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## Cell-cell interactions in the gastrointestinal tumour-microenvironment

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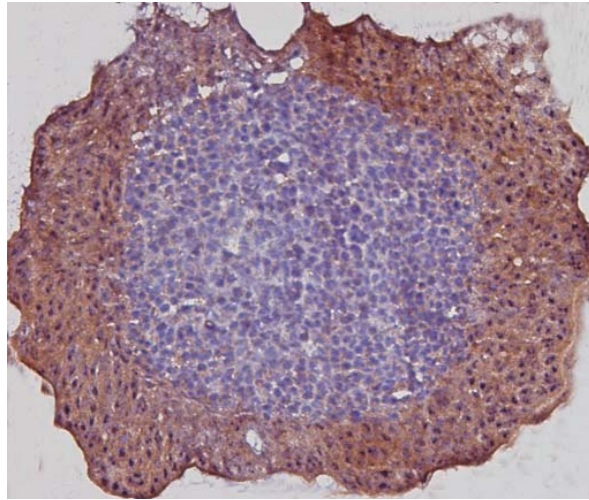
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## Full-colour illustrations



*HT29 spheroid stained for laminin (chapter 12)*

Chapter 3

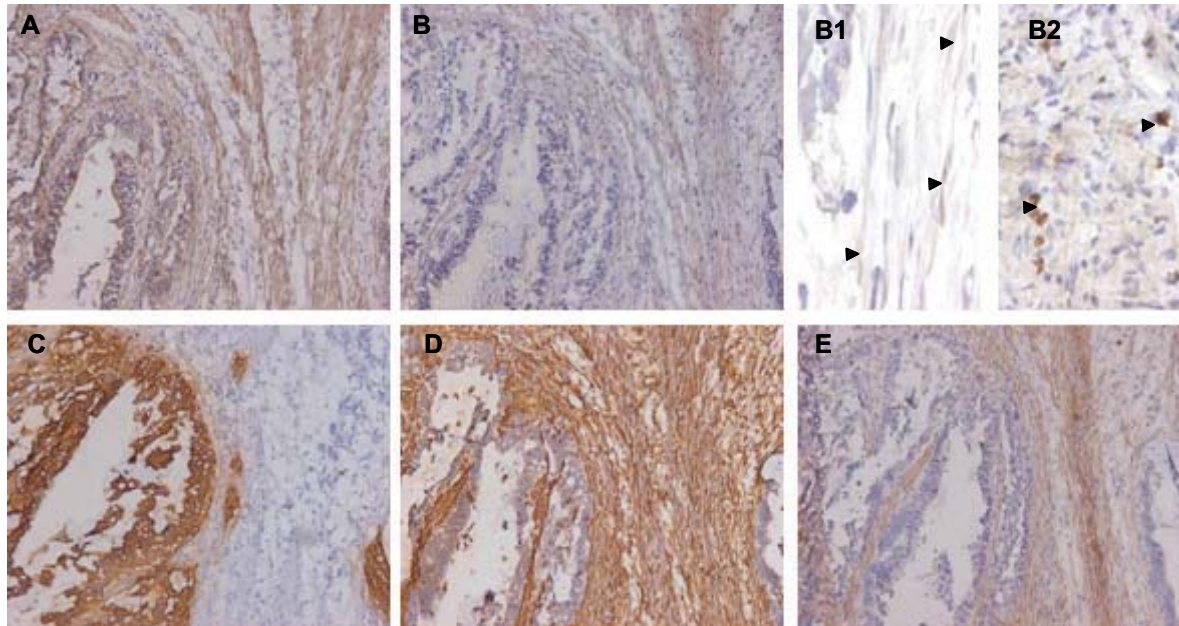


Figure 2. Cellular localisation of active TGF- $\beta$ 1 in gastric cancer. Immunohistochemical staining of gastric carcinomas (sequential frozen sections). Staining pattern for active TGF- $\beta$ 1 (A) corresponds to phospho-smad 2 staining (B). Inserts B1 and B2 show respectively nuclear localisation (arrowheads) of p-smad-2 in the myofibroblasts and malignant cells. As shown by staining for pan-Cytokeratin (C, epithelial marker), Vimentin (Vim, D, mesenchymal marker) and Smooth Muscle Actin (SMA, E smooth muscle/myofibroblast marker) TGF- $\beta$ 1 activity is observed in malignant cells and in Vim<sup>+</sup>/SMA<sup>+</sup> cells (myofibroblasts). Magnification 200x, B1-B2 630x.

