Serum endostatin levels are elevated in colorectal cancer and correlate with invasion and systemic inflammatory markers

T Kantola\textsuperscript{1,2,3}, J P Väyrynen\textsuperscript{1,2,3}, K Klintrup\textsuperscript{2,3,4}, J Mäkelä\textsuperscript{2,3,4}, S M Karppinen\textsuperscript{5}, T Pihlajaniemi\textsuperscript{5}, H Autio-Harmainen\textsuperscript{1,2,3}, T J Karttunen\textsuperscript{1,2,3}, M J Mäkinen\textsuperscript{1,2,3} and A Tuomisto\textsuperscript{*1,2,3}

\textsuperscript{1}Department of Pathology, University of Oulu, POB 5000, 90014 Oulu, Finland; \textsuperscript{2}Oulu University Hospital, POB 21, 90029 Oulu, Finland; \textsuperscript{3}Medical Research Center Oulu, POB 21, 90029 Oulu, Finland; \textsuperscript{4}Department of Surgery, University of Oulu, POB 5000, 90014 Oulu, Finland and \textsuperscript{5}Faculty of Biochemistry and Molecular Medicine and Biocenter Oulu, University of Oulu, POB 5400, 90014 Oulu, Finland

Background: Endostatin, a fragment of collagen XVIII, is an endogenous angiogenesis inhibitor with anti-tumour functions. However, elevated circulating endostatin concentrations have been found in several human cancers including colorectal cancer (CRC).

Methods: Serum endostatin levels were measured by enzyme-linked immunoassay from a series of 143 patients with CRC and from 84 controls, and correlated with detailed clinicopathological features of CRC, serum leukocyte differential count and C-reactive protein (CRP) levels.

Results: Patients with CRC had higher serum endostatin levels than the controls ($P = 0.005$), and high levels associated with age, tumour invasion through the muscularis propria and poor differentiation, but not with metastases. Endostatin levels showed a positive correlation with the markers of systemic inflammatory response and a negative correlation with the densities of tumour-infiltrating mast cells and dendritic cells. Collagen XVIII was expressed in tumour stroma most strikingly in blood vessels and capillaries, and in the muscle layer of the bowel wall.

Conclusions: Elevated endostatin levels in CRC correlate with systemic inflammation and invasion through the muscularis propria. Increased endostatin level may be a result of invasion-related cleavage of collagen XVIII expressed in the bowel wall. The negative correlations between serum endostatin and intratumoural mast cells and immature dendritic cells may reflect angiogenesis inhibition by endostatin.
invasion (Wilson et al, 2003; Folkman, 2006). Endostatin binds to several cell membrane proteins including α5β1 and αvβ3 integrins (Rehn et al, 2001; Faye et al, 2009), glycans (Karumanchi et al, 2001), and VEGF receptors 1, 2 and 3 (Kim et al, 2002; Koijima et al, 2008). It inhibits the activation of pro-MMP-2, pro-MMP-9 and proMMP-13 and the catalytic activity of MMP-2 and membrane type -1 MMP (Kim et al, 2000; Nyberg et al, 2003). The broad range of the molecular targets of endostatin suggests that it can affect the behaviour of the cells via numerous pathways. The extensive influence of endostatin on endothelial cells is based on its effect on gene expression: By using genome-wide microarray analysis, Abdolali et al, 2004 showed that endostatin treatment of cultured human endothelial cells resulted in significant changes in 12% of the genes analysed.

