Phenotypic change of muscularis mucosae in early invasive colorectal adenocarcinoma

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Abstract

***Background*—Invasive colorectal adenocarcinomas have bundles of eosinophilic spindle cells, which are regarded as myofibroblasts, in their desmoplastic stroma, some of which are continuous with the muscularis mucosa.**

***Aim*—To investigate the relation between the eosinophilic spindle cells and the muscularis mucosa based on their cytoskeletal phenotypes in early invasive colorectal adenocarcinoma.**

***Methods*—Formalin fixed, paraffin wax embedded tissues of 17 early invasive colorectal adenocarcinomas were immunostained for α-smooth muscle actin (α-SMA), desmin, and vimentin.**

***Results*—The phenotype of the muscularis mucosa was α-SMA positive, desmin positive, and vimentin weakly positive, whereas the eosinophilic spindle cells showed a decreased degree of immunoreactivity for α-SMA and desmin in particular, and an increased degree of immunoreactivity for vimentin. The degree of phenotypic difference between the muscularis mucosa and the eosinophilic spindle cells was greater in the eosinophilic spindle cells in the centre of the invasive area that were not continuous with the muscularis mucosa than in the eosinophilic spindle cells continuous with the muscularis mucosa.**

***Conclusions*—These findings suggest that the smooth muscle cells of the muscularis mucosa change their phenotype to become eosinophilic spindle cells, namely myofibroblasts, in the early invasive area of colorectal adenocarcinoma.**

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Keywords: colorectal adenocarcinoma; muscularis mucosa; myofibroblast; cytoskeletal phenotype

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The routine histological examination of invasive colorectal adenocarcinoma often reveals bundles of eosinophilic spindle cells in the desmoplastic stroma around carcinomatous glands. The desmoplastic stroma of invasive carcinoma is characterised by the infiltration of myofibroblasts,[1–2] and the eosinophilic spindle cells are also regarded as myofibroblasts.

The derivation of myofibroblasts is controversial, and local fibroblasts, smooth muscle cells, and pericytes are all thought to be candidate precursors of myofibroblasts, except in certain pathological situations in which specialised cells (interstitial cells of the pulmonary alveolar septa, glomerular mesangial cells, hepatic perisinusoidal cells, etc) undergo myofibroblastic change.[1 3] In general, local fibroblasts are regarded as the most likely candidate as the precursor of myofibroblasts.[1 3] However, in early invasive colorectal adenocarcinoma, bundles of the eosinophilic spindle cells are often continuous with the muscularis mucosa at the periphery of the invasive area (fig 1A). This finding is attractive from the aspect of the derivation of myofibroblasts, as well as the role of the muscularis mucosa in the early invasive process of colorectal adenocarcinoma. On the other hand, when bundles of the eosinophilic spindle cells are seen at the early invasive front of colorectal adenocarcinoma, it may not be easy to differentiate them from the muscularis mucosa remaining at the invasive front. Therefore, it is important for the precise histological diagnosis of the early invasion of colorectal adenocarcinomas to elucidate the relation between the eosinophilic spindle cells and the muscularis mucosa.

Cytoskeletal proteins are useful differentiation markers for the identification of phenotypes of stromal cells, and their use has revealed the phenotypic heterogeneity of fibroblasts/myofibroblasts.[1 4] Therefore, in our study, we investigated immunohistochemically the cytoskeletal phenotypes—the expression of α-smooth muscle actin (α-SMA), desmin, and vimentin—of the eosinophilic spindle cells in the early invasive area of colorectal adenocarcinoma, and compared this with the phenotype of the muscularis mucosa to determine the relation between them.

Methods

We selected from the files of the department of pathology, Saitama Medical School, 17 colorectal adenocarcinomas in which the invasion was limited to the submucosa and in which continuity between the muscularis mucosa and bundles of the eosinophilic spindle cells was seen at least in part. All had been endoscopically or surgically resected at Saitama Medical School Hospital or its related hospitals.

