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The Plasminogen System in Microdissected Colonic Mucosa Distant from an Isolated Adenoma

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Abstract In the colon, the urokinase-type plasminogen activator (uPA), its receptor (uPAR), and plasminogen activator inhibitors, PAI-1 and PAI-2, are implicated in the transition from mucosa to adenoma and tumour progression. However, expression in the mucosa adjacent, or distant, to an adenoma has not yet been investigated. Three biopsies from mucosae adjacent (20 cm, ipsilateral) and distant (contralateral) to an isolated tubular adenoma were analysed in 14 patients and 8 controls. Laser microdissection isolated stromal and epithelial crypt components, and quantitative RT-PCR analyses of uPA, uPAR, PAI-1 and PAI-2 mRNA levels were performed. Among controls, no significant differences in the markers were noted. With left colon isolated tubular adenoma, uPA, uPAR, and PAI-2 mRNA levels were significantly increased in the adjacent mucosal stroma compared to epithelial crypt levels ($p<0.05$). In right colon adenoma, the mRNA levels of these 3 molecular markers were significantly increased only in the adjacent mucosal stromal samples ($p<0.05$). Isolated tubular adenoma in the colon increases significantly the mRNA levels of 3 proteolysis-associated molecular markers in the stromal, but not in the epithelial, components of adjacent mucosa. These results suggest the presence of regional and dynamic interactions in apparently non-involved mucosae.

Keywords Urokinase-type plasminogen activators · Plasminogen activator inhibitors · Laser microdissection · Tubular adenoma · Colonic stroma · Colonic epithelium

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Introduction

Colorectal cancer is the third most common cancer worldwide and the second most frequent cause of cancer-related deaths [1]. There is now strong evidence that most, if not all, colorectal carcinomas develop from precursor non-invasive adenomatous polyps. The transition from an adenomatous polyp to a malignant invasive lesion correlates with three independent features: polyp size, histological type and severity of epithelial dysplasia, the highest transition risk being in large villous adenomas with severe dysplasia [2]. It is currently viewed that genetic alterations, including mutations in proto-oncogenes, in tumour suppressor and DNA mismatch repair genes, underlie initiation of adenomas and progression to carcinomas [3]. Some of these genetic lesions have been shown to affect the morphogenetic signalling pathways [4]. In addition, epigenetic environmental information expressed by stromal mesenchymal and/or inflammatory cell types may participate in inducing switches in cellular epithelial phenotypes and thus contribute to tumour progression [5, 6].

Breakdown of extracellular matrices (ECM), considered as a key step in cancer invasion and initiation of metastases, can be accomplished by the concerted action of proteolytic enzymes, including the serine protease plasmin, and a number of matrix metalloproteinases. It is now recognized that the plasminogen activation system (PA) can modulate both proteolytic-dependent and–independent processes.

including cell proliferation, cell migration and ECM degradation. The urokinase plasminogen activation system (PA) comprises uPA, its receptor uPAR (CD87) and two endogenous inhibitors PAI-1 and PAI-2 [7]. Besides proteolysis, the PA system can activate a number of cascade pathways, such as the FAK and ERK/MAPK signaling, thus affecting cell adhesion, migration, proliferation as well as epithelial cell plasticity [8, 9]. Paradoxically, the primary endogenous plasminogen activator inhibitor, PAI-1, has been found active in cell-matrix "de-adhesion" [10] and uPA and PAI-1 levels in tissues of various tumour types may predict adverse patient outcome [11]. Recent advances in our understanding of the mechanisms of cancer invasion and metastasis have led us to reconsider the evolving role of proteolytic activities in tissue remodelling [12].

We previously showed that expression of uPA mRNA was stromal and adjacent to foci of epithelial dysplasia in large size colorectal adenomas (larger than 1 cm in size).Both uPAR and PAI-1 mRNAs could be found by in situ hybridization in focalised subpopulations of stromal cells [13]. We then showed that PAI-1 and uPA protein levels were higher in adenomas than in adjacent mucosae, but the uPA levels correlated only with the grade of epithelial dysplasia [14]. Additional results suggested that PAI-1 could be an indicator for adenoma recurrence following polypectomy [15].

Moreover, it is now recognized that initiation or progression of tumours requires complex and collaborative interactions between premalignant or malignant cells and a variety of stromal cell types recruited and activated at both primary and secondary tumour tissue sites. As recently pointed out, genetically altered epithelial cells may interact with their adjacent stroma to form a supportive and permissive environment and tumour stage may depend on the nature and extent of stromal activation [16].