Elevated circulating endostatin concentrations have been found in several human cancers. Despite the acknowledged antiangiogenic functions of endostatin, higher serum endostatin levels are associated with poor differentiation and advanced stage in CRC, gastric cancer and bladder cancer (Li et al, 2012; Szarvas et al, 2012) and with poor prognosis of the patient in bladder cancer, non-small cell lung cancer, gastric cancer and soft tissue sarcoma (Feldman et al, 2001; Suzuki et al, 2002; Woo et al, 2006; Szarvas et al, 2012). The effect of endostatin on endothelial cells depends on the length of exposure (Li et al, 2005), the type of endothelial cells (Schmidt et al, 2004) and the type of growth factor inducing endothelial cell proliferation (Delaney et al, 2006). Finally, the composition of the extracellular matrix with which the cells are in contact modifies the effect of endostatin (Delaney et al, 2006). Interestingly, endostatin has a tumour-specific optimal inhibition concentration, higher and lower dosages having less inhibitory effect (Celik et al, 2005; Tjin Tham Sjin et al, 2006). All in all, endostatin is associated with several fundamental aspects of cancer including tumour cell differentiation, cancer angiogenesis and lymphangiogenesis, and inflammatory cell infiltration (Brideau et al, 2007; Seppinen and Pihlajaniemi, 2011).

In this study, we aimed to enlighten the significance of serum endostatin levels in CRC patients. We measured systemic endostatin levels in 143 CRC patients and 84 healthy controls matched for age and gender and correlated the endostatin levels with local inflammatory cell densities in paired TMEs. Serum endostatin levels were determined by sex-matched controls. Serum endostatin levels were measured from serum samples of 148 CRC patients and 84 age- and sex-matched controls. Serum endostatin levels were determined by using the commercial Quantikine Human Endostatin Immunoassay (R&D Systems, Minneapolis, MN, USA) according to the manufacturer’s instructions. Previously produced recombinant human endostatin (Rehn et al, 2001) was used as a control in the assay. Colour intensity of the samples was measured with a Victor3 plate reader (PerkinElmer, Waltham, MA, USA). All the assays were performed in duplicate and the mean values were used as the final concentration. Finally, endostatin measures of 143 CRC patients, 113 CRC patients without RT/CRT and 84 controls were included for the analyses. One of the samples was ignored because of having an endostatin value above the standard curve and four samples were left aside because the measured duplicates differed from each other by at least 20%.

Follow-up. All CRC patients who underwent surgery were followed up for tumour recurrence at regular intervals for up to 5 years. For disease-free survival (DFS) analyses, the time to the end point was calculated from the date of diagnosis of CRC until the date of locoregional or systemic CRC recurrence. The DFS analysis included 81.4% (92 out of 113) of the patients, while excluded patients (18.6%, 21 out of 113) underwent palliative operation. The median follow-up time was 51.5 months (range 0.1–60 months). For cancer-specific survival (CSS) and overall survival (OS) analyses, all 113 patients were included, with a median follow-up of 57.9 months (range 0.1–60 months). The OS for all patients was 65.5% (74 out of 113).

Statistical analysis. Normally distributed continuous variables are presented as mean (standard deviation, s.d.), whereas other continuous variables are presented as median (interquartile range). IBM SPSS Statistics 19 was used for statistical analysis (IBM, Chicago, IL, USA). Statistical significances of the differences in serum endostatin levels between the different study groups and age, gender, stage, grade and tumour location categories were
analysed by Mann–Whitney U-test or Kruskal–Wallis test. Univariate correlations are presented as Pearson correlation coefficients. A multiple linear regression analysis using stepwise method was performed to analyse the independent association of serum endostatin to the clinicopathological features of the cancer. Kaplan–Meier curves were used to visualise the differences of DFS, CSS and OS for patient groups stratified based on serum endostatin levels, and differences between groups were evaluated by the log rank test. The serum endostatin cutoff value (172 ng ml$^{-1}$) for survival analyses was obtained from receiver operating characteristics (ROC) analysis, in which optimal cutoff scores for serum endostatin levels in discriminating CRC patients from healthy controls were defined. In all the tests, a $P$-value less than 0.05 was considered statistically significant.

