten CK7-positive cells occurring in the boundary epithelium; *positive*: ten or more CK7-positive cells occurring in the boundary epithelium that could be easily observed under low magnification (40x). Boundary cells were defined as the adenomatous epithelial cells immediately adjacent to the junction of normal mucosa with tissue of different differentiation (e.g., adenoma ― normal mucosa junction; carcinoma ― normal mucosa junction; adenoma ― carcinoma junction). The other parts of nonneoplastic mucosa were defined as background mucosa.

**Statistical analysis**

Differences in the degree of expression of a positive boundary pattern in the subgroups of Ca and Ap were analyzed using the $\chi^2$ test. A value of $p \leqq 0.05$ was considered significant.

Table 1 Clinicopathologic features of primary adenocarcinomas of the Colon

| Features | cCa (n = 86) | mCa (n=10) | tCa (n = 34) | Total Ca (n =130) |
|---|---|---|---|---|
| Patients'age (years) | | | | |
| Mean ± SD | 61.5±15.1 | 60.5±7.1 | 64.8±13.3 | 62.3±14.2 |
| Range | 21-86 | 50-73 | 28-82 | 21-86 |
| Male: female ratio | 47:39 | 7:3 | 15:19 | 69:61 |
| Size (cm) | | | | |
| Mean ± SD | 4.1±1.6 | 5.3±1.9 | 1.4±0.5 | 3.5±1.9 |
| Range | 0.7-8.5 | 3.5-9 | 0.5-9 | 0.5-9 |
| CK7+ (n;%) | 84;97.7 | 2;20 | 21;61.8 | 107;82.3 |

cCa: common adenocarcinoma; mCa: mucinous carcinoma; tCa: adenoma with malignant transformation; CK7+: CK7 positive immunoactivity in boundary epithelium

Table 2 Clinicopathologic features of Primary Adenomas of the Colon

| Features | Tubular adenoma (n = 40) | Villotubular adenoma (n = 40) | Villous adenoma (n =25) |
|---|---|---|---|
| Patients' age (years) | | | |
| Mean±SD | 56.1±18.7 | 67.5 ± 11.0 | 62.1 ± 15.0 |
| Range | 21-81 | 44-81 | 32-85 |
| Male: female ratio | 16:24 | 19:21 | 13:12 |
| CK7+ (n;%) | 32;80 | 34;85 | 22;88 |

CK7+: CK7 positive immunoactivity in boundary epithelium

## RESULTS

**Clinical and Individual Characteristics of the Patients**

After excluding the cases that showed CK7-positive immunoreactivity in neoplastic and/or nonneoplastic parts, or loss of morphologic orientation, 130 cases of Ca and 105 cases of Ap were extracted. Apart from common adenocarcinoma (cCa) (WHO adenocarcinoma, ICD 8140/3), ten mucinous carcinomas (mCa) and 34 cases of focally malignant transformation from adenomatous polyps (tCa) were included in the Ca group. None of the patients had received chemotherapy or radiotherapy before biopsy or surgery. The mean age of patients was 62.3±14.2 years (range 21-86 years) for the Ca group (Table 1) and 61.9±15.9 years (range 21-85 years) for the Ap group (Table 2). The cases included in the Ca group had a male/female ratio of 1.13 (69/61), whereas that of the Ap patients was 0.84 (48/57). Six patients in the Ca group had additional tumors (one each of adenocarcinoma of the prostate, squamous cell carcinoma of the esophagus, bronchoalveolar carcinoma of the lung, polyposis of the colon, adenocarcinoma of the lung, low grade noninvasive papillary urothelial carcinoma)