Serial sections were cut from formalin fixed, paraffin wax embedded samples of each lesion for histological examination with haematoxylin and eosin staining (H&E) and for immunohistochemical analysis using the indirect staining method. For immunohistochemistry of cytoskeletal proteins we used the following monoclonal antibodies (clone; source; dilution in parenthesis): anti-α-SMA (1A4; Dako, Glostrup, Denmark; 1/25), antidesmin (D33; Immunotech, Marseille, France; 1/25), and antivimentin (V9; Immunotech; 1/100). Antigen retrieval by autoclaving at 121°C for five

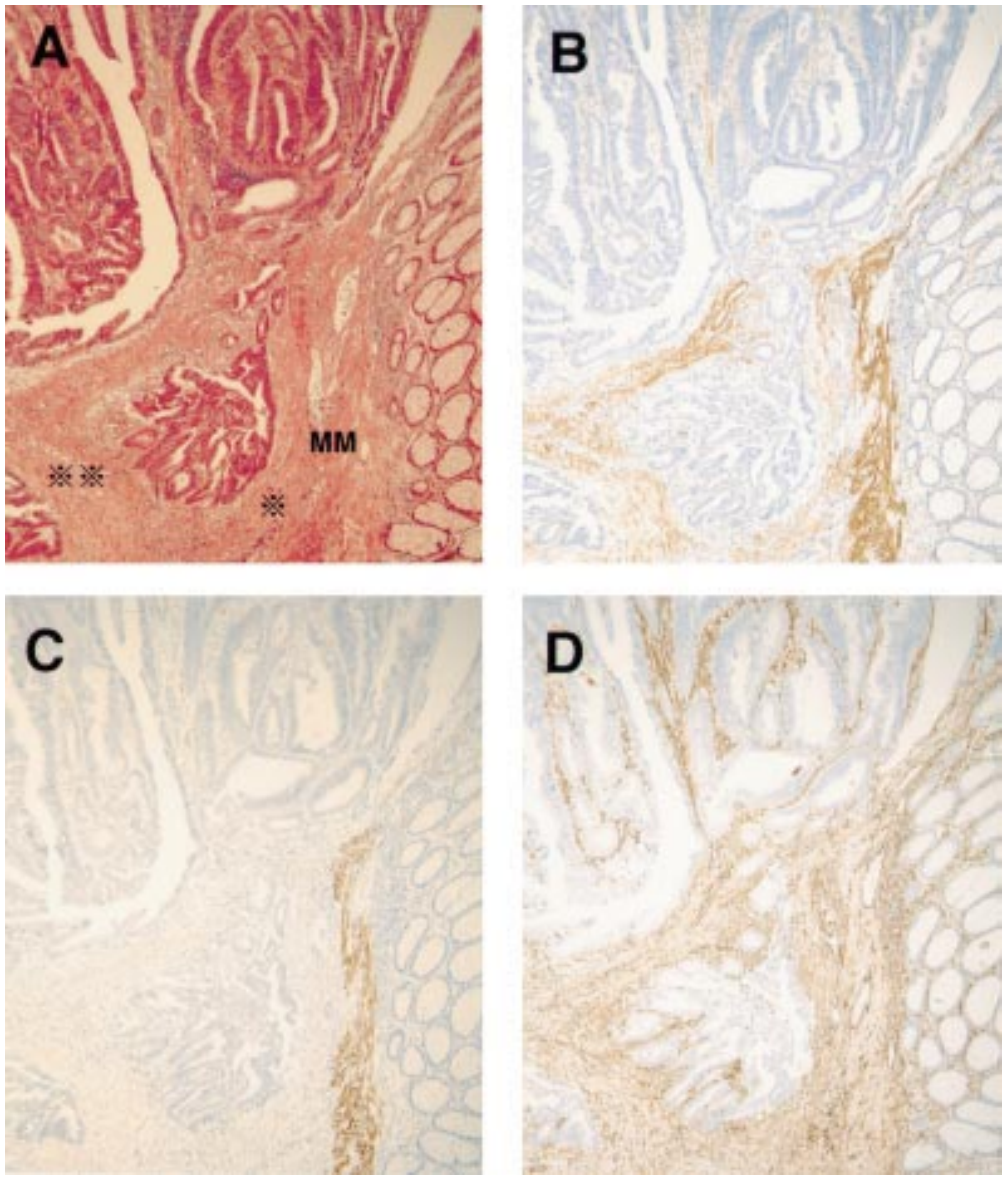

Figure 1 Bundles of eosinophilic spindle cells around the carcinomatous glands in the early invasive area of a colonic adenocarcinoma (A), showing the eosinophilic spindle cells (marked with one asterisk-like symbol) continuous with the muscularis mucosa (MM), and those (marked with two symbols) in the centre of the invasive area (haematoxylin and eosin). Immunohistochemically, the muscularis mucosa, the eosinophilic spindle cells continuous with the muscularis mucosa, and those in the centre of the invasive area are all positive for α-smooth muscle actin (α-SMA), although the muscularis mucosa is the most intensely positive (B). The muscularis mucosa is also positive for desmin; however, the eosinophilic spindle cells are almost negative (C). The positivity of vimentin is less intense in the muscularis mucosa than in the eosinophilic spindle cells (D).

minutes in citrate buffer was performed before the immunostaining of desmin and vimentin. Negative control sections were incubated without primary antibodies. Internal positive controls were normal submucosal fibroblasts for vimentin, and the muscularis propria for α-SMA and desmin, if available.

The immunohistochemical expression of each cytoskeletal protein was evaluated in three areas of each carcinoma that were defined as follows (fig 1A): (1) the muscularis mucosa, (2) bundles of the eosinophilic spindle cells continuous with the muscularis mucosa, and (3) bundles of the eosinophilic spindle cells in the centre of the invasive area that were not always continuous with the muscularis mucosa.