## RESULTS

### Demographic characteristics and serum endostatin in CRC

The characteristics of CRC patients and healthy controls are presented in Table 1 and the preoperative serum endostatin levels in Table 2. Because radiation is known to induce microvascular damage and changes in microvascular density (Seemann et al, 2012) as well as to cause a pronounced fibroblastic reaction (Nagtegaal et al, 2002b), we first evaluated the effect of preoperative RT/CRT on endostatin levels of CRC patients. For this, we divided the CRC patient group into three subgroups (Table 1). The patients receiving RT/CRT had similar endostatin levels to patients not receiving RT/CRT and matched for tumour stage and location (RT/CRT control group) (Table 2; median 154.1 ng ml$^{-1}$ vs 150.3 ng ml$^{-1}$, $P = 0.833$). Although preoperative RT/CRT did not affect serum endostatin levels, we excluded the RT/CRT group from the subsequent analyses because of the known effects of RT/CRT on local characteristics of the tumour (Nagtegaal et al, 2002a; Seemann et al, 2012).

Serum endostatin levels were significantly increased in CRC patients without RT/CRT compared with healthy controls (Figure 1A; Table 2; median 151.1 ng ml$^{-1}$ vs 136.1, $P = 0.005$). A ROC analysis was conducted to test the feasibility of serum endostatin in discriminating CRC patients without preoperative RT/CRT from healthy controls. It yielded an area under the curve (AUC) of 0.618 (95% confidence interval 0.539–0.696). Using a cutoff value of 172 ng ml$^{-1}$, discriminating specificity was 0.655 and sensitivity 0.798.

Endostatin serum levels were similar in females and males (Table 3). The endostatin levels were significantly higher in elderly patients ($\geq 65$ years) compared with younger ($< 65$ years) patients ($P = 0.014$). In the controls, the effect of age on serum endostatin concentration was similar and even more distinct ($P = 7.4 \times 10^{-10}$).

### Serum endostatin levels in relation to clinicopathological parameters

The relationships between serum endostatin levels and clinicopathological variables of CRC are presented in Table 3. TNM stage I patients had significantly lower serum endostatin levels compared with more advanced stages ($P = 0.014$). Deeper local invasion (T) was associated with a trend towards higher serum levels of endostatin ($P = 0.055$), and invasion through the muscularis propria (T1-2 vs T3-4) was associated with higher serum endostatin concentrations ($P = 0.007$; Table 3; Figure 1B). The presence of regional ($P = 0.802$) or distant ($P = 0.790$) metastases did not have a significant effect on serum endostatin concentrations. WHO grade 3 tumours showed a tendency towards higher endostatin levels compared with grade 1 or 2 tumours.