In this report, we sought to investigate whether the presence of an adenomatous lesion could affect the expression levels of uPA, uPAR, PAI-1 and PAI-2 mRNAs in adjacent ipsilateral (20 cm from the adenoma) or distant contralateral mucosa biopsies. Laser-mediated microdissection was performed on frozen sections to isolate and analyse selectively epithelial and stromal tissue samples. We show here that combining laser-based microdissection and real-time PCR allows the quantitative assessment of regional dynamic interactions affecting components of the PA system which take place in the early stages of an initial adenomatous lesion.

Materials and Methods

Patients and Tissue Samples

The first group comprises 4 men and 3 women (mean age 74.3±5.6 years) each presenting with colorectal adenoma in the left colon. The second group included 3 men and 4 women (mean age 76.1±5.2 years), but each with an adenoma in the right colon. The third group included 4 women and 4 men (mean age 75.2±4.8 years), free of colorectal neoplasia, polyps or inflammatory bowel disease who underwent colonoscopy for irritable bowel syndrome and were defined as the control group.

Adenomas were harvested during colonoscopy and histology sections were examined by two experienced pathologists. Histological type and grade of dysplasia in the adenoma were determined according to the criteria of the World Health Organization [17] and of the National Polyp Study Group [18]. Low-grade dysplasia corresponded to mild and moderate dysplasia, and high-grade to severe dysplasia. We biopsied macroscopically normal colorectal mucosa in all patients with adenoma. Three biopsies were done within an area of 1 cm^2 ipsilateral to the adenoma at a minimal distance of 20 cm from the adenoma; these biopsies were then defined as adjacent, non-involved mucosa as illustrated in Fig. 1. Three other biopsies were also performed contralateral to the adenoma and were defined as distant non-involved mucosa. In the control group, three biopsies were done in the right colon and three in the left colon. All biopsies were taken with the same type of forceps in a standardized technique to obtain approximately identical sampling volumes. Immediately after endoscopic removal, the biopsies were divided in two. One part was frozen in liquid nitrogen-cooled 2-methylbutane and stored at –80°C and the remaining part was referred for routine histological examination.

The study protocol was approved by the Ethics Committee of the Faculty of Medicine of the University of Lausanne. All patients gave their written informed consent.

Laser Capture Microdissection

The Freshly frozen colorectal mucosal biopsies were cut into 15-μm thick sections with a cryostat, then dried for 30 min at room temperature. The sections were fixed within IHC zinc fixative Plasmigen for 5 min, and then stained with Mayer's haemalum (1 g heamatoxylin in 1000 mlH2O DEPC, 200 mg sodium iodate, 50 g potash alum, 50 g chloral hydrate, 1 g citric acid) for 5 min. They were rinsed in tap water DEPC then air dried and placed at room temperature for 10 min. The slides used for laser-capture microdissection (LCM) were provided with a *5*-μm thick, clear polyethylene membrane incubated for 30 min in an ultraviolet chamber to reduce electrostatic charges and eliminate Rnases. Using a Leica LMD apparatus (Leica Microsystems, Mannheim, Germany), we determined the laser power and the aperture for each sample. The LCM procedure started with the epithelial cells of the crypts (Fig. 2), which were collected in an Eppendorf tube

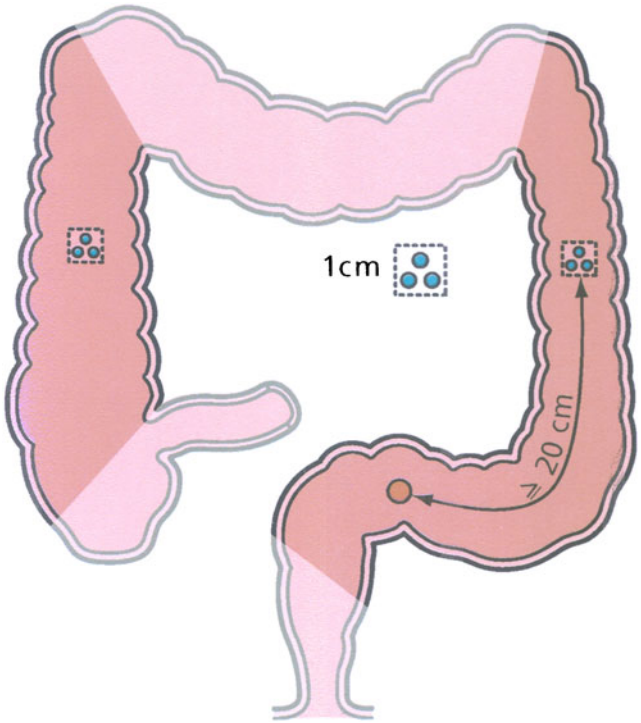

Fig. 1 Diagram of the colon with the adenoma (*red point*) and the biopsies (*blue points*) 20 cm away

