Available online at www.sciencedirect.com
ScienceDirect
Journal of Nutritional Biochemistry 22 (2011) 634–641
Journal of Nutritional Biochemistry

Up-regulation of the novel proinflammatory adipokines lipocalin-2, chitinase-3 like-1 and osteopontin as well as angiogenic-related factors in visceral adipose tissue of patients with colon cancer☆,☆☆

Victoria Catalán[a,b], Javier Gómez-Ambrosi[a,b], Amaia Rodríguez[a,b], Beatriz Ramírez[a,b], Camilo Silva[b,c], Fernando Rotellar[b,d], José L. Hernández-Lizoain[d], Jorge Baixauli[d], Victor Valentí[d], Fernando Pardo[d], Javier Salvador[b,c], Gema Frühbeck[a,b,c,*]

[a]*Metabolic Research Laboratory, Clínica Universidad de Navarra, 31008 Pamplona, Spain*
[b]*CIBER Fisiopatología de la Obesidad y Nutrición, Instituto de Salud Carlos III, Spain*
[c]*Department of Endocrinology, Clínica Universidad de Navarra, 31008 Pamplona, Spain*
[d]*Department Surgery, Clínica Universidad de Navarra, 31008 Pamplona, Spain*

Received 23 September 2009; received in revised form 12 April 2010; accepted 15 April 2010

Abstract

Background: Obesity is widely recognised as an important risk factor for colorectal cancer (CC).
Aim: The study aimed to evaluate the effect of CC on circulating concentrations and gene expression levels of inflammatory and angiogenesis-related factors in human visceral adipose tissue (VAT).
Methods: VAT biopsies were obtained from 18 healthy individuals and 11 patients with CC. Real-time polymerase chain reactions were performed to quantify gene expression levels and zymographic analyses were used to determine the activity of matrix metalloproteinases (MMPs).
Results: Patients with CC exhibited increased mRNA expression levels of lipocalin-2 (P=.014), osteopontin (P=.027), tumor necrosis factor-α (TNF-α) (P=.016) and chitinase-3 like-1 (P=.006) compared to control subjects in VAT. Gene expression levels of hypoxia-inducible factor-1 α, vascular endothelial growth factor and MMP-2 were significantly higher (P<.05) in VAT of patients with CC. The expression of insulin-like growth factor I, insulin growth factor binding protein 3 and MMP-9 followed the same trend, although no significant differences were reached. The enzymatic activity of MMP-9 was increased (P<.001) in patients with CC. Furthermore, individuals with CC showed increased (P<.05) circulating concentrations of the inflammatory markers interleukin-6, tumour necrosis factor α and hepatocyte growth factor, whereas levels of the anti-inflammatory adipokine adiponectin were decreased (P<.01).
Conclusion: These findings represent the first observation that mRNA levels of the novel inflammatory factors lipocalin-2, chitinase-3 like-1 and osteopontin are increased in human VAT of subjects with CC. This observation together with the up-regulation of angiogenic factors suggests that adipokines secreted by VAT may be involved in the development of colon cancer.

Keywords: Visceral adipose tissue; Colon cancer; Inflammation; Angiogenesis; Gene expression

1. Introduction

The incidence of obesity and its associated disorders is increasing at an accelerating and alarming rate world wide [1]. Noteworthily, excess body weight is widely recognised as an important risk factor for the development of some common cancers, as is the case with colorectal and breast cancer [2–5]. In most studies obesity, defined as a body mass index (BMI) >30 kg/m^2, has been consistently associated with a higher risk of colorectal cancer both in men (relative risk of 1.5 to 2.0) and women (relative risk of 1.2–1.5) compared with a low or normal BMI [3,6–9]. Gender differences have been observed consistently across different studies [5]. One plausible explanation relies on the fact that central adiposity is a stronger predictor of colon cancer risk than peripheral adiposity or general overweight. In this regard, waist circumference and waist-to-hip ratio, indicators of abdominal obesity, have been strongly associated with colon cancer risk in men and women [10,11].