## Table 1. Characteristics of the patients with CRC and the controls

|                     | All CRC patients ($n = 143$) | CRC patients without RT/CRT ($n = 113$) | CRC patients with RT/CRT ($n = 30$) | CRC patients RT/CRT control group* ($n = 31$) | Healthy controls ($n = 84$) |
|---------------------|-----------------------------|----------------------------------------|------------------------------------|-----------------------------------------------|-----------------------------|
| **Age, mean (s.d.)**| 67.0 (11.3)                 | 68.0 (11.3)                            | 63.5 (10.7)                        | 68.1 (10.4)                                  | 66.9 (10.3)                 |
| **Gender**          |                             |                                        |                                    |                                               |                             |
| Male                | 77 (53.8%)                  | 56 (49.6%)                             | 21 (70%)                           | 20 (64.5%)                                   | 44 (52.4%)                  |
| Female              | 66 (46.2%)                  | 57 (50.4%)                             | 9 (30%)                            | 11 (35.5%)                                   | 40 (47.6%)                  |
| **Preoperative RT/CRT** |                           |                                        |                                    |                                               |                             |
| Yes                 | 30 (21%)                    | 0 (0%)                                 | 30 (100%)                          | 0 (0%)                                       | 31 (100%)                   |
| No                  | 113 (79%)                   | 113 (100%)                             | 0 (0%)                             | 31 (100%)                                    |                             |
| **Tumour location** |                             |                                        |                                    |                                               |                             |
| Proximal colon      | 48 (33.6%)                  | 48 (42.5%)                             | 0 (0%)                             | 0 (0%)                                       | 31 (100%)                   |
| Distal colon        | 27 (18.9%)                  | 27 (23.9%)                             | 0 (0%)                             | 0 (0%)                                       | 0 (0%)                      |
| Rectum              | 68 (47.6%)                  | 38 (33.6%)                             | 30 (100%)                          | 0 (0%)                                       |                             |
| **WHO grade**       |                             |                                        |                                    |                                               |                             |
| Grade 1             | 20 (14%)                    | 15 (13.3%)                             | 5 (16.7%)                          | 5 (16.1%)                                    |                              |
| Grade 2             | 105 (73.4%)                 | 84 (74.3%)                             | 21 (70%)                           | 24 (77.4%)                                   |                              |
| Grade 3             | 18 (12.6%)                  | 14 (12.4%)                             | 4 (13.3%)                          | 2 (6.5%)                                      |                              |
| **TNM stage**       |                             |                                        |                                    |                                               |                             |
| Stage I             | 25 (17.6%)                  | 18 (16.1%)                             | 7 (23.3%)                          | 8 (25.8%)                                    |                              |
| Stage II            | 54 (38.0%)                  | 45 (40.2%)                             | 9 (30%)                            | 8 (25.8%)                                    |                              |
| Stage III           | 45 (31.7%)                  | 31 (27.7%)                             | 14 (46.7%)                         | 14 (45.2%)                                   |                              |
| Stage IV            | 18 (12.7%)                  | 18 (16.1%)                             | 0 (0%)                             | 1 (3.2%)                                      |                              |

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Abbreviations: CRC — colorectal cancer; RT/CRT — radiotherapy or chemoradiotherapy; s.d. — standard deviation; TNM — tumour, node, metastasis; WHO — World Health Organization. *Without RT/CRT.
Endostatin has been suggested to modulate inflammatory reactions and tumour infiltration of leukocytes (Brideau et al, 2007), and thus we evaluated the associations between serum endostatin levels and local inflammatory cell densities in CRC tissue (Table 4). Of the studied inflammatory cells, CD1a+ DCs in tumour stroma, CD83+ DCs at the invasive front and mast cells in tumour stroma showed a negative correlation with serum endostatin levels.

**Correlation of serum endostatin and systemic inflammation markers.** We evaluated the correlations between serum endostatin levels, blood leukocyte counts and CRP concentration in patients with CRC. Of the studied parameters, CRP levels, NLR, total leucocyte count and neutrophil count showed a positive correlation with the serum endostatin levels (Table 5), and endostatin levels were higher in patients with moderate or high mGPS score as compared with those with low mGPS (Table 6).

**Multivariate analyses.** Next, a multiple linear regression analysis was performed to analyse the independent associations between serum endostatin levels and clinicopathological features of the cancer as well as immune cell infiltrates and systemic inflammatory cell counts. The variables analysed included age, gender, BMI, tumour location, distant metastases, nodal metastases, invasion through the muscularis propria, tumour-destructing peritumoural inflammatory infiltrate and necrosis. Of these factors, the

### Table 2. Serum endostatin levels in CRC patients compared with healthy controls

| Group                      | N   | Endostatin (serum) ng ml⁻¹, median (IQR) | P-value |
|----------------------------|-----|-----------------------------------------|---------|
| Healthy controls           | 84  | 136.1 (109.0–166.4)                     |         |
| All CRC patients           | 143 | 151.1 (125.5–194.1)                     |         |
| CRC patients without RT/CRT| 113 | 151.1 (125.6–195.4)                     |         |
| CRC patients with RT/CRT   | 30  | 154.1 (117.5–189.0)                     |         |
| RT/CRT control group       | 31  | 150.3 (131.0–174.4)                     |         |

Abbreviations: CRC = colorectal cancer; RT/CRT = radiotherapy or chemoradiotherapy; IQR = interquartile range. The P-values are for Mann-Whitney test.