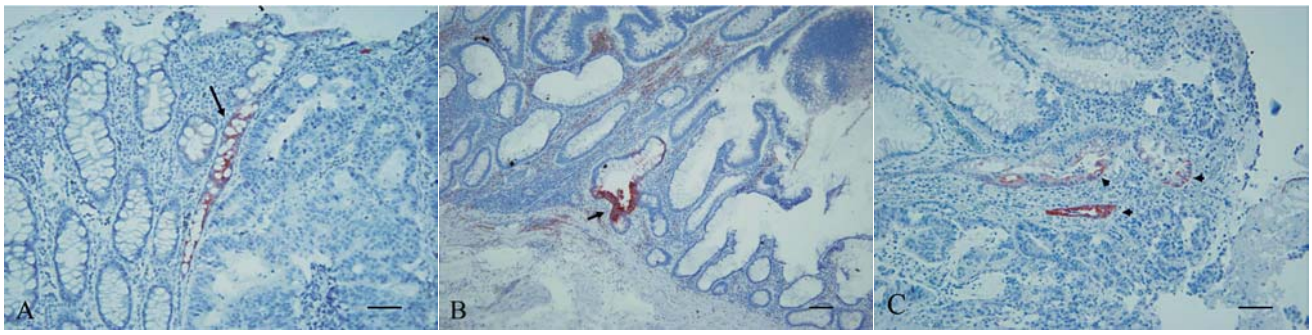


Fig. 1 Characteristic CK7-positive boundary cells in different transitional regions: (A) CK7+ boundary cells (brown, long arrow) between normal (left side) and adenocarcinoma (right side). (B) CK7+ boundary cells (short arrow) between normal (right side) and adenoma (left side) junction. (C) In case of adenocarcinoma arising from adenoma, the CK7-positive adenomatous cells (arrow head) were identified between adenoma (upper side) and carcinoma (lower side) (Scale bar: 200 $\mu$m).

before diagnosis of colon adenocarcinoma. Of the patients in the Ap group, only one had a previous history of cancer (squamous cell carcinoma of the anus). Schistosomiasis was noted at the time of diagnosis of colon adenocarcinoma in one case. One patient in the Ap group had a family history of cancer (father: died, cause unavailable; mother: died of colon adenocarcinoma; elder sister: leukemia under treatment; younger sister: adenocarcinoma of colon after surgical treatment) while one patient in the Ca group had an associated history (familial adenomatous polyposis).

**CK7 Expression Profiles**

Normal-appearing nonneoplastic large intestinal mucosa was universally negative for CK7 expression by immunohistochemistry. Eighty-four cases (97.7%) in the cCa group showed a positive boundary pattern, of which the prominent feature was a sharp transition from CK7-negative carcinomatous epithelium to sparse CK7-positive adenomatous epithelium (boundary epithelium) then to CK7-negative remaining normal-appearing mucosa (Fig. 1A). Of the ten primary mucinous colon carcinomas, eight cases (80%) did not show this boundary pattern, which was in marked contrast to the cCa group, where only two cases (2.3%; 2/96) of tumors with mucinous foci occupying less than half of the tumor mass were negative ($p < 0.05$). Of the tumors in the tCa group, the cases with CK7-positive boundary features expressed the staining either in the adenomatous cells at the normal appearing mucosa — adenoma junction (Fig. 1B), carcinoma — adenoma junction (Fig. 1C) or both junctions (Table 3). The overall CK7-positive boundary immunoreactivity patterns of the cCa group did not appear to correlate with the age and sex of the patients, nor the size, differentiation status, or stage of the tumors ($p > 0.05$). Eighty-eight cases (83.8%) in the Ap group showed similar boundary patterns that were mainly located in the adenomatous cells near the adenomatous — normal-appearing mucosa junction. There was no obvious difference in the expression rate between the three histopathological types ($p > 0.05$) (Table 2).

Table 3 CK7 boundary patterns of colon adenomas with adenocarcinoma transformation

| Expression of CK7 boundary pattern | Tubular adenoma (n =12) | Villotubular adenoma (n =17) | Villous adenoma (n =5) |
|---|---|---|---|
| Positive (n;%) | 10;83.3 | 9;53 | 2;40 |
| Negative (n;%) | 2;16.7 | 8;47 | 3;60 |

No lesion in our collected cases that was diagnosed as primary benign nonadenomatous tumor, inflammatory bowel disease or secondary metastatic carcinoma to the colon had such boundary CK7 reactivity.

## DISCUSSION

Cytokeratins are intracellular intermediate filaments that are critical in the maintenance of the cytoskeleton and are selectively expressed during the course of development and differentiation in different types of epithelium[5]. They can be classified into types I (CK9-23) and II (CK1-8), and there is heterodimerization of one type I keratin with a type II keratin, the nature of the combination varying within epithelial compartments[10].