The mechanisms linking obesity and colon cancer are unclear but the obesity-associated low-grade chronic inflammation is widely accepted as an important factor in colon cancer pathogenesis [12–15].

☆ Funding: This work was supported by FIS, PI061458, PI09/02330 and PI081146 from the Spanish Instituto de Salud Carlos III, Ministerio de Sanidad y Consumo, by the Department of Health (20/2005 and 3/2006), by the Department of Education of the Gobierno de Navarra of Spain (res228/2008) and by Fundación Mutua Madrileña.
☆☆ Disclosure statement: The authors have nothing to disclose.
* Corresponding author. Department of Endocrinology, Clínica Universidad de Navarra, 31008 Pamplona, Spain. Tel.: +34 948 255400x4484; fax: +34 948 296500.
E-mail address: gfruhbeck@unav.es (G. Frühbeck).

0955-2863/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2010.04.015

It has been described that the tumoural microenvironment contains inflammatory cells that secrete cytokines, growth factors, metalloproteinases, and reactive oxygen species, which can induce DNA damage and chromosomal instability, thereby favouring carcinogenesis [15]. In this respect, adipose tissue constitutes a highly active endocrine organ [16,17] that secretes a variety of proteins collectively called adipokines directly involved not only in the regulation of whole-body metabolism but also in inflammatory and immune responses [18]. Noteworthy, novel adipokines related to inflammation and insulin resistance with emerging roles in tumour development have been recently described [19–21]. Lipocalin-2 (LCN2) is known to promote breast, ovarian and oesophageal cancer progression [22–24], while osteopontin (OPN) is reportedly involved in the regulation of cell signalling that controls tumour progression and metastasis [25]. Furthermore, a strong association between elevated plasma concentrations of chitinase-3 like-1 (YKL-40) and the incidence of gastrointestinal cancer has been found [26]. On the other hand, adiponectin, an anti-inflammatory and insulin-sensitizing hormone secreted by adipocytes, is inversely associated with visceral adiposity and it has been recently described that its levels are negatively correlated with the risk of malignancies associated with obesity [27,28].

The importance of angiogenesis for the growth of solid tumours [29,30] as well as for the development of obesity [31,32] is well recognised. Growing adipocytes produce a wide variety of angiogenic factors including leptin, vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), insulin-like growth factor I (IGF-I), tumour necrosis factor α (TNF-α), interleukin (IL)-6, IL-8, IL1-β or tissue factor (TF) [31]. In agreement with this, our group has shown that the members of the VEGF family play a role in adipose tissue expansion that takes place in obesity [33,34]. In addition, in expanding adipose tissue, clusters of enlarged adipocytes become distant from the vasculature, leading to local areas of hypoxia [35,36] and, therefore, to the secretion of hypoxia-inducible factor-1 α (HIF-1α), a key factor for vascular growth and remodelling that dysregulates the production of adipokines [37]. In this context, an increased expression of matrix-metabolising enzymes is observed in the inflammatory state of obesity at the same time as being a hallmark of many tumour processes [38]. Human adipose tissue releases matrix metalloproteinase (MMP)-2 and MMP-9 during adipocyte differentiation suggesting a pivotal role in the remodelling that occurs during the development of obesity as well as in the invasion process of tumour cells [39].

Chronic hyperinsulinaemia associated to obesity is another postulated mechanism involved in cancer development through the growth-promoting effect of elevated levels of insulin [40]. Hyperinsulinemia reduces the production of insulin-like growth factor binding proteins (IGFBPs) which modulate bioavailability of IGF-I, in both the circulation and the cellular microenvironment with resultant increased levels of free IGF-I. Due to its mitogenic and anti-apoptotic functions, IGF-I has been associated with an increased risk of colorectal and prostate cancers [41].