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**Figure 1. Serum endostatin in colorectal cancer and association with primary tumour invasion.** (A) CRC patients had higher serum endostatin levels than age- and sex-matched healthy controls. (B) Tumour invasion through the muscularis propria associated with higher serum endostatin concentrations. (C–F) Collagen XVIII immunohistochemistry. (C) Blood vessels and capillary structures (arrows) show strong collagen XVIII expression, whereas no explicit staining could be found in carcinoma cells (asterisks). Collagen XVIII localised into the basement membranes surrounding the invasive tumour cell islets in some CRC cases (arrowheads). (D) In some CRC cases, collagen XVIII localised around myofibroblasts in desmoplastic tumour stroma. (E) In the bowel wall smooth muscle, collagen XVIII was detected in the muscle layer. (F) Higher magnification reveals collagen XVIII expression between muscle cells corresponding the location of basal laminae. Scale bars: c,d,f, 100 μm, e, 200 μm.
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### DISCUSSION

Angiogenesis regulatory proteins are important modifiers of tumour growth and invasion, representing potential biomarkers for diagnostic and prognostic assessment and potential targets for CRC therapy. There is also a linkage between tumour-associated inflammatory reactions and tumour angiogenesis (Mantovani et al, 2008). Thus, it would be of importance to understand the interactions and mutual regulation of these complex systems. In this study, serum endostatin levels in CRC were first analysed in healthy controls in ROC analysis. Especially, the elderly appeared to have high serum endostatin levels in the absence of CRC. The future study, serum endostatin levels were correlated with blood leucocyte counts and inflammatory cell densities in CRC tissue.

We found that preoperative serum levels of endostatin are increased in CRC patients compared with healthy controls. A similar association has been reported in several human cancers and recently also in gastrointestinal cancers, including CRC, gastric cancer and hepatocellular cancer (Li et al, 2012). It has also been shown that the removal of a primary colorectal tumour leads to a decrease in serum endostatin levels (Wu et al, 2005). However, our study suggests that endostatin is unlikely to prove to be a valuable tool in CRC diagnosis or follow-up, because of relatively low AUC (0.618) in discriminating the patients from controls. In the bowel wall, collagen XVIII was most strikingly expressed in the muscle layer between muscle cells corresponding to the location of basal laminae structures (Figure 1F).