previously filled with 50 μl of lysis buffer (Buffer RLT, Qiagen Rneasy) and 1% β-mercaptoethanol. The Eppendorf tubes were changed every hour to prevent evaporation and stored on ice. Using the same slides, laser-assisted microdissection was then applied to isolate stromal components cf. Fig. 2.

Real-Time Quantitative Polymerase Chain Reaction (RT PCR)

We selected 30 crypts (~ 300 cells) for microdissection. The adjacent stroma was microdissected to obtain the same volume. Selected crypts and stromal cells were immersed in 50 μl lysis buffer. The total RNA was extracted using Rneasy Mini Kits (Qiagen, Hilden, Germany), as described by the manufacturer, followed by an on-column Dnase treatment using an RNase-Free DNase Set (Qiagen, Hilden, Germany). The RNA was then concentrated using a Microcon (Millipore AB, Sundlbyberg, Sweden) by bringing the total volume of RNA to 500 μl, then centrifuging on Microcon-100 spin at 500 g for 20 min at 4°C. We then added 500 μl of cold water and centrifuged at 500 g for 22 min at 4°C. The Microcon was inverted and centrifuged at 1000 g for 3 min at 4°C.

First-strand cDNA was synthesised by using Sensiscript reverse transcriptase (Qiagen, Hilden, Germany). The total RNA was set on ice, and the following reagents were added: 1 μl enzyme (Sensiscript RT, Qiagen, Hilden, Germany), 2 μl oligo-dT, 2 μl deoxynucleotide triphosphate , 2 μl 10x buffer, 1 μl Rnase inhibitor added to Rnase free water to have a total reaction volume of 20 μl. The reaction was allowed to proceed for 60 min at 37°C, then adding 30 μl Rnase free water was added.

The sequences of uPA, uPAR, PAI-1 and PAI-2 intron overlapping primers were as follows: for uPA 5′-GCAG CCATCCCGGACTATAC–3′ (forward primer 886),

3′-TCGTTATACATCGAGGGCAGG–5′ (reverse primer 916); for uPAR, 5′-CCTGGAGCTTGAAATCTGCC–3′ (forward primer 474), 3′-CATGGGTGCTGTTCCCCT T–5’ (reverse primer 541), for PAI-1, 5’–CCGCCTCTTCCACAAATCAG–3’ (forward primer 627), 3’-GCCATCATGGGCAGAGA–5’ (reverse primer 677); for PAI-2, 5’-GCAGGCACAAGCTGCAGATA–3’ (forward primer 285), 3’-GAGCTGAGAGAGCGGAA GGAT–5’ (reverse primer 335).

Each PCR assay was repeated 2 times for each cDNA sample. The 25-μl total PCR volume consisted of 1 μl cDNA, 12.5 μl Sybr green PCR mastermix 2x (Eurogentec, Belgium), 3.5 μl water and 4 μl each of forward/reverse primers at 5 μM (MWG-Biotech, Ebersberg, Germany). After denaturation for 4 min at 95°C, the PCR assays were carried out for 60 cycles, with denaturation at 95°C for 15 s and annealing and extension at 60°C for 1 min. To monitor amplification of possible contaminating DNA samples, distilled water served as a negative control. The results were normalized with GAPDH.

Statistical Analysis

All the results were expressed as the mean±standard deviation of at least three independent experiments. Statistical analysis used a two-tailed paired *t* test. Probability values <0.05 were considered significant. Statistics were performed with StatView 5.0.1 (SAS Institute Inc.,Cary, NC USA).

Results

Histopathologic Evaluation

Half of the adenomas were classified as low grade and the other half as high grade. Resected polyps were all tubular adenomas. Carcinomas were not found. Adjacent and macroscopically normal distant colonic tissues were also examined. All these biopsies were microscopically normal. The extent of stromal inflammatory cells was compared among the samples and no significant differences could be recognized.