The degree of immunostaining at the above defined areas was evaluated according to the following analogue scale: almost all cells positive (+++), loss of positivity of some cells (++), a few scattered positive cells (+), and negative (−). For statistical analysis, we assigned each scale a score from 3 to 0 in the same order. When the evaluation of immunostaining took an intermediate scale such as +++/++ or ++/+++, we assigned it a mean score; namely, 2.5. The statistical difference between the scores of each area was tested using the Friedman test ($p < 0.01$).

Using the same samples, we also evaluated the cytoskeletal protein expression of pericryptal/intercryptal fibroblasts in the normal mucosae and of small arteries in the normal submucosae.

Table 1 Immunostaining scores for α-smooth muscle actin (α-SMA)

Case	Muscularis mucosa	ESC continuous with muscularis mucosa	ESC in the centre of the invasive area
1	3	2	0
2	3	2	2
3	3	2	1.5
4	3	3	2
5	3	3	2
6	3	2.5	2.5
7	3	0.5	1.5
8	3	3	2.5
9	3	2.5	2.5
10	3	2.5	2
11	3	3	3
12	3	3	3
13	3	1.5	2
14	3	2.5	1.5
15	3	2	1.5
16	3	1.5	0.5
17	3	2	2
Mean (SD)	3.0 (0.0)	2.3 (0.7)	1.9 (0.8)

ESC, eosinophilic spindle cells.

Results

The phenotype of the muscularis mucosa was α-SMA positive, desmin positive, and vimentin weakly positive (fig 1B–D). In comparison with the muscularis mucosa, a decreased degree of immunoreactivity for α-SMA (fig 1B) and, especially, for desmin (fig 1C), and an increased degree of immunoreactivity for vimentin (fig 1D) were demonstrated in the eosinophilic spindle cells, the degree of which was more evident in the eosinophilic spindle cells in the centre of the invasive area than in those continuous with the muscularis mucosa (tables 1–3). The statistical differences of immunostaining scores among the muscularis mucosa, the eosinophilic spindle cells continuous with the muscularis mucosa, and those in the centre of the invasive area were significant ($p < 0.01$) for α-SMA, desmin, and vimentin.