A series of reports have discussed both CK7 and CK20 expression patterns in neoplasms of different origins[7,8,11-13]. Adenocarcinoma of the colon is generally considered to be immunopositive for CK20 and negative for CK7. Although several recent studies have indicated a higher

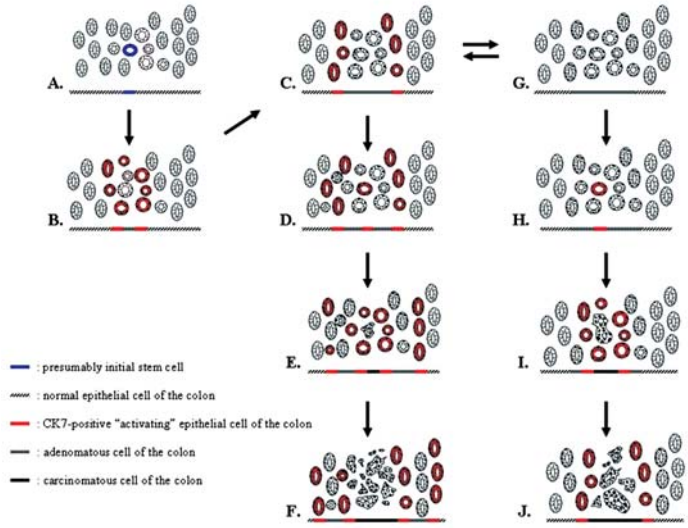


Fig. 2 "Ripple Model". Presumably, the tumor development initiates from a single pocket of epithelial stem or dedifferentiated cells (blue glands in A). Via possible cell dedifferentiation and clonal expansion, the boundary epithelial cells around the primary stem or dedifferentiated cells become "activating" (red glands in B) and then transform into adenomatous cells, and the activating wave moves along the junctional regions between normal-appearing colon mucosa and adenoma. The transformed adenomatous cells might proliferate/expand (C) or go into "static" adenomatous cells (G). As accumulation of genetic loss and/or mutation, or environmental factors stimulation, a progressed sentinel adenomatous cell becomes "activating" (D or H) and then transforms into carcinomatous cell (E or I). The boundary cells are activated and then sequel to carcinomatous cells as well as advanced cellular expansion (F or J). In this model, the "activating" CK7-positive cells that locate over the periphery of the transforming mass present an expansion/ripple wave.

percentage of CK7 expression in rectal lesions[12,14], we know of no previous description of the phenomenon of CK7 expression in the boundary epithelium. The specific pattern of CK7 expression in the boundary epithelium did not occur in primary benign nonadenomatous tumors, inflammatory bowel disease or carcinoma metastatic to the colorectal area, which led us to suggest that it was frequently associated with primary neoplasia rather than being a secondary reactive phenomenon. In comparison with the boundary epithelium, the uninvolved mucosa directly above or adjacent to primary colon lesions or metastatic tumors from non-large bowel primary sites (called "transitional mucosa" in previous associated reports) revealed mucin expression and alteration of mucosal thickness that was considered secondary reactive change[15-19].

A characteristic CK7 expression pattern, similar to our observations in colorectum, is also found in the boundary mucosa of the neoplastic stomach[2]. Review articles have suggested that malignancy could arise either from stem cells because of a maturation arrest, or from a dedifferentiation of mature cells that regain the ability to proliferate[20,21]. It has been suggested that a decisive step for gastric carcinogenesis is dedifferentiation, defining a backward change of differentiated epithelial cells into a fetal-like phenotype. Such a dedifferentiation phenomenon has been described in a number of experimental studies on tissue or cultured cells[21-23]. The possible pathways of the switch between differentiation and neoplastic growth with involvement of $\beta$-catenin accumulation, transforming growth factor (TGF)-$\alpha$ and epidermal growth factor (EGF) have been mentioned in human stomach[2], hair follicle[24] and in transgenic mice[25,26]. Several studies imply that epithelial neoplasms are clonally derived; that is, a single transformed cell is the ancestor of all cells that compose the neoplasm[1,27].