In the control group, all the samples were classified as normal.

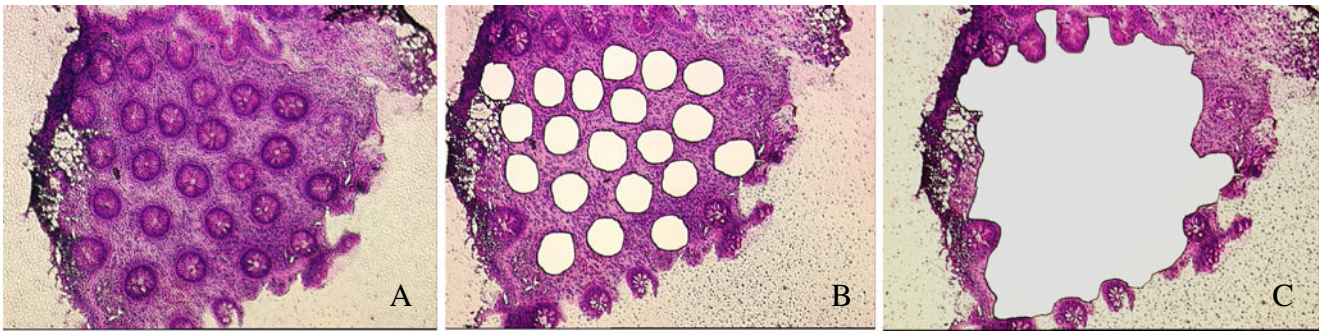

Fig. 2 **a** Histological preparation for LCM of normal colonic tissue:, **b** Harvesting of colonic crypts by LCM and **c** Harvesting of stroma around the crypts (×20)

uPA, uPAR, PAI-1 and PAI-2 mRNA Ratio Expression in Normal Colonic Tissue Ipsilateral or Contralateral to a Left Adenoma

As shown in Fig. 3, uPA, uPAR, and PAI-2 mRNA concentrations within the microdissected stromal part of the ipsilateral normal colonic tissue were significantly increased ($p<0.05$) when compared to those found in the epithelial crypts. These changes were not observed in the contralateral normal colonic tissue or in the PAI-1 ipsilateral stroma. Moreover, the mRNA concentrations found in the ipsilateral and contralateral epithelial crypts did not significantly differ.

uPA, uPAR, PAI-1 and PAI-2 mRNA Ratio Expression in Normal Colonic Tissue Ipsilateral or Contralateral to a Right Adenoma

Figure 4 shows that the uPA, PAI-1 and PAI-2 mRNA ratio expression in the stroma of the right colon was significantly increased ($p<0.05$) which was not observed in the ipsilateral or in the contralateral part of the colon, with no