Taken together, obesity-associated dysregulation of adipokine production and secretion is likely to contribute both to mutagenesis and tumour progression. The aim of the present study was to analyse the gene expression profile of key inflammatory and angiogenesis-related factors as well as relevant adipokines in human visceral adipose tissue (VAT) from volunteers with and without colon cancer (CC) as well as the potential impact of CC on their circulating concentrations.

2. Materials and methods

2.1. Patient selection

2.1.1. Subjects

Twenty-nine samples of VAT from 11 patients with CC and 18 volunteers without CC were used to analyse the effects of this pathology on the gene expression levels of specific inflammatory and angiogenesis-related factors. In addition, the activity of MMP-2 and MMP-9 in VAT was assessed in these groups of samples. Volunteers for the study were recruited among male patients attending the Departments of Endocrinology and Surgery of the Clínica Universidad de Navarra.

2.1.2. Sample collection

The control samples were collected via a laparoscopic approach from patients undergoing either Nissen fundoplication (for hiatus hernia repair in lean [LN] volunteers) or Roux-en-Y gastric bypass (for obesity treatment in obese [OB] subjects). Unless for the specific disease originating the surgical intervention, these subjects were healthy, were not on medication, and had no signs or clinical symptoms of cancer, liver alteration or type 2 diabetes mellitus. The CC samples were obtained from patients who underwent curative resection for primary colon carcinoma. None of the patients received preoperative and/or postoperative adjuvant chemo and/or radiotherapy, as these modalities were not part of the standard treatment regimen at that time. Tissue samples were immediately frozen in liquid nitrogen and stored at −80°C for subsequent analysis.

The study was approved, from an ethical and scientific standpoint, by the Hospital's Ethical Committee responsible for research and the written informed consent of participants was obtained.

2.2. Blood assays

Plasma samples were obtained by venipuncture after an overnight fast. Glucose was analysed based on enzymatic spectrophotometric reactions by an automated analyzer (Hitachi Modular P800, Roche, Basel, Switzerland). Insulin was measured by means of an enzyme-amplified chemiluminescence assay (IMMULITE, Diagnostic Products, Los Angeles, CA, USA). Insulin resistance and sensitivity was calculated using respectively, the Homeostatic Model Assessment (HOMA) [fasting glucose (mmol/L)×fasting insulin (μU/ml)/22.5] and quantitative insulin sensitivity check index (QUICKI) [1/(log fasting insulin (μU/ml)+log fasting glucose (mg/dl)] indices [42,43]. High-sensitivity C-reactive protein (CRP) concentrations were quantified using the Tina-quant CRP (Latex) ultrasensitive assay (Roche). Leptin was measured by a double-antibody RIA method (Linco Research, St. Charles, MO, USA). Intra- and interassay coefficients of variation were 5.0 and 4.5%, respectively. Adiponectin concentrations were assessed using a commercially available ELISA kit (R&D Systems Europe, Abingdon, UK) according to the manufacturer's instructions with intra- and interassay coefficients of variation of 3.7% and 6.5%.

2.3. Multiplex immunoassays

The circulating concentrations of different inflammatory markers was quantified with a Luminex 200 platform (Luminex, Austin, TX, USA) using microsphere-based multiplexing technology. The human serum adipokine immunoassay kit used (Millipore Iberica, Madrid, Spain) was composed by analyte-specific components for the simultaneous measurement of the following human cytokines [interleukin (IL)-6, IL-8, TNF-α, monocyte chemoattractant protein-1 (MCP-1), HGF and nerve growth factor (NGF)]. The standard curve was calculated using a five-parametric-curve fitting method, and results were analysed using the Luminex IS software 2.3 (Luminex). Intra-assay precision ranged from 1.4% to 7.9%, while interassay precision was <21%. Calibrators, controls and samples were run in duplicate throughout the study.

