Differential expression of high molecular weight caldesmon in colorectal pericryptal fibroblasts and tumour stroma

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Abstract

Aim—To investigate an extent of smooth muscle differentiation of pericryptal fibroblasts.

Methods—Expression of high molecular weight caldesmon (*h*-CD) and α smooth muscle actin was investigated in 123 invasive colorectal adenocarcinomas and their surrounding non-neoplastic tissues.

Results—The monoclonal antibody to *h*-CD, which showed predominantly positive immunostaining in well differentiated smooth muscle cells, recognised pericryptal fibroblasts, smooth muscle cells, and pericytes, but was not reactive to myofibroblasts. Antibody to α smooth muscle actin recognised not only pericryptal fibroblasts, smooth muscle cells, and pericytes but also myofibroblasts.

Conclusions—Pericryptal fibroblasts show greater smooth muscle differentiation than myofibroblasts and there is a possibility that they are well differentiated smooth muscle cells; *h*-CD is an excellent marker to discriminate pericryptal fibroblasts from myofibroblasts.

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Keywords: high molecular weight caldesmon; pericryptal fibroblast; colon; rectum

Pericryptal fibroblasts play an important role in the progression of colorectal epithelial neoplasms and non-neoplastic disorders.¹⁻³ It is

suggested that myofibroblasts in the stroma of colorectal carcinomas originate mainly from pericryptal fibroblasts.⁴ However, there have been no reports on the relation between pericryptal fibroblasts and myofibroblasts in the colon and rectum. To investigate the extent of smooth muscle differentiation in pericryptal fibroblasts, we examined expression of two smooth muscle markers, high molecular weight caldesmon (*h*-CD) and α smooth muscle actin, in colorectal mucosa.

Methods

We examined 123 surgically resected primary colorectal adenocarcinomas and their adjacent tissues. The monoclonal antibodies used were clone *h*-CD (Dakopatts) which recognises *h*-CD, and clone 1A4 (Dakopatts) which reacts with α smooth muscle actin. Immunohistochemical studies were performed by the streptavidin-biotin method using the Histofine SAB-PO(M) kit (Nichirei). In the present immunohistochemical study for *h*-CD, deparaffinised tissue sections in 10 mM citrate buffer (pH 6.0) received heat treatment in a microwave oven for 15 minutes before the primary antibody reaction. The antibody against *h*-CD was diluted to 1:50.

Results

In all the cases examined, *h*-CD was positive in pericryptal fibroblasts in the adjacent mucosa (fig 1A) and also positive for vascular smooth muscle cells, smooth muscle cells of muscularis mucosa and muscularis propria, and pericytes, but was negative in myofibroblasts in the desmoplastic tumour stroma (fig 1B); no *h*-CD positive cells were detected except smooth muscle cells and pericytes. In contrast, α smooth muscle actin was positive not only in pericryptal fibroblasts (fig 2A), vascular smooth muscle cells, smooth muscle cells of muscularis mucosa and muscularis propria (parenchymal smooth muscle cells), and pericytes, but also in myofibroblasts (fig 2B). The remaining stromal spindle cells were negative for *h*-CD and α smooth muscle actin. (Note: figs 1A and 2A, and 1B and 2B are from the same respective sites.)

Discussion

Pericryptal fibroblasts decrease in number in the sequence: adenoma, intramucosal carcinoma, submucosal invasive adenocarcinoma.¹ In ulcerative colitis, dysplastic epithelium transforms into adenocarcinoma, and pericryptal fibroblasts decrease in number.³ On the

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Figure 1 The representative case of immunostaining for high molecular weight caldesmon (*h*-CD) in colorectal normal and carcinoma tissues. (A) Pericryptal fibroblasts (arrows) are positive for *h*-CD. (B) Myofibroblasts in the desmoplastic stroma are negative for *h*-CD. T, carcinoma; M, muscularis propria.

Figure 2 The representative case of immunostaining for a smooth muscle actin (ASMA) in colorectal normal and carcinoma tissues. (A) Pericryptal fibroblasts (arrows) are positive for ASMA. (B) Myofibroblasts in the desmoplastic stroma are also positive. T, carcinoma; M, muscularis propria.

other hand, myofibroblasts are abundant in colorectal cancer stroma, and it is suggested that they are derived from pericryptal fibroblasts.⁴ In the previous studies concerning pericryptal fibroblasts, clone 1A4 and HHF-35 were used for a marker for pericryptal fibroblasts¹⁻³; the latter clone recognises muscle specific but not smooth muscle specific actin. Furthermore, these two antibodies unfortunately react not only with pericryptal fibroblasts but also with myofibroblasts, smooth muscle cells, and pericytes. To detect pericryptal fibroblasts more precisely, another novel marker should be used. In our previous study concerning myofibroblasts at the tumour border of colorectal adenocarcinomas, we performed immunostaining for α smooth muscle actin and *h*-CD in colorectal carcinomas, in order to differentiate myofibroblasts from smooth muscle cells objectively—because myofibroblasts expressed α smooth muscle actin and not *h*-CD, whereas smooth muscle cells were positive for both α smooth muscle actin and *h*-CD.^{5,6}

Caldesmon was originally purified from gizzard smooth muscle as a major calmodulin binding protein which also interacts with actin filaments.⁷ Two molecular weight forms of caldesmon are distributed in a wide range of tissues and cells.⁸ It is reported that *h*-CD, molecular weight 120–150 kDa, is associated

with smooth muscle contraction and localised exclusively in smooth muscle cells⁷; the other caldesmon, low molecular weight caldesmon (MW 77–80 kDa), is also detected in myofibroblasts and non-muscle cells.⁸

In this journal about 12 years ago,⁹ a monoclonal antibody PR2D3 was described, which reacted with the cell membrane of pericryptal fibroblasts, smooth muscle cells throughout the body, and myofibroblasts in Wharton's jelly (myofibroblasts in the desmoplastic stroma of carcinomas were not examined); it did not react with fibroblasts or with cardiac and striated muscle cells. The protein which the antibody recognised was of 140 kDa molecular weight.⁹ There is a possibility that this 140 kDa protein is identical to *h*-CD.

These data, and our present study, suggest that pericryptal fibroblasts share a common differentiation process with vascular and parenchymal smooth muscle cells and that they are more akin to differentiated smooth muscle cells than to myofibroblasts.

In conclusion, our findings indicate that *h*-CD is an excellent marker for pericryptal fibroblasts, and there is a possibility that these cells are in fact well differentiated smooth muscle cells.

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