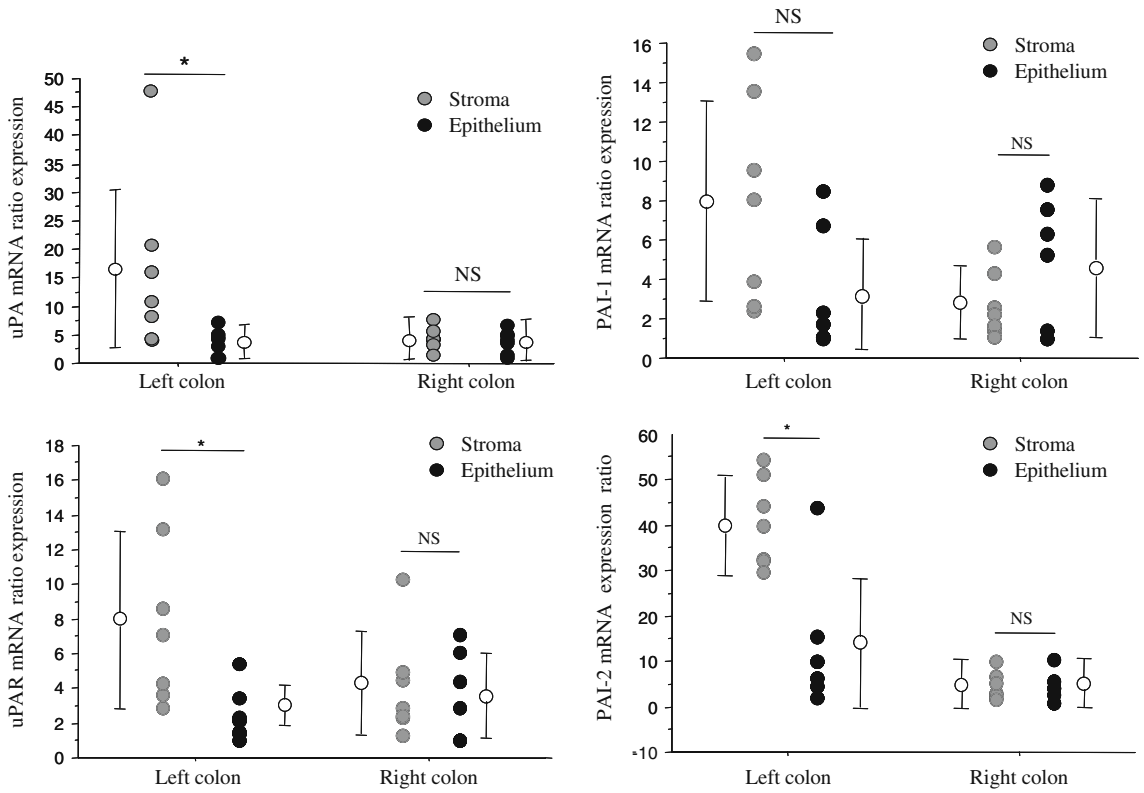

Fig. 3 uPA, uPAR, PAI-1 and PAI-2 mRNA expression in ipsilateral and contralateral normal colonic tissue in patients with left colonic adenoma
* $p<0.05$

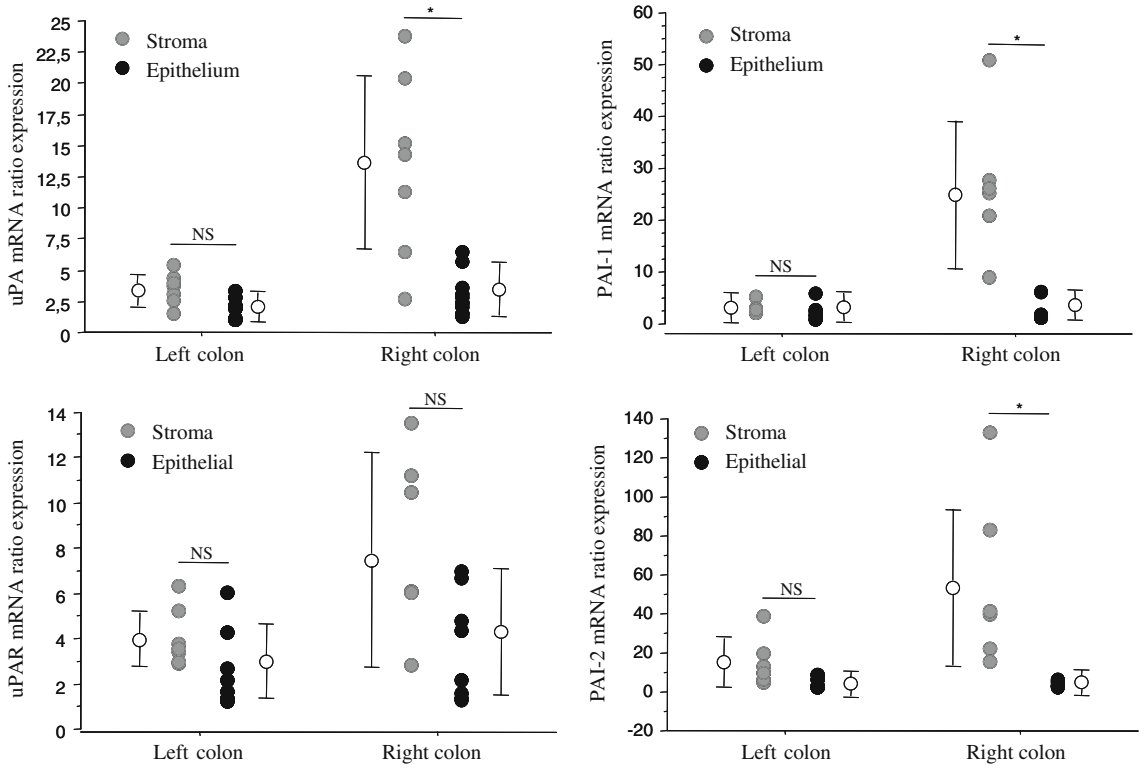

Fig. 4 uPA, uPAR, PAI-1 and PAI-2 mRNA expression in ipsilateral and contralateral normal colonic tissue in patients with right colonic adenoma *$p<0.05$

significantly lower mRNA ratio expression compared to the right colon.

uPA, uPAR, PAI-1 and PAI-2 mRNA Concentrations in Normal Colonic Tissue in Control Patients

Table 1 shows that mRNA concentrations found in the epithelial crypts did not significantly differ between the right and the left colon. There were also no significant differences between the mRNA concentrations in the stromal part of the left and right colon.