## Chapter 3

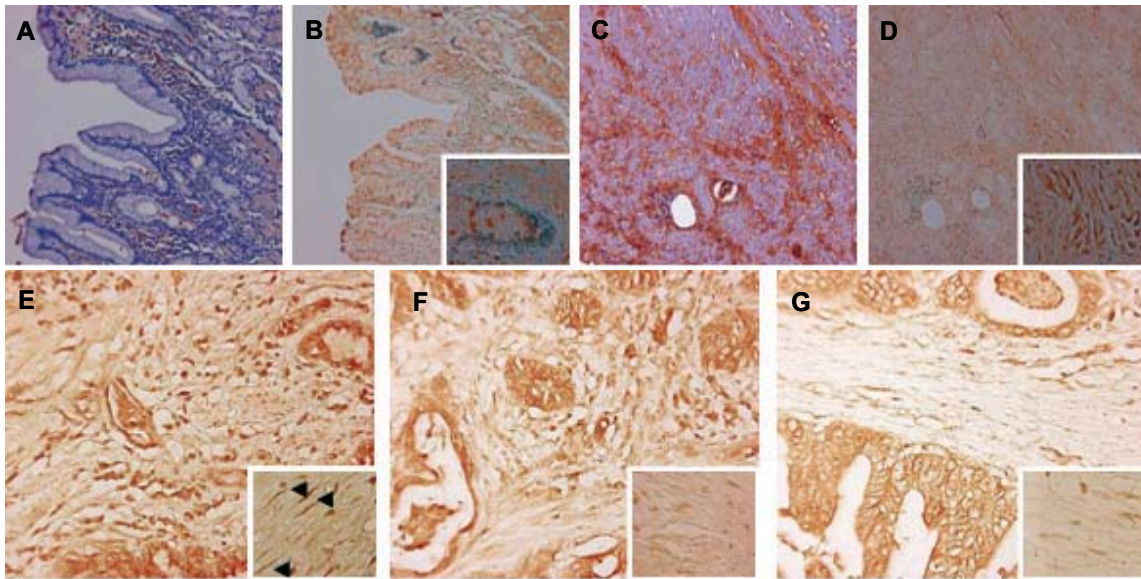


Figure 3. Total TGF- $\beta$  and p-smad-2 staining on paraffin embedded gastric cancer tissue sections.

Staining on normal gastric mucosa shows some staining for total TGF- $\beta$  (A) and almost no staining for p-smad-2 (B, insert 400x). Both are strongly increased in corresponding tumour tissue (C, total TGF- $\beta$ 1, D, p-smad-2, insert 400x). Figure E-G shows p-smad 2 staining in 3 different gastric carcinomas with high (81.3 pg/mg, E), median (21.1 pg/mg, F) and low active TGF- $\beta$ 1 levels (1.6 pg/mg, G). A strong decrease in nuclear staining (inserts E-G, magnification 630x, arrowheads indicate intense nuclear staining in myofibroblasts in E) is observed with especially in myofibroblasts (staining for pan-Cytokeratin, Vimentin and Smooth Muscle Actin on sequential sections, not shown). Magnification 200x.

