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Midkine is up-regulated in both cancerous and inflamed bowel, reflecting lymph node metastasis in colorectal cancer and clinical activity of ulcerative colitis

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ABSTRACT

Midkine is a multifunctional cytokine and growth factor displaying proinflammatory and protumorigenic activity. Its association with bowel diseases has not been fully elucidated. Our purpose was to delineate midkine expression pattern by RT-qPCR in inflamed/cancerous bowel (n = 208) and whole blood (n = 150) in colorectal cancer (CRC), Crohn's disease (CD), and ulcerative colitis (UC) and to evaluate midkine dynamics in early postoperative period following colorectal surgery. The expression of midkine was significantly up-regulated in stage III CRC and independently associated with lymph node metastasis. The expression of midkine in whole blood was up-regulated solely in N1 CRC. Midkine expression in cancer-free tissue (CRC) was also elevated and dependent on CRC advancement. In IBD, inflammation increased the bowel expression of midkine solely in UC, in a manner proportional to the disease clinical activity. Large and small bowel differed with respect to the expression of midkine in quiescent tissue (higher in small bowel) and to its correlation pattern with chemokines (in a large bowel) and angiogenic factors and cell cycle regulators (in a small bowel). Circulating midkine and its expression in whole blood dropped directly following colorectal surgery; however, the concentration of midkine in serum was restored on postoperative day three.

Midkine is involved in bowel inflammation in UC and lymph node metastasis in CRC, rendering midkine an attractive target for their treatment. Owing to midkine elevation in early postoperative period and its overexpression in tumor-adjacent tissue, targeting midkine might be considered also as a prevention of CRC recurrence following curative tumor resection.

1. Introduction

Colorectal cancer (CRC) remains one of the most prevalent cancers worldwide [1]. Chronic bowel inflammation in course of Crohn's disease (CD) or ulcerative colitis (UC), two dominant types of inflammatory bowel disease (IBD), is one of the major risk factors predisposing for colon carcinogenesis [2]. Mechanistically, it is hypothesized that inflammation increases the turnover of colonic cells and creates highly mutagenic environment enriched in reactive oxygen and nitrogen species. Supporting the link between inflammation and carcinogenesis, mediators of inflammation are frequently overexpressed in both inflamed and neoplastic bowel [3]. Cytokines contribute to carcinogenesis by induction and perpetuation of inflammatory response as well as by promotion of angiogenesis, an integral component of the pathogenesis of CRC and IBD [4,5]. Consequently, cytokines are viewed as promising targets for the treatment of both pathologies [6,7].

Midkine is a cytokine and heparin-binding growth factor of versatile biological activities [8–11]. Among others, midkine contributes to chronic inflammation by promoting tissue infiltration with neutrophils and monocytes and by inducing expression of

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proinflammatory cytokines [8–10]. Midkine displays a pro-tumorigenic character by triggering neoplastic transformation, promoting tumor growth and angiogenesis, and by facilitating dissemination of cancer cells. Midkine is transiently expressed during mid-gestation but in adult organisms its expression is low and limited to several organs. However, the expression of midkine is resumed during carcinogenesis, inflammation, and tissue repair and remodeling [8–10]. Serum concentrations of midkine are elevated in numerous pathological conditions, particularly these of inflammatory background [12]. An increase in circulating midkine reflects the advancement of CRC [13] and the clinical activity of IBD [14,15]. Moreover, it is correlated with the degree of bowel inflammation as assessed by endoscopy in UC [14]. The overexpression of midkine on protein [16,17] and mRNA [16,18] level has been demonstrated in neoplastic colon. However, findings concerning midkine in a bowel inflammation are limited to UC [19,20]. Neither the expression of midkine in CD nor its possible association with the severity of IBD and the advancement of CRC has been investigated. Also midkine expression in whole blood of CRC and IBD patients remains unknown. To supplement the present knowledge on midkine in bowel diseases, we conducted comprehensive analysis of midkine expression in whole blood and bowel tissue with respect to the disease advancement/severity and the co-expression of chemokines, angiogenic factors, and cell cycle regulatory proteins. Additionally, we analyzed the changes in the serum concentrations of midkine as well as its whole blood expression in the perioperative period following colorectal surgery.