2.4. RNA extraction and real-time polymerase chain reaction

2.4.1. RNA extraction

RNA isolation from adipose tissue was performed by homogenisation with an ULTRA-TURRAX T 25 basic (IKA Werke GmbH, Staufen, Germany) using QIAzol Reagent (Qiagen, Valencia, CA, USA). Samples were purified with the RNeasy Lipid Tissue Mini Kit (Qiagen) according to the manufacturer's directions and treated with DNase I (RNase-free DNase Set, Qiagen) in order to remove any trace of genomic DNA. For first strand cDNA synthesis constant amounts of 2 μg of total RNA were reverse transcribed in a 40-μL final volume using random hexamers (Roche) as primers and 400 units of M-MLV reverse transcriptase (Invitrogen, Carlsbad, CA, USA) [33].

2.4.2. Real-time polymerase chain reaction

The transcript levels for adiponectin (*ADIPOQ*), *HIF1A*, *IGF1*, *IGFBP3*, *LCN2*, *MMP9*, *MMP2*, *OPN*, *TNF*, vascular endothelial growth factor A (*VEGFA*), and *YKL40* were quantified by Real-Time polymerase chain reaction (PCR) (7300 Real Time PCR System, Applied Biosystem, Foster City, CA, USA). Primers and probes (Table 1) were designed using the software *Primer Express 2.0* (Applied Biosystems) and purchased from Genosys (Sigma, Madrid, Spain). Primers or TaqMan probes encompassing fragments of the areas from the extremes of two exons were designed to ensure the detection of the corresponding transcript avoiding genomic DNA amplification. The cDNA was amplified at the following conditions: 95°C for 10 min, followed by 45 cycles of 15 s at 95°C and 1 min at 59°C, using the TaqMan Universal PCR Master Mix (Applied Biosystems). The primer and probe concentrations for gene amplification were 300 and 200 nmol/L, respectively. All results were normalised to the levels of the ribosomal *18S* rRNA (Applied Biosystems) and relative quantification was calculated using the ΔΔCt formula [44]. Relative mRNA expression was expressed as fold expression over the calibrator sample (average of gene expression corresponding to the control group) as

previously described [45]. All samples were run in triplicate and the average values were calculated.

2.5. Gelatin zymography

MMP-2 and MMP-9 gelatinolytic activity was measured as previously described [46,47]. Briefly, protein extracts of 15 μg from each sample were run in duplicate in 10% SDS-PAGE containing 0.1% gelatin (Sigma). After the electrophoresis, gels were washed in 2.5% Triton X-100 (Sigma) for 45 min and, subsequently, incubated overnight at 37°C in enzyme development buffer (Invitrogen). Subsequently, gels were fixed in 50% (vol/vol) methanol and 7% (vol/vol) acetic acid (Sigma) for 15 min and then stained for 1 h in GelCode Blue Stain Reagent (Pierce, Rockford, IL, USA). Finally, the gels were rinsed in distilled water. MMP-2 and MMP-9 were identified based on their molecular weight and the Quantity One software (Bio-Rad) was used for densitometric analysis of the zymographic activities.

2.6. Statistical analysis

Data are presented as mean±standard error of the mean (S.E.M.). Differences between the control and CC groups were assessed by two-tailed unpaired Student's *t* tests. Due to their non-normal distribution gene expression levels were logarithmically transformed. The normal distribution of the other variables was adequate for the use of parametric tests. Pearson's correlation coefficients (*r*) were used to analyse the association between variables. The calculations were performed using the SPSS/Windows version 15.0 statistical package (SPSS, Chicago, IL, USA). $P<.05$ was considered statistically significant.