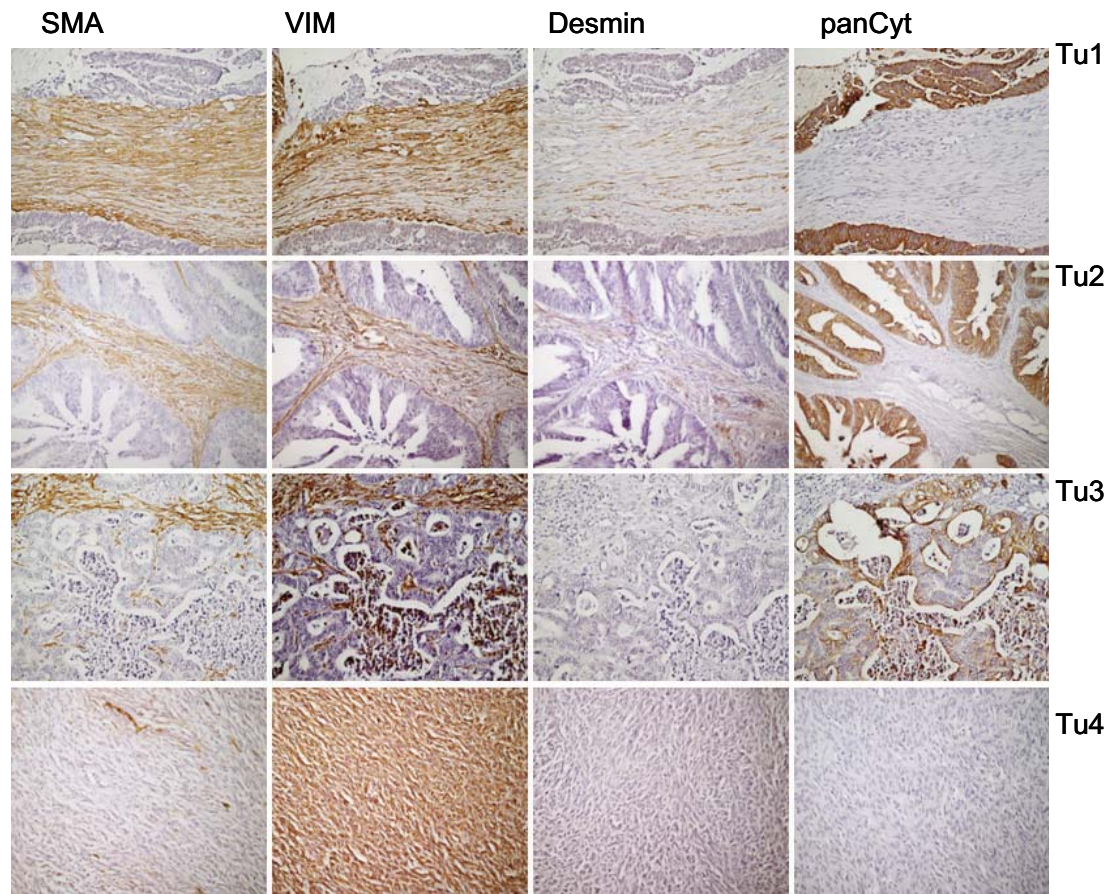


Figure 3F. Immunohistochemistry on Tu1-Tu4 samples, SMA = smooth muscle actin, myofibroblast and smooth muscle cell marker; Vim = vimentin, stromal cell marker; desmin, smooth muscle cell marker; pan-cytokeratin, epithelial marker).

