

Reduced representation of collagen and reticular fibers in the lamina propria of human rectal mucosa 10 cm and 20 cm away from the malignant tumor

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Introduction

Recent studies have revealed that the initiation, progression and metastasis of the malignant disease depend on the interplay between malignant cells and elements of the tumor stroma, particularly fibroblasts and extracellular matrix (1). However, there is a little evidence about changes of stromal components in the remote surrounding of the malignant tumor. Lately, it has been noted that lamina propria at the distance 10 cm and 20 cm away from the rectal adenocarcinoma shows reduced cellularity, tissue edema and disorganization of the extracellular matrix (2). The aim of our study was to investigate the representation of collagen and reticular fibers in the extracellular matrix of the lamina propria in the rectal mucosa 10cm and 20 cm away from the malignant tumor using an objective morphometric approach.

Materials and Methods

Tissue samples of the rectal mucosa were endoscopically collected during examination of 23 patients suffering from adenocarcinoma. Control samples were taken from 10 healthy individuals with family history of the intestinal malignancy, within active endoscopy screening procedure. The biopsies of the rectal mucosa were routinely processed to Paraplast. Masson trichrome technique and Gomori's silver impregnation were used for demonstration of collagen and reticular fibers, respectively. Microphotographs of the slides were acquired with a digital camera Leica DFC295 connected to a light microscope Leica DM4000 B LED (Leica, Hüllhorst, Germany). A random selection of 15 microscopic fields per slide (5 fields in subepithelial region, 5 fields between the crypts of the lamina propria and 5 fields above lamina muscularis mucosae) was assessed at the magnification of x400. In each microphotograph, region of interest which included lamina propria and excluded crypts was drawn (Figure 1). Collagen fibers were identified using Color Picker Threshold plugin within open source software for digital image analysis Icy (Figure 2) (3). The density of collagen fibers in rectal lamina propria was expressed as an area occupied by collagen fibers divided by the area of the lamina propria selected with an imaging processor. The density of reticular fibers in the lamina propria of healthy individuals as well 10 cm and 20 cm away from malignant tumor was determined in the same manner as described for collagen fibers. Results were statistically analyzed with Student's T-test.

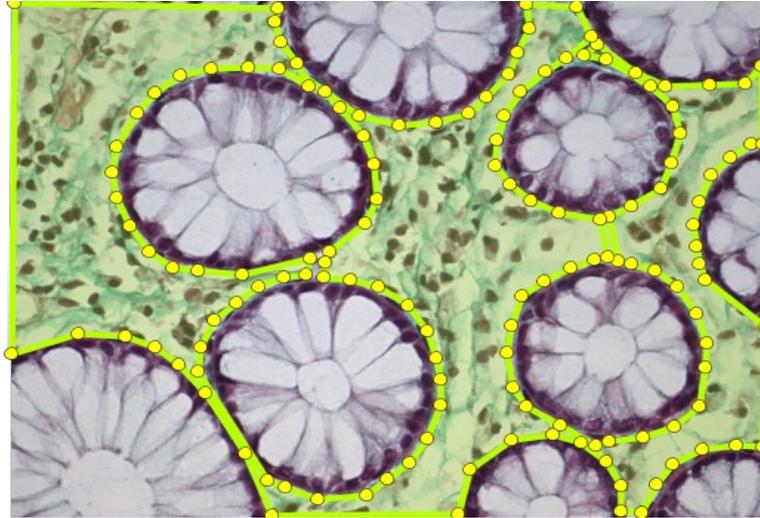


Figure 1. Cross section of the lamina propria of human rectal mucosa 20 cm away from the malignant tumor. Female, 62 years old. Region of interest which includes lamina propria, but excludes crypts drawn in software Icy. Masson trichrome. x 400

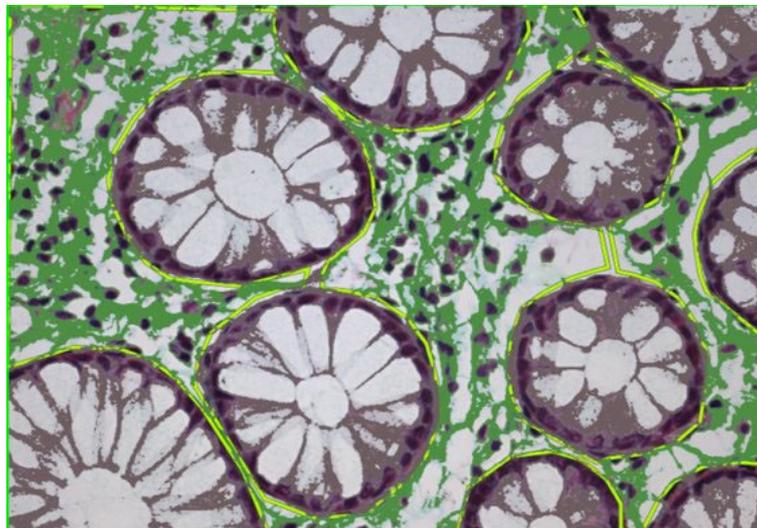


Figure 2. Cross section of the lamina propria of human rectal mucosa 20 cm away from the malignant tumor. Female, 62 years old. Collagen fibers detected using Color Picker Threshold plugin within software Icy. Masson trichrome. x 400

Results and Discussion

The representation of collagen fibers in the lamina propria of the remote rectal mucosa 10 cm and 20 cm away from the adenocarcinoma was significantly lower (41.31 ± 10.89 and 45.68 ± 12.22 , respectively; $P < 0.01$) in comparison to healthy individuals (58.16 ± 7.37). The representation of collagen was significantly lower ($P < 0.05$) in the lamina propria at the distance of 10 cm away from the malignant tumor compared with the representation at the distance of 20 cm. Similarly, the representation of reticular fibers was significantly decreased in the lamina propria of rectal mucosa 10 cm and 20 cm away from the tumor in comparison to the tissue of healthy individuals.

Collagen is the most represented component of the extracellular matrix and affects proliferation and surviving of tumor cells, as well as the formation of metastasis (4). Possible mechanisms involved in reduction of collagen fibers inside of the tumor could be increased destruction of collagen with the enzymes produced by tumor cells or inflammatory cells, changed synthetic activity of the fibroblasts or relative reduction of collagen representation due to tissue edema (5). Our study shows that significant reduction of collagen and reticular fibers could also be observed at the distance 10 cm and 20 cm from primary lesion. Described changes in representation of collagen and reticular fibers could represent consequence of the growing tumor or initial changes of the intestinal mucosa which provides adequate microenvironment for tumor progression.

Conclusion

The reduction of collagen and reticular fibers is present not only in the stroma of the tumor, but also in the lamina propria of the rectal mucosa 10 cm and 20 cm away from the malignant lesion.

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