Discussion

In human colon adenomas, we and others have previously shown that high levels of certain PA members are associated with the early stages of colonic tumour development [13, 14]. It is our understanding that these studies were restricted to comparing values obtained from adenomatous versus adjacent mucosa full tissue extracts. It is not known whether a single adenomatous lesion can induce a stromal, gradient-like, activation in the adjacent mucosa.

In the present study, LCM combined with quantitative RT-PCR was applied to the differential analyses of uPA, uPAR, PAI-1 and PAI-2 mRNAs expressed by epithelial crypts and their stromal tissue environment adjacent to and distant from an adenoma. LCM allows for selective tissue sampling and has been used to screen for mRNA transcripts expressed by colonic adenomatous crypts [19] by colorectal carcinoma cells [20], or to characterise the global gene expression profiles between small and large adenomas [21]. Using gene arrays and LCM, Sugiyama et al. reported that genes contributing to the invasive and metastatic processes

Table 1 uPA, uPAR, PAI-1 and PAI-2 mRNA expression ratio in control patients

	Left colon (n=8)		Right colon (n=8)		P value
	Crypts	Stroma	Crypts	Stroma	
uPA	5.31±1.52	7.21±4.51	4.82±1.91	6.52±1.63	0.16
uPAR	25.12±1.41	37.87±16.41	19.45±8.32	28.45±16.56	0.32
PAI-1	6.81±4.53	12.87±8.41	7.41±5.86	7.98±5.76	0.24
PAI-2	73.26±36.41	89.13±39.45	65.17±29.46	69.81±23.75	0.42

were increased in the reactive stromal compartment of colorectal cancer [22]. LCM methodology is labour-intensive and our results were obtained from a relatively small number of patient and control samples. However, they clearly indicate that the mere presence of a colonic tubular adenoma can induce a stromal activation as expressed by a significant increase in the levels of four main members of the PA system, i.e. uPA, uPAR, PAI-1 and PAI-2 mRNAs. This activation could be detected as far as twenty centimeters away from the adenoma (as shown in Fig. 1), which was located either on the left or right side of the colon. Activation was bound solely to the stromal components within the microdissected tissue samples, as no significant differences in uPA, uPAR, PAI-1 and PAI-2 mRNA expression could be found in either ipsilateral or contralateral micro-dissected epithelial crypts. Moreover, stromal activation was not associated with the left or the right colonic side, but was instead linked to the presence of a tubular adenoma. Results in control cases corroborated these findings.

The regulation of PA-dependent activities implies a complex interplay between premalignant epithelial cells, reactive stromal cells and components of extracellular matrices. Using uPA, uPAR and PAI-1 expression and tissue localization, we have previously shown that clustered interactions take place between epithelial adenomatous crypts and stromal components [13]. Interestingly, we show here that this activation can extend outside of the lesion, through the stroma of colonic mucosa distant from the altered epithelial cells of adenomatous crypts. Activated (myo)fibroblasts and inflammatory cell infiltrates can secrete a mix of extracellular factors which may diffuse out of an adenomatous lesion to activate distant stromal cells in a paracrine mode. Ethical considerations limited biopsy sampling to one site, 20 cm away from the adenoma, but our data in contralateral versus ipsilateral stromal tissues indicate that upregulated PA-associated activation may decline in a gradient fashion.

It has long been recognized that inflammation can be linked to the development of dysplasia and to malignant progression [23]. An array of pro-inflammatory mediators, including cytokines and chemokines, can be secreted within and around tumours. The PA system takes part in the inflammatory processes [24] and uPA has been implicated in the early stages of colonic tumour development [25]. In experimental murine models, the induction of an inflammatory bowel disease accelerates adenoma formation [26] whereas a uPA deficiency diminishes their frequency and impairs leukocyte infiltration. [27] The molecular pathways involved in cell survival and proliferation and linking inflammatory cells to altered epithelial cells may be regulated by the tissue context in which tumour cells may or may not progress [28]. Due to the limited number of tissue samples analysed histologically, no significant differences could be found regarding the extent of inflammatory cells infiltrating mucosae at sites adjacent versus distant to the adenomas.