Table 2 Immunostaining scores for desmin

Case	Muscularis mucosa	ESC continuous with muscularis mucosa	ESC in the centre of the invasive area
1	2	0.5	0
2	1.5	0.5	0.5
3	3	1.5	0.5
4	3	1	0.5
5	2.5	1.5	0
6	2.5	1.5	0.5
7	2.5	0	0
8	3	0.5	0
9	3	0.5	1.5
10	2.5	1.5	0.5
11	2.5	0.5	0.5
12	3	2.5	0.5
13	2	0.5	0.5
14	2	2	0.5
15	2	1	0.5
16	2	1.5	0
17	3	1	0
Mean (SD)	2.5 (0.5)	1.1 (0.7)	0.4 (0.4)

ESC, eosinophilic spindle cells.

Table 3 Immunostaining scores for vimentin

Case	Muscularis mucosa	ESC continuous with muscularis mucosa	ESC in the centre of the invasive area
1	1.5	2	3
2	1	2.5	2.5
3	1	1.5	2.5
4	1	1.5	1.5
5	1	2	2
6	1	2	2
7	1	2	3
8	1.5	1.5	2
9	1.5	2.5	2.5
10	1	2	2
11	1.5	2.5	2.5
12	1.5	1.5	2
13	0.5	0.5	0.5
14	0.5	0.5	1.5
15	0.5	1	1.5
16	0.5	1.5	1.5
17	0.5	3	2.5
Mean (SD)	1.0 (0.4)	1.8 (0.7)	2.1 (0.6)

ESC, eosinophilic spindle cells.

In the normal colorectal mucosae, most pericryptal and intercryptal fibroblasts had the phenotype of α-SMA positive and/or vimentin positive, and desmin negative. A part of the α-SMA positive pericryptal fibroblasts was continuous with the muscularis mucosa at the crypt bases (fig 2A). Some of these pericryptal fibroblasts expressed both α-SMA and desmin only at the crypt bases (fig 2A and B). In the normal submucosae, fibroblasts expressed only vimentin, and the media of the small arteries was α-SMA positive, desmin partly positive, and vimentin positive. The muscularis propria was α-SMA positive, desmin positive, and vimentin negative or weakly positive.

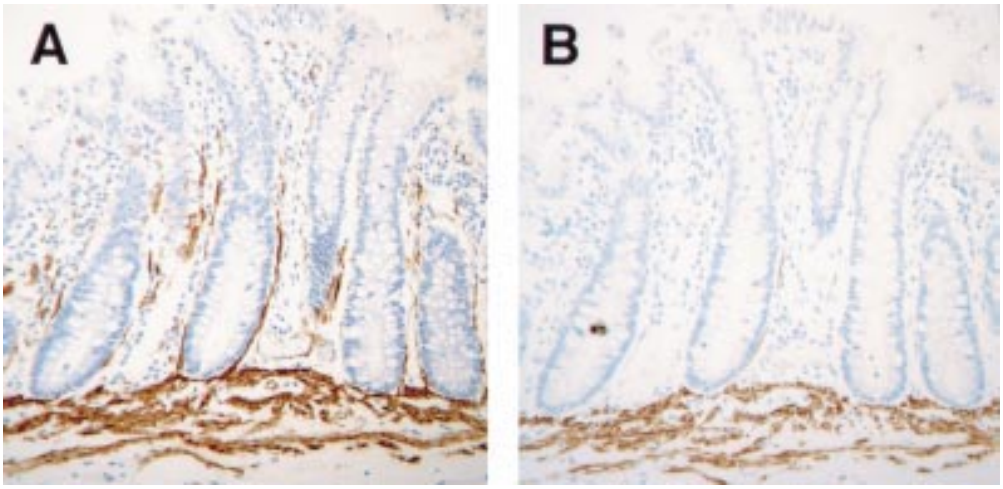

Figure 2 In normal colorectal mucosa, α-smooth muscle actin (α-SMA) positive pericryptal fibroblasts are continuous with the muscularis mucosa at the crypt bases (A). Some of those pericryptal fibroblasts are also positive for desmin (B).