### Table 3. Serum endostatin levels in relation to clinical and pathological characteristics of CRCs

| Characteristics                  | Endostatin (ng mL⁻¹, median [IQR]) | P-value |
|----------------------------------|-----------------------------------|---------|
| **Gender**                       |                                   |         |
| Male patients                    | 148.7 (122.0–183.3)               | 0.503   |
| Female patients                  | 154.2 (125.6–201.9)               | 0.205   |
| Male controls                    | 121.4 (107.1–166.9)               |         |
| Female controls                  | 146.5 (113.9–166.4)               |         |
| **Age**                          |                                   |         |
| Patients <65 years (n = 40)      | 138.6 (105.3–170.4)               | 0.014   |
| Patients ≥65 years (n = 73)      | 159.7 (135.7–201.3)               |         |
| Controls <65 years (n = 36)      | 108.1 (101.2–127.0)               |         |
| Controls ≥65 years (n = 48)      | 154.2 (137.7–179.1)               | 7.4 E-10|
| **TNM Stage**                    |                                   |         |
| Stage I (n = 18)                 | 132.6 (103.1–149.8)               | 0.014   |
| Stage II (n = 45)                | 164.4 (135.7–222.0)               |         |
| Stage III (n = 31)               | 149.0 (125.7–173.4)               |         |
| Stage IV (n = 18)                | 159.7 (108.9–211.3)               |         |
| **TNM classes T1-T4**            |                                   |         |
| T1 (n = 5)                       | 147.9 (109.4–156.2)               | 0.055   |
| T2 (n = 17)                      | 136.2 (103.9–154.1)               |         |
| T3 (n = 82)                      | 159.5 (130.2–204.6)               |         |
| T4 (n = 9)                       | 172.7 (98.2–195.4)                |         |
| **TNM classes T1-T2 vs T3-T4**   |                                   |         |
| T1-T2 (n = 22)                   | 139.1 (104.1–154.1)               | 0.007   |
| T3-T4 (n = 91)                   | 159.7 (127.7–203.9)               |         |
| **TNM classes N0-N2**            |                                   |         |
| N0 (n = 67)                      | 150.3 (125.1–198.9)               | 0.802   |
| N1 (n = 26)                      | 153.6 (118.8–173.7)               |         |
| N2 (n = 19)                      | 149.0 (138.1–203.9)               |         |
| **TNM classes M0-M1**            |                                   |         |
| M0 (n = 95)                      | 150.3 (127.0–190.2)               | 0.790   |
| M1 (n = 18)                      | 159.7 (108.9–211.3)               |         |
| **WHO Grade 1–3**               |                                   |         |
| Grade 1 (n = 15)                 | 125.1 (106.9–179.4)               | 0.064   |
| Grade 2 (n = 84)                 | 152.1 (128.4–188.7)               |         |
| Grade 3 (n = 14)                 | 180.4 (133.9–279.8)               |         |
| **WHO Grade 1–2/3**              |                                   |         |
| Grade 1–2 (n = 99)               | 149.0 (125.1–184.2)               | 0.055   |
| Grade 3 (n = 14)                 | 180.4 (133.9–279.8)               |         |
| **Tumour location**              |                                   |         |
| Proximal colon (n = 48)          | 160.3 (130.0–212.1)               | 0.162   |
| Distal colon (n = 27)            | 154.1 (115.1–172.7)               |         |
| Rectum (n = 38)                  | 150.0 (124.1–176.9)               |         |
| **Necrosis**                     |                                   |         |
| None or rare (n = 67)            | 149.0 (118.4–184.2)               | 0.769   |
| Frequent small (n = 29)          | 154.1 (131.5–195.4)               |         |
| Broad (n = 17)                   | 153.1 (109.3–212.7)               |         |

Abbreviations: CRC = colorectal cancer; IQR = interquartile range; TNM = tumour, node, metastasis; WHO = World Health Organization. The P-values are for Mann–Whitney or Kruskal–Wallis test.

Collagen XVIII immunohistochemistry. We used immunohistochemistry to assess the expression patterns of collagen XVIII, the precursor molecule of endostatin. In CRC specimens, collagen XVIII expression mainly localised to the endothelial cells of the blood vessels (Figure 1C). No explicit positivity could be found in carcinoma cells. Collagen XVIII also localised into basement membrane structures surrounding invasive tumour cell islets (Figure 1C) and around myofibroblasts in some desmoplastic tumour stroma areas (Figure 1D). We graded the quantity of positive blood vessels at tumour stroma and invasive front but found no correlation with systemic endostatin levels (data not shown). In the bowel wall, collagen XVIII was most strikingly expressed in the muscle layer between musclecells corresponding to the location of basal laminae structures (Figure 1E and F).

In a second model, in addition to the variables above, we included the inflammatory markers, that is, the counts of stromal mast cells, peritumoural mature DCs, stromal immature DCs, serum CRP, and blood leucocytes, lymphocytes, neutrophils and NLR, which showed correlation with serum endostatin in univariate analysis. This analysis indicated that blood NLR and patient age were positively and stromal immature DC count negatively associated with serum endostatin.