Chapter 5

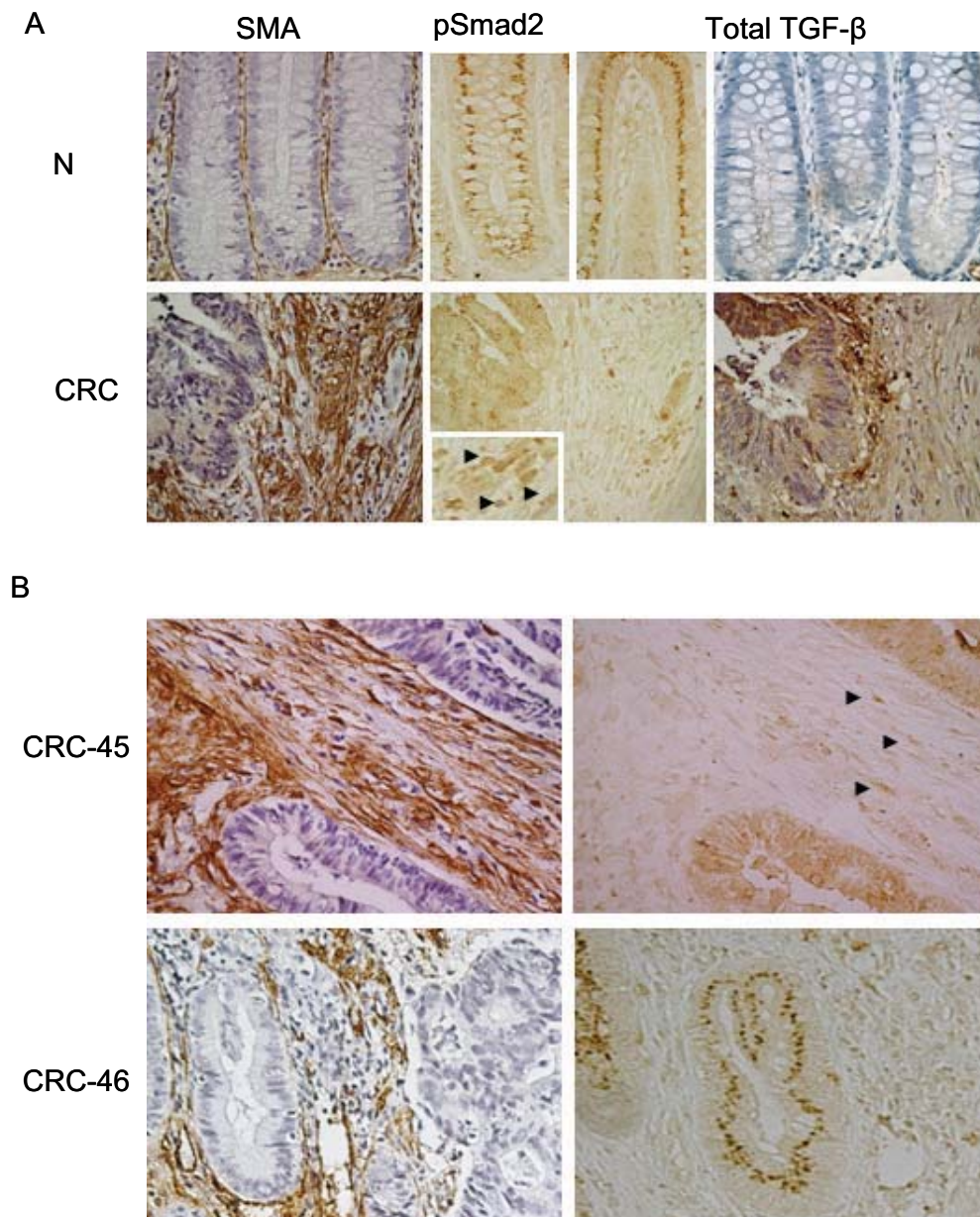
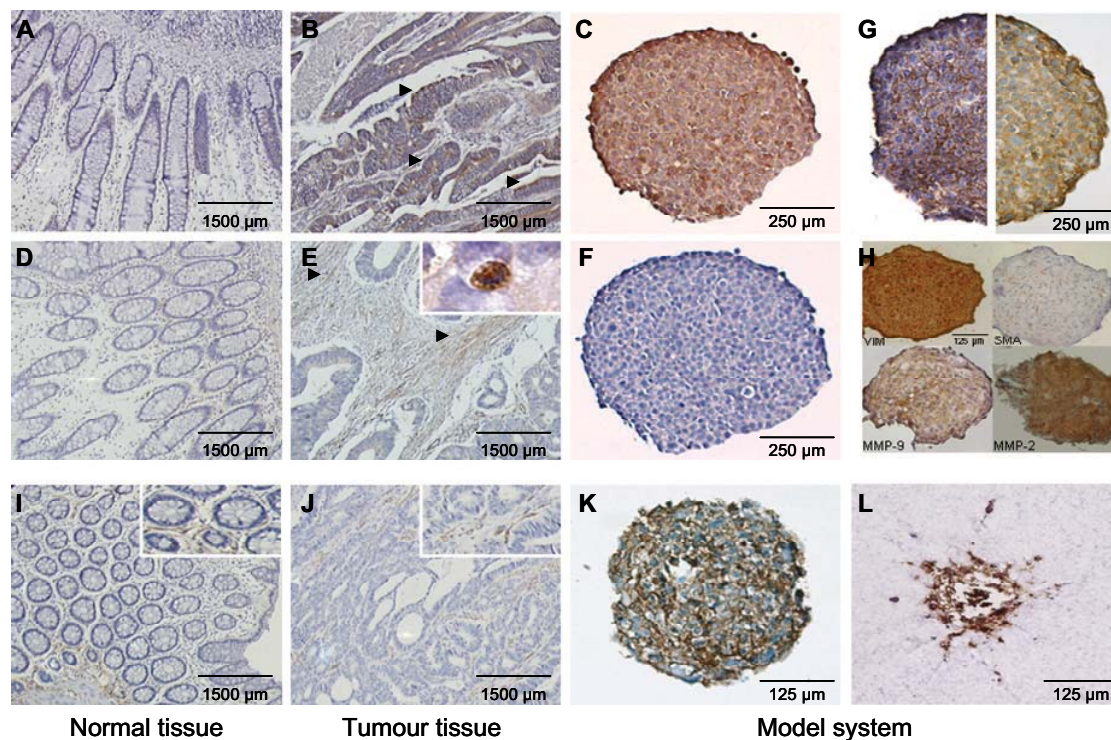