Discussion

In our study, stromal cells in the early invasive area, which we referred to as bundles of eosinophilic spindle cells on routine H&E preparations and which were partly continuous with the muscularis mucosa, showed relatively preserved α-SMA expression, loss of desmin expression, and gain of vimentin expression. These cytoskeletal features are intermediate between those of the muscularis mucosa and those of the stromal myofibroblasts reported previously in invasive colorectal carcinoma (α-SMA positive, vimentin positive, and desmin negative).[2] In addition, the degrees of desmin loss and vimentin gain were more evident in the eosinophilic spindle cells distant from the muscularis mucosa than in those continuous with the muscularis mucosa. These findings suggest that the smooth muscle cells of the muscularis mucosa could change their phenotype and be converted to eosinophilic spindle cells, namely myofibroblasts, in early invasive colorectal adenocarcinoma. This concept is supported by the electron microscopic observations of Ohtani and Sasano, who suggested a muscularis mucosa or muscularis propria origin of myofibroblasts in the stroma of invasive colorectal carcinoma.[5]

In certain pathological settings, such as the pathogenesis of atherosclerosis[6] and the process of airway wall remodelling in asthma,[7] changes or modulations of vascular or airway smooth muscle cells from the contractile phenotype to the synthetic one have been reported to play an important role and to be implicated in matrix production. Moreover, smooth muscle cells of the synthetic phenotype are regarded as representing myofibroblasts derived from smooth muscle cells of the contractile phenotype.[7] Similarly, considering the formation of desmoplastic stroma during carcinoma invasion,[1 2] it is conceivable that myofibroblasts derived from the muscularis mucosa—phenotypically altered smooth muscle cells—play a role in desmoplastic stroma generation through matrix production. In this sense, the muscularis mucosa might not be a barrier against carcinoma invasion but instead may play an active role in carcinoma invasion.

In normal colorectal mucosae, pericryptal fibroblasts were shown to migrate from the crypt base to the surface.[8] They are α-SMA positive and regarded as myofibroblasts.[9 10] We found that some of these pericryptal myofibroblasts were continuous with the muscularis mucosa and expressed desmin as well as α-SMA at the crypt bases, losing desmin expression at the upper pericryptal zone. This observation suggests a muscularis mucosa origin of pericryptal myofibroblasts, with a phenotypic change from the crypt base to the surface. This could be regarded as the normal counterpart of the phenotypic change of the muscularis mucosa to eosinophilic spindle cells in the stroma of early invasive carcinoma, although some pericryptal myofibroblasts themselves might contribute to the formation of eosinophilic spindle cells.

Vascular smooth muscle cells and pericytes have been implicated as candidate precursors of myofibroblasts in carcinomatous stroma.[1 3 11] In spite of the similarity between the cytoskeletal features of submucosal vascular smooth muscle cells and those of eosinophilic spindle cells, the contiguity of the eosinophilic spindle cells with the vasculature was not evident in our study. Therefore, the vasculature appears to contribute little as a source of myofibroblasts, at least in the early phase of invasive colorectal adenocarcinoma.

In the routine histological diagnosis of colorectal adenocarcinomas, it is sometimes necessary to discriminate bundles of eosinophilic spindle cells from the muscularis mucosa to determine early invasion. Considering the phenotypic change of the muscularis mucosa described above, immunohistochemistry of cytoskeletal proteins can be useful. In fact, desmin immunohistochemistry is recommended to identify the muscularis mucosa remaining in the invasive front (M I Ikegami, 1999, personal communication).

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