High levels of uPA and PAI-1 are recognized as indicators of a poor prognosis in most cancers, including colon cancer. It has been hypothesized that this correlation may be explained by the capacity of PAI-1 to protect the tumour cells from proteolysis at points of invasion [29]. Interestingly, our previous findings suggested that PAI-1, and not uPA, levels found in adenomas and adjacent mucosae could be indicators of postpolypectomy tumour recurrence [14]. The link between PAI-1 levels, poor patient prognosis and possible adenoma recurrence may reflect, in addition to PAI-1 activity as a uPA inhibitor, the existence of dynamic interactions among cells and ECM components, including signalling events, and possibly taking place at a distance from a premalignant or malignant site. In addition, whereas stromal cells are generally considered as genetically stable, epigenetic and chromosomal abnormalities have been described as contributing to the genesis of colorectal adenomas [30]. In contrast to PAI-1, the level of PAI-2(SERPINB2), another uPA inhibitor, has been linked, experimentally and clinically, with prolonged survival and decreased tumour growth [31]. In the present study, both PAI-2 mRNA levels were significantly increased within the micro-dissected stroma of adjacent ipsilateral mucosa. Key structural, topologic and functional differences, identified between the two inhibitors, may relate to this apparently paradoxic in situ expression.PAI-2 expression is known to be upregulated by many inflammatory mediators. It has been proposed that extracellular PAI-2 might compete for the binding of PAI-1 to uPA, thus controlling plasmin generation [31].

In summary, these findings indicate for the first time that a colonic adenoma, wherever it may develop, is associated with a PA-reactive inflammatory stroma extending outside of the lesion in the adjacent mucosae, presumably in a gradient-like mode. Such a reactive PA-associated micro-environment is likely to be tissue context-dependent and to have a critical role in either promoting or inhibiting the emergence of recurrent or additional lesions.

Since multiple parallel pathways to sporadic colorectal cancer have now been recognized, [32] our data on proteolytic-associated markers in tubular adenoma may help in completing the molecular profile associated with this precursor lesion.

References

1. Parkin DM, Bray F, Ferlay J, Pisani P (2001) Estimating the world cancer burden: Globocan 2000. Int J Cancer 94(2):153–156