Figure 1. Immunohistochemistry on CRC tissue samples. Normal colonic mucosa displays a single layer of SMA positive myofibroblasts along the crypt axis, which show no nuclear staining for pSmad2, in contrast to epithelial cells, which show a gradient in staining from bottom to top of the crypt. Total TGF- $\beta$  is present in low levels in normal mucosa, whereas it is strongly increased in CRC (A). Strongly increased SMA expression and a shift from epithelial to mesenchymal nuclear accumulation of pSmad2 occurs in CRC, although some samples, like CRC-46, still show nuclear accumulation of pSmad2 in malignant cells next to myofibroblasts (B).

## Chapter 6



*Figure 2. Immunohistochemical staining of normal and tumour colon tissues and 3-dimensional cell culture models showing the resemblance between patients derived tissue and in vitro cell culture models. VEGF expression was very low in normal colon tissue (A) and strongly increased in colon tumour cells (B, arrowheads). HT29 colon cancer spheroids strongly express VEGF (C). MMP-9 was hardly detectable in normal colon tissue (D) and strongly expressed in stromal cells in colorectal cancer (E), especially in neutrophils (enlarged insert, 630x). HT29 spheroids were negative for MMP-9 (F), and produced VEGF binding HSPGs (G, left) and the ECM molecule laminin (G, right). Fibroblast spheroids (H) were positive for vimentin (VIM) and for SMA. MMP-9 was expressed, but MMP-2 was more abundant. CD34 showed minor staining in normal colon except for the submucosal area (I, insert). In tumours many neo-angiogenic CD34 positive vessels are present in the proximity of tumour cells (J, insert, 630x). HUVEC spheroids were positive for CD31 (K) while sprouting endothelial cells showed additional positivity for the angiogenesis marker CD105 (L).*