We found that preoperative serum levels of endostatin are increased in CRC patients compared with healthy controls. A similar association has been reported in several human cancers and recently also in gastrointestinal cancers, including CRC, gastric cancer and hepatocellular cancer (Li et al, 2012). It has also been shown that the removal of a primary colorectal tumour leads to a decrease in serum endostatin levels (Wu et al, 2004; Peeters et al, 2005). However, our study suggests that endostatin is unlikely to prove to be a valuable tool in CRC diagnosis or follow-up, because of relatively low AUC (0.618) in discriminating the patients from healthy controls in ROC analysis. Especially, the elderly appeared to have high serum endostatin levels in the absence of CRC. The mechanism of such age-related increase in endostatin levels is unknown, but it might be associated with age-related increase in cardiovascular disease and the elevated endostatin levels in these patients (Mitsuma et al, 2007; Carlsson et al, 2013).

Li et al, 2012 have reported that increased serum endostatin levels correlate with CRC progression. Our results also showed a trend towards elevated serum endostatin levels in the presence of deeper local invasion of the tumour (T classification) in univariate analysis (Table 3; P = 0.055) and significant correlation between higher serum endostatin levels and the invasion of the cancer tissue through the muscularis propria (T1–2 vs T3–4) in univariate (Table 3; P = 0.007) and multivariate analysis (Table 7; P = 0.032).
The presence of metastases did not further increase the serum endostatin levels in our study.

Immunohistochemical analysis indicated that the CRC cells do not express collagen XVIII, the source of endostatin, whereas blood vessels and capillary structures showed strong collagen XVIII positivity (Figure 1C). In some tumour areas, collagen XVIII also localised around myofibroblastic stromal cells and basement membrane structures encircling invasive tumour cell islets. In the bowel wall, collagen XVIII expression was most prominent around smooth muscle cells of the muscle layer (Figure 1E). This suggests that the increase of serum endostatin levels may result from the degradation of collagen XVIII and the release of endostatin, when

![Figure 2. Kaplan–Meier survival analyses of patients with CRC.](image)

(A) Serum endostatin level had no effect on disease-free survival, (B) cancer-specific survival or (C) overall survival.

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**Table 4. Correlation of serum endostatin levels with local areal density of inflammatory cells in CRC specimens**

| Invasive front | Pearson r | P-value |
|----------------|-----------|---------|
| CD3            | −0.004    | 0.966   |
| CD8            | 0.074     | 0.434   |
| FoxP3          | −0.023    | 0.810   |
| CD68           | 0.132     | 0.164   |
| CD83           | −0.189    | 0.047   |
| CD1a           | −0.160    | 0.090   |
| Mast cell typtase | −0.103 | 0.276   |
| Neutrophil elastase | 0.084 | 0.378   |

| Tumour stroma  | Pearson r | P-value |
|----------------|-----------|---------|
| CD3            | 0.059     | 0.537   |
| CD8            | 0.159     | 0.092   |
| FoxP3          | 0.095     | 0.317   |
| CD68           | 0.023     | 0.805   |
| CD83           | −0.140    | 0.145   |
| CD1a           | −0.281    | 0.003   |
| Mast cell typtase | −0.214 | 0.023   |
| Neutrophil elastase | 2.2E-4 | 0.998   |

| Intraepithelial | Pearson r | P-value |
|-----------------|-----------|---------|
| CD3             | 0.038     | 0.690   |
| CD8             | 0.042     | 0.671   |

**Table 5. Correlations between serum endostatin and CRP concentrations, and peripheral blood white blood cell counts and the NLR**

|               | Endostatin Pearson r | P-value |
|---------------|----------------------|---------|
| CRP           | 0.274                | 0.003   |
| NLR           | 0.283                | 0.003   |
| Leukocytes    | 0.199                | 0.035   |
| Lymphocytes   | −0.074               | 0.436   |
| Monocytes     | 0.144                | 0.130   |
| Neutrophils   | 0.298                | 0.001   |

**Table 6. Difference in endostatin levels within mGPS**

| mGPS | Endostatin ng ml⁻¹, median (IQR) | P-value |
|------|----------------------------------|---------|
| 0 (n = 88)   | 148.2 (121.3–178.1)             |         |
| 1–2 (n = 25) | 180.4 (141.7–222.0)             | 0.017   |

Abbreviations: CRP = C-reactive protein; NLR = neutrophil/lymphocyte ratio. Numbers indicate Pearson correlation coefficients (r) for logarithmically transformed variables.