2. O'Brien MJ, Winawer SJ, Zauber AG, Gottlieb LS, Sternberg SS, Diaz B et al (1990) The national polyp study. Patient and polyp characteristics associated with high-grade dysplasia in colorectal adenomas. Gastroenterology 98(2):371–379
3. Vogelstein B, Kinzler KW (2004) Cancer genes and the pathways they control. Nat Med 10(8):789–799
4. Gregorieff A, Clevers H (2005) Wnt signaling in the intestinal epithelium: from endoderm to cancer. Genes Dev 19(8):877–890
5. Galon J, Fridman WH, Pages F (2007) The adaptive immunologic microenvironment in colorectal cancer: a novel perspective. Cancer Res 67(5):1883–1886
6. van den Brink GR, Offerhaus GJ (2007) The morphogenetic code and colon cancer development. Cancer Cell 11(2):109–117
7. Dano K, Behrendt N, Hoyer-Hansen G, Johnsen M, Lund LR, Ploug M et al (2005) Plasminogen activation and cancer. Thromb Haemost 93(4):676–681
8. Mazzieri R, Blasi F (2005) The urokinase receptor and the regulation of cell proliferation. Thromb Haemost 93(4):641–646
9. Yang L, Avila H, Wang H, Trevino J, Gallick GE, Kitadai Y et al (2006) Plasticity in urokinase-type plasminogen activator receptor (uPAR) display in colon cancer yields metastable subpopulations oscillating in cell surface uPAR density–implications in tumor progression. Cancer Res 66(16):7957–7967
10. Czekay RP, Aertgeerts K, Curriden SA, Loskutoff DJ (2003) Plasminogen activator inhibitor-1 detaches cells from extracellular matrices by inactivating integrins. J Cell Biol 160(5):781–791
11. Duffy MJ (2005) Predictive markers in breast and other cancers: a review. Clin Chem 51(3):494–503
12. Duffy MJ, McGowan PM, Gallagher WM (2008) Cancer invasion and metastasis: changing views. J Pathol 214(3):283–293
13. Sordat I, Chaubert P, Protiva P, Guillou L, Mazzucchelli L, Saraga E et al (1997) In situ stromal expression of the urokinase/plasmin system correlates with epithelial dysplasia in colorectal adenomas. Am J Pathol 150(1):283–295
14. Protiva P, Sordat I, Chaubert P, Saraga E, Tran-Thang C, Sordat B et al (1998) Alterations in plasminogen activation correlate with epithelial cell dysplasia grading in colorectal adenomas. Br J Cancer 77(2):297–304
15. Dorta G, Chaubert P, Benhattar J, Saraga E, Protiva P, Blum AL, Sordat B (1998) PAI-1 is an indicator for adenoma reccurence after polypectomy. Gastroenterology 114:A585
16. Mueller MM, Fusenig NE (2004) Friends or foes—bipolar effects of the tumour stroma in cancer. Nat Rev Cancer 4(11):839–849
17. Jass JR, Sobin LH, Watanabe H (1990) The World Health Organization's histologic classification of gastrointestinal tumors. A commentary on the second edition. Cancer 66(10):2162–2167
18. Winawer SJ, Zauber AG, O'Brien MJ, Gottlieb LS, Sternberg SS, Stewart ET et al (1992) The national polyp study. Design, methods, and characteristics of patients with newly diagnosed polyps. The national polyp study workgroup. Cancer 70(5 Suppl):1236–1245
19. Lechner S, Muller-Ladner U, Renke B, Scholmerich J, Ruschoff J, Kullmann F (2003) Gene expression pattern of laser microdissected colonic crypts of adenomas with low grade dysplasia. Gut 52(8):1148–1153
20. Danjoux M, Guimbaud R, Al Saati T, Meggetto F, Carrere N, Portier G et al (2006) Contribution of microdissection for the detection of microsatellite instability in colorectal cancer. Hum Pathol 37(3):361–368
21. Sabates-Bellver J, Van der Flier LG, de Palo M, Cattaneo E, Maake C, Rehrauer H et al (2007) Transcriptome profile of human colorectal adenomas. Mol Cancer Res 5(12):1263–1275
22. Sugiyama Y, Farrow B, Murillo C, Li J, Watanabe H, Sugiyama K et al (2005) Analysis of differential gene expression patterns in colon cancer and cancer stroma using microdissected tissues. Gastroenterology 128(2):480–486
23. Tlsty TD, Coussens LM (2006) Tumor stroma and regulation of cancer development. Annu Rev Pathol 1:119–150
24. Del Rosso M, Fibbi G, Pucci M, Margheri F, Serrati S (2008) The plasminogen activation system in inflammation. Front Biosci 13:4667–4686
25. Sier CF, Verspaget HW, Griffioen G, Verheijen JH, Quax PH, Dooijewaard G et al (1991) Imbalance of plasminogen activators and their inhibitors in human colorectal neoplasia. Implications of urokinase in colorectal carcinogenesis. Gastroenterology 101 (6):1522–1528
26. Huang EH, Park JC, Appelman H, Weinberg AD, Banerjee M, Logsdon CD et al (2006) Induction of inflammatory bowel disease accelerates adenoma formation in Min +/-mice. Surgery 139 (6):782–788
27. Ploplis VA, Tipton H, Menchen H, Castellino FJ (2007) A urokinase-type plasminogen activator deficiency diminishes the frequency of intestinal adenomas in ApcMin/+ mice. J Pathol 213 (3):266–274
28. Balkwill F, Coussens LM (2004) Cancer: an inflammatory link. Nature 431(7007):405–406
29. Sidenius N, Blasi F (2003) The urokinase plasminogen activator system in cancer: recent advances and implication for prognosis and therapy. Cancer Metastasis Rev 22(2-3):205–222
30. Ishiguro K, Yoshida T, Yagishita H, Numata Y, Okayasu T (2006) Epithelial and stromal genetic instability contributes to genesis of colorectal adenomas. Gut 55(5):695–702
31. Croucher DR, Saunders DN, Lobov S, Ranson M (2008) Revisiting the biological roles of PAI2 (SERPINB2) in cancer. Nat Rev Cancer 8(7):535–545
32. Issa JP (2008) Colon cancer: it's CIN or CIMP. Clin Cancer Res 14(19):5939–5940