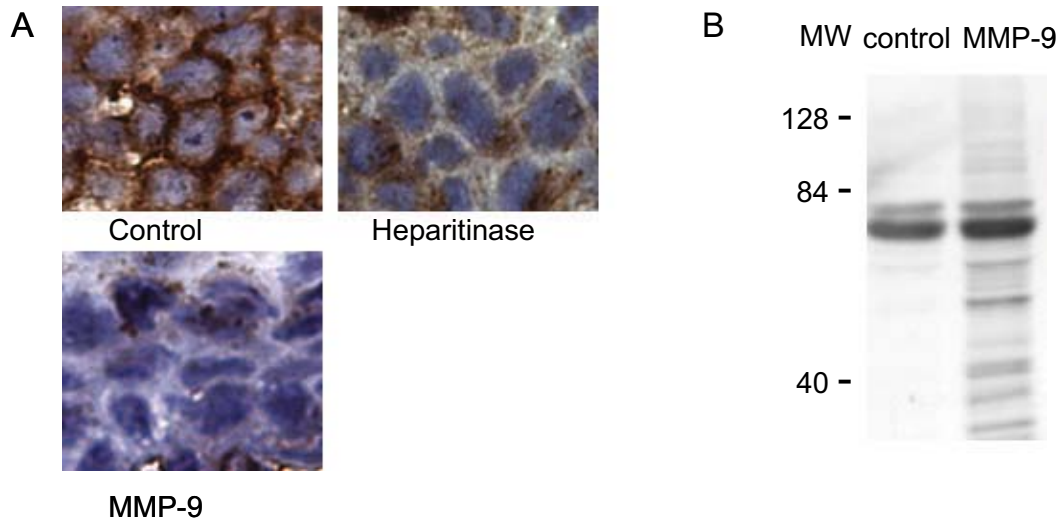


Figure 4. A) Immunohistochemical staining for HSPGs with monoclonal antibody 10E4 of HT29 spheroids after treatment with MMP-9 or heparitinase resulting in decreased extracellular localization of HSPGs (magnification 630x). B) Western blot of medium from HT29 spheroids treated with MMP-9 and subsequently stained with monoclonal antibody 3G10, specifically recognizing epitopes on HSPGs after cleavage.

### Chapter 7

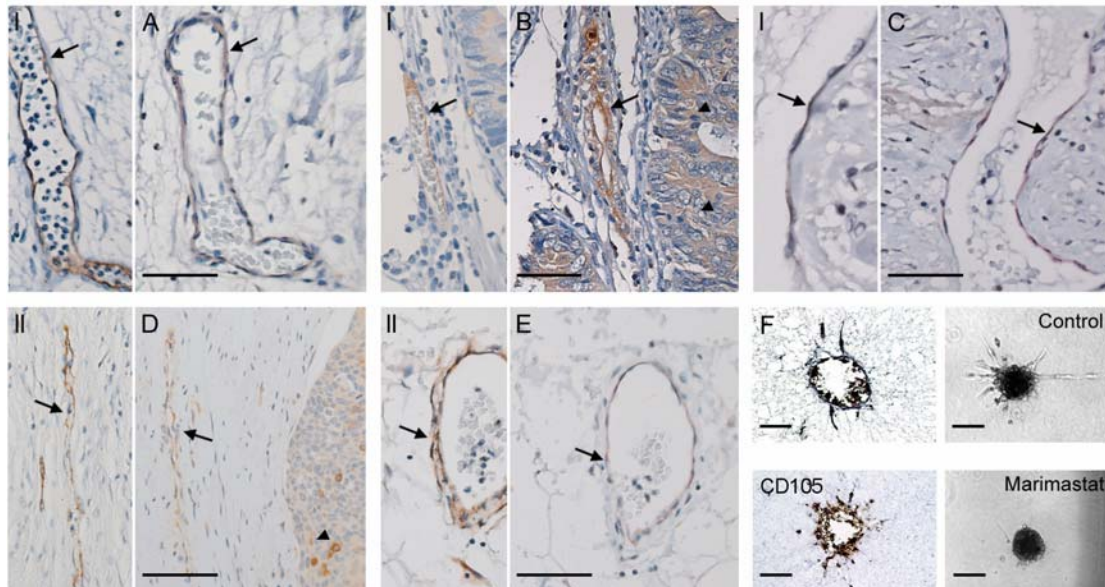


Figure 1. Immunohistochemical staining of endothelial cells for MMP-7, indicated by arrows in gastric cancer (A), colonic cancer (B), breast cancer (C), cervical cancer (D), prostate cancer (E), and in in vitro sprouting HUVEC cells (F). Inserts I and II indicate respectively CD34 and CD105 staining in sequential section from the same tissue. The inserts in (F) show VEGF-induced endothelial cell sprouting in control and marimastat-treated HUVEC spheroids. Arrowheads indicate epithelial cell staining. Bars correspond with 100  $\mu$ m in (A-E) and with 300  $\mu$ m in (F).