In the 34 cases within the tCa group that were diagnosed as adenocarcinoma arising from adenoma, it was possible to study different stages in the same tumor specimen including both adenomatous and carcinomatous regions of individual tumors. The CK7-positive cells in the tCa group, similar to those in the cCa group, were situated in boundary regions between normal appearing mucosa, adenoma and carcinoma junctions. This specific phenomenon revealed that the CK7-positive adenomatous cells mainly locate in the neoplasia during progression or within transforming areas which, together with our observation that most of the CK7-positive boundary cells are low-grade adenomatous epithelial cells, might indicate that these "activating" adenomatous cells precede some events involved in colorectal tumorigenesis. The characteristic location of these CK7-positive cells, CK7-associated proteins or gene products may be related to one or more stages in the genetic model for colorectal tumorigenesis[28], particularly those steps between early to intermediate adenoma. CK7, as one of the type II keratins that are encoded on chromosome 12q,[29], and the relatively low-grade adenomatous dysplasia in most of the CK7-positive boundary cells, suggest the possibility that CK7-associated proteins or the tumorigenic stage involved might have something to do with alteration of genes located on chromosome 12 (for example the *ras* gene located on chromosome 12p) that result in the colorectal tumorigenesis event transitioning between early to intermediate adenoma. Similar findings were described by Chen and Wang who showed that acquisition of CK7 expression occurs early in small intes-

tinal tumorigenesis, i.e., at the adenomatous stage, and are also highlighted by observations in studies of CK7 and CK20[30].

Although it is still controversial that mucinous colorectal carcinoma is associated with a relatively poor prognosis, mucinous carcinoma of the colorectum is considered a distinct entity with recent studies describing a number of clinicopathological parameters (such as localization, prevalence in different countries and age groups, association with hereditary nonpolyposis colorectal cancer, and inflammatory processes) and genetic alterations (e.g., frequency of mutation in Ki-ras and p53 genes, level of MUC2 expression) that differentiate mucinous from nonmucinous tumors[31]. The almost negative CK7 immunoreactivity in the boundary epithelium of mucinous tumors in our present analysis may indirectly support such evidence of a separate "mucinous pathway of carcinogenesis"[31].

Nearly 30 years ago, Nowell suggested that adenoma progression presumably resulted from successive waves of clonal expansion[32]. The generally accepted genetic model for colorectal tumorigenesis suggests that it occurs in three stages through a series of genetic alterations involving oncogenes (ras) and tumor suppressor genes (particularly FAP, DCC, and p53) that take more than ten years[28,33]. However, these hypotheses, models or observations are based on the biochemical or molecular examination of homogenized tissues and there have been few opportunities to observe the clonal development of colorectal tumors directly in human epithelia[34]. In this paper we present a hypothesized "ripple model" (Fig. 2) to try to interpret the presumed pattern of clonal expansion in the development of colorectal tumorigenesis in terms of the specific CK7 boundary features representing a possible cell dedifferentiation[20-27] and clonal expansion[32].

As we try to develop a "ripple model" to explain the relationship between CK7 immunohistochemical findings and the pathogenesis of colorectal neoplasia, however, more questions remain unanswered. First, little is known about the normal function of any of the CK7-associated proteins or structures implicated in colorectal tumorigenesis, a prerequisite for understanding the biochemical and physiologic effects of the CK7-positive boundary cells[5]. Second, it is not known which point or stage of the colorectal genetic model involves these CK7-positive adenomatous cells of boundary regions, nor the exact route(s) of transduction of the possible "waves" in the ripple model as depicted in Fig. 2, the role of that route(s) in tumorigenesis or whether the progression to CK7-positive boundary cells is inexorable or can be arrested by nonsurgical means and, lastly, the possibility of the "ripple model" being applied to tumorigenesis of other organs.

Although the precise meaning of these CK7-positive adenomatous cells in the boundary region cannot yet be determined, our hypothesized model may enable us to predict the results of several future experiments, especially the genetic/molecular relationship between CK7 and early adenoma development in the genetic model for colorectal tumorigenesis. Further identification of genetic and molecular changes characteristic of the CK7-positive boundary cells will help in understanding the etiology of colorectal tumors and possibly establish useful markers or targets for clinical diagnosis and therapy.

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