Abbreviations: IQR = interquartile range; mGPS = modified Glasgow prognostic score. P-value is for Mann–Whitney test.
Table 7. Multiple linear regression model of serum endostatin level in colorectal cancer patients

| Independent         | Beta | P-value |
|---------------------|------|---------|
| **Model 1**         |      |         |
| Age                 | 0.291| 0.001   |
| Grade               | 0.239| 0.008   |
| Invasion through muscularis propria | 0.192 | 0.032   |
| **Model 2**         |      |         |
| Blood NLR           | 0.231| 0.013   |
| Age                 | 0.298| 0.001   |
| CD1a^+ cell count, stromal | -0.282 | 0.002   |

Abbreviation: NLR — neutrophil/lymphocyte ratio.

The effect of endostatin on mast cells has earlier been reported by Brideau et al., 2007 using a carcinogen-induced skin tumour-igenesis model in J4 mice overexpressing endostatin in their keratinocytes. Elevated endostatin levels in J4 mice reduced the number of VEGF-C-producing mast cells in the tumour tissue, in addition to which endostatin inhibited mast cell migration and adhesion in vitro. Brideau et al. 2007 also detected reduced numbers of peritumoural lymphatic vessels in J4 mice, suggesting an inhibitory effect of endostatin on lymphangiogenesis, at least partially resulting from the effect of endostatin on VEGF-C-expressing mast cells. Potential mechanisms of the direct interactions between mast cell and endostatin remain hypothetical. Potentially, endostatin may interact with integrins z5z1 and zvz3, expressed by both mast cells (Columbo et al., 1995; Columbo and Bochner, 2001) and DCs (Jancic et al., 1998; Skoberne et al., 2005), which also serve as binding partners for endostatin in endothelial cells (Rehn et al., 2001).

In patients with CRC, we detected positive correlations between serum endostatin and markers of systemic inflammation, including serum CRP, blood NLR and mGPS. In recent years, it has become apparent, that systemic inflammation in patients with cancer predicts poor outcome independently of tumour stage (McMillan, 2013). The relationships between serum endostatin and systemic inflammation have not been studied in patients with cancer, and the mechanisms linking endostatin and systemic inflammation are unknown. As discussed below, the numbers of tumour-associated leukocytes did not show correlation with endostatin levels (Table 4). This suggests that other mechanisms are involved, such as products released to the circulation as cancer cells invade.

In conclusion, our results suggest that elevated endostatin levels in CRC may be released by invading cancer cells cleaving endostatin from collagen XVIII. Endostatin levels correlated with markers of systemic inflammation but the mechanisms remain speculative. There were negative correlations between serum endostatin levels and the numbers of intratumoural mast cells and DCs, which could reflect higher endostatin concentrations inhibiting angiogenesis, being related to a decreased number of angiogenesis-related cell types, or signifying a direct inhibitory effect of endostatin on mast cells and DCs.

**ACKNOWLEDGEMENTS**

We thank Ms. Riitta Vuento and Ms Aila White for their expert assistance, and Dr. Ritva Heljasvaara and Dr. Valerio IZZI for the critical reading of the manuscript. This work was supported by grants from Academy of Finland (24300234, 259872 and 251314), Emil Aaltonen Foundation, Finnish Cancer Foundation, Ida Montin Foundation, Oulu University Scholarship Foundation, Orion-Farmos Research Foundation, the Sigrid Juselius Foundation Northern Finland Cancer Foundation, and Vatsataluntutkimussäätiö.

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