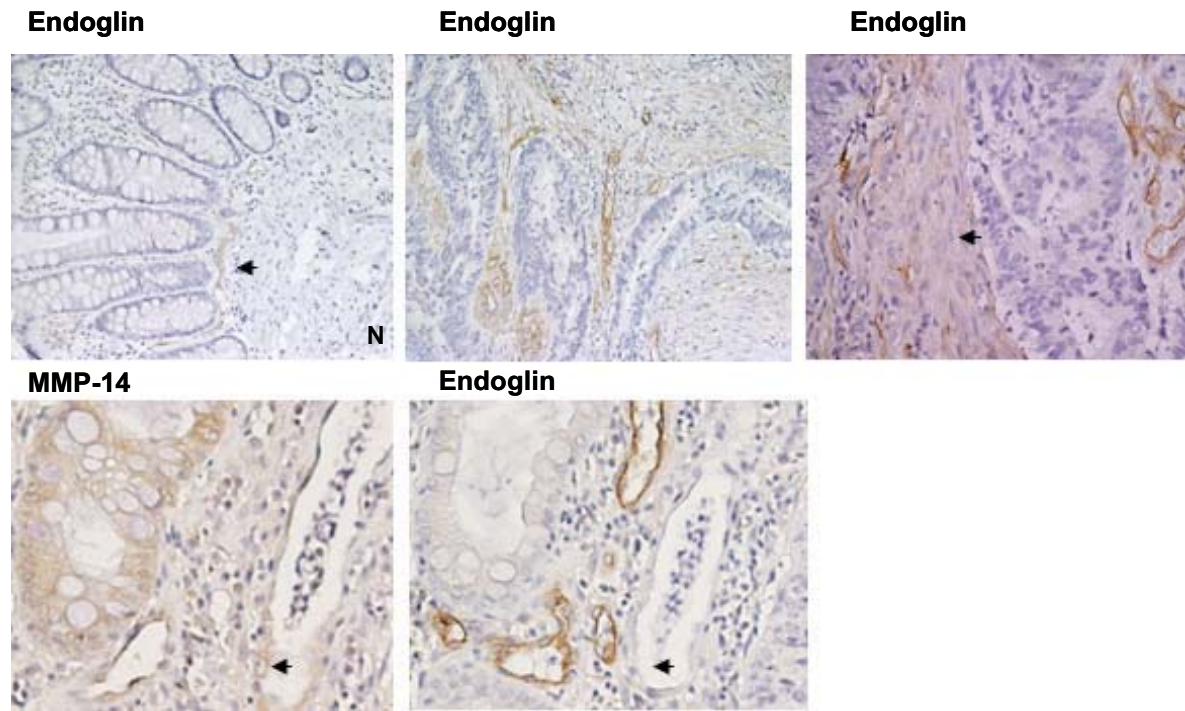


Figure 2 Endoglin (CD105) immunohistochemistry on normal mucosa (N, left panel) and colorectal cancer specimens. The lower panels show staining on sequential sections for Endoglin and MMP-14.

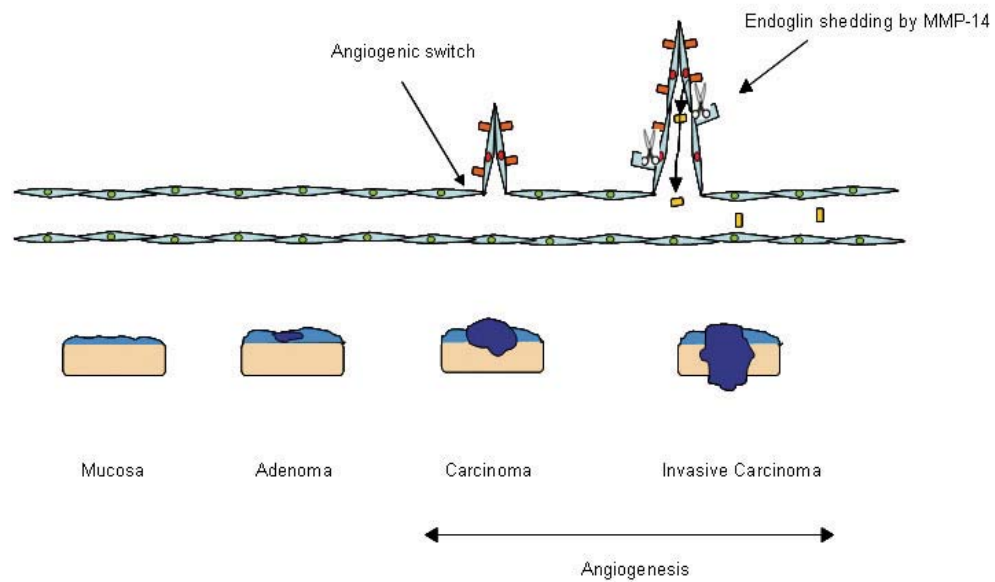


Figure 6 Proposed mechanism of Endoglin expression and generation of sEndoglin in colorectal cancer.