2. Materials and methods

2.1. Patients

For the present study, 208 bowel tissue specimens were obtained from 104 individuals: 50 with histologically confirmed CRC, two with massive adenomas, and 52 with IBD (30 with CD and 22 with UC). CRC and adenoma patients were admitted to the Dept. of Gastrointestinal and General Surgery of Wroclaw Medical University or to the First Department of Oncological Surgery of Lower Silesian Oncology Center, Wroclaw, Poland. IBD patients were admitted to the First Dept. and Clinic of General, Gastroenterological and Endocrinological Surgery of Wroclaw Medical University because of the ineffectiveness of continued non-operative therapy, occurrence of the disease complications (perforation, fistule, abscess, obstruction) or to complete further stages of previous surgery procedure. Altogether, we examined 50 tumor specimens (large colon), two adenomas (large colon), 44 specimens of inflamed tissue (18 sampled from small and 26 from large bowel) and 112 of macroscopically normal tissue (40 sampled from small and 72 from large bowel: 50 as tumor-free resection margins from CRC patients). Resected tumors were staged pathologically according to UICC TNM. The disease stage distribution is given in Table 1. For the assessment of clinical activity of CD, the Crohn's Disease Activity Index (CDAI) combining the evaluation of vital parameters, clinical findings, and medical history was applied. For the assessment of clinical activity of UC, the Rachmilewitz Index (RI), encompassing stool frequency, number of stools with blood, general well-being, abdominal pain/cramp, fever, extraintestinal manifestations, and laboratory tests (erythrocyte sedimentation rate and hemoglobin concentration) was applied. CDAI $\geqslant$ 150 and RI $\geqslant$ 6 were indicative of, respectively, active CD and UC. Subject distribution based on gender (females/males) in CRC and IBD cogorts was, respectively, 17/33 and 20/32, whereas mean age was 67 yrs. (range: 33–91) and 36.3 yrs. (range: 19–64).

Whole blood for midkine gene expression analysis was collected from 135 individuals: 34 with CRC, 43 with IBD (35 with UC: 14 in remission and 21 with active disease and 44 with CD: 21 in remission and 23 with active disease) and 58 controls. Subject distribution based on gender (females/males) in CRC, IBD, and healthy control cohorts was, respectively, 7/27 17/26, and 21/37. Mean age was, respectively, 62.1 yrs. (range: 33–91) 36.4 yrs. (range: 19–61), and 39.9 yrs. (range: 18–78).

In order to evaluate changes in midkine in response to bowel surgery, whole blood was collected preoperatively and 24 h post-surgery from 15 patients (for mRNA analysis) and serum samples prior surgery and at 24, 48, and 72 h post incision from 12 patients (for circulating midkine determination). Within study period, all

Table 1
Sequences and efficiencies of primers used in current study.

Symbol	Gene name; function of encoded protein	Accession no.	Primer sequence 5′ → 3′ (forward/reverse)	Amp. size	E [%]
PPIA[a]	Peptidylprolyl isomerase A; protein folding	NM_021130.3	F: ggcaaatgctggacccaacaca R: tgctggtcttgccattcctgga	161bp	104.6
RPLP0[a]	Ribosomal protein, large, P0; component of 60S subunit	NM_001002.3	F: tggtcatccagcaggtgttcga R: acagacactggcaacattgcgg	119bp	106.4
RPS23[a]	Ribosomal protein S23; component of 40S subunit	NM_001025.4	F: aggaagtgtgtaagggtccagc R: caccaacagcatgacctttgcg	142bp	106.9
FGF2	Basic fibroblast growth factor	NM_002006.4	F: tctatcaaaggagtgtgtgctaacc R: tgcccagttcgtttcagtgc	179bp	105.2
CXCL8[a]	Interleukine 8, chemokine	NM_000584.3	F: gagagtgattgagagtggaccac R: cacaaccctctgcacccagttt	112bp	102.9
MDK[a]	Midkine; neurite growth-promoting factor 2; pro-tumorigenic cytokine	NM_001012334.2	F: gctacaatgctcagtgccagga R: cttggcgtctagtccttttccct	109bp	100.8
VEGF-A[a]	Vascular endothelial growth factor A	NM_001025366.2	F: ttgccttgctgctctacctcca R: gatggcagtagctgcgctgata	126bp	99.3
CCL2	Monocyte chemoattractant protein (MCP)-1; chemokine	NM_002982.3	F: tctgtgcctgctgctcatag R: acttgctgctggtgattcttc	155bp	104.2
CCL4[a]	Macrophage inflammatory protein (MIP)-1β; chemokine	NM_002984.2	F: ggtcatacacgtactcctggac R: gcttcctcgcaactttgtggtag	140bp	97.5
TP53[a]	p53 protein; cell cycle regulator	NM_001126118.1	F: cctcagcatcttatccgagtgg R: tggatggtggtacagtcagagc	128bp	102.7
CDKN1A	$p21^{CIP1/WAF1}$ protein; cell cycle regulator	NM_001291549.1	F: agaccagcatgacagatttc R: actgagactaaggcagaaga	144bp	100.9

Amp., amplicon; E, efficiency.
[a] Primer sequences were as proposed by Origene (www.origene.com). The remaining primers were designed using Beacon Designer Probe/Primer Design Software (BioRad) and validated *in silico* by Blast analysis. Forward and reverse primer sequences are denoted by "F" and "R", respectively.

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