Table 1
Sequences of the primers and TaqMan probes

Gene (GenBank accession)	Oligonucleotide sequence (5′-3′)
ADIPOQ (NM_004797)	
Forward	GGAGATCCAGGTCTTATTGGTCCTA
Reverse	CCTTGGATTCCCGGAAAGC
TaqMan Probe	FAM-ACATCGGTGAAACCGGAGTACCCGG-TAMRA
HIF1A (NM_001530)	
Forward	AACTAGCCGAGGAAGAACTATGAACA
Reverse	TACCCACACTGAGGTTGGTTACTGT
TaqMan Probe	FAM-AACATGGAAGGTATTGCACTGCACAGGC-TAMRA
IGF1 (NM_001111283)	
Forward	GCCAAGTCAGCTCGCTCTGT
Reverse	TTTCCTTCTCTGAGACTTCGTGTTC
TaqMan Probe	FAM-TGCCCAAGACCCAGAAGTATCAGCCC-TAMRA
IGFBP3 (NM_001013398)	
Forward	AACTTCTCCTCCGAGTCC
Reverse	ACACTGAATCACCTGAAGT
TaqMan Probe	FAM-AATATGGTCCCTGCCGTAGAG-TAMRA
LCN2 (NM_005564)	
Forward	CCCAGCCCCACCTCTGA
Reverse	CTTCCCCTGGAATTGGTTGTC
TaqMan Probe	FAM-CAAGGTCCCTCTGCAGCAGAACTTCCA-TAMRA
MMP2 (NM_004530)	
Forward	CCATTTTGATGACGATGAGCTATG
Reverse	GTTGTACTCCTTGCCATTGAACAA
TaqMan Probe	FAM-CTTGGGAGAAGGCCAAGTGGTCCGT-TAMRA
MMP9 (NM_004994)	
Forward	GCCCGGACCAAGGATACAGT
Reverse	CCCCTCAGTGAAGCGGTACA
TaqMan Probe	FAM-ACGCGCTGGGCTTAGATCATTCCTCA-TAMRA
OPN (NM_000582)	
Forward	CATCCAGTACCCTGATGCTACAGA
Reverse	GGCCTTGTATGCACCATTCAA
TaqMan Probe	FAM-ACATCACCTCACACATGGAAAGCGAGGA-TAMRA
TNF (NM_00594)	
Forward	CCCCAGGGACCTCTCTCTAATC
Reverse	ACATGGGCTACAGGCTTGTCA
TaqMan Probe	FAM-CCTCTGGCCCAGGCAGTCAGATCAT-TAMRA
VEGFA (NM_001025250)	
Forward	CAGCACAACAAATGTGAATGCA
Reverse	ACACGTCTGCGGATCTTGTACA
TaqMan Probe	FAM-AATCCCTGTGGGCCTTGCTCAGAGC-TAMRA
YKL40 (NM_001276)	
Forward	GGACGGAGAGACAAACAGCATT
Reverse	CCTGGCTGGGCTTCCTTTAT
TaqMan Probe	FAM-CCACCCTAATCAAGGAAATGAAGGCC-TAMRA

IGFBP3, insulin-like growth factor binding protein 3; VEGFA, vascular endothelial growth factor A.

3. Results

3.1. Patient characteristics

The biochemical and hormonal characteristics of the subjects included in the study are shown in Table 2. No significant differences were detected between groups in BMI, which was in the overweight range and accompanied by no statistically significant differences in leptin concentrations. Patients with CC were significantly older ($P<.05$) than the control volunteers and exhibited higher insulin sensitivity than the control individuals as evidenced by the lower HOMA ($P<.05$) and higher QUICKI ($P<.01$) indices. Circulating concentrations of the inflammatory markers IL-6 ($P=.007$), TNF-α ($P=.012$) and HGF ($P=.034$) were significantly increased in patients with CC, whereas circulating levels of adiponectin ($P=.009$) were decreased in CC patients compared to the control group (Table 2).

3.2. Patients with colon cancer show increased gene expression levels of inflammatory markers in visceral adipose tissue

3.2.1. Gene expression levels of inflammatory markers

To address the relevance of inflammation, gene expression levels of *ADIPOQ*, *LCN2*, *OPN*, *TNF* and *YKL40* were measured in the VAT of CC patients compared to that of control volunteers. Expression levels of *LCN2* ($P=.014$), *YKL40* ($P=.006$), *OPN* ($P=.027$) and *TNF* ($P=.016$) were significantly up-regulated in CC patients (Fig. 1). As expected, an opposite tendency was observed in *ADIPOQ* mRNA expression levels, which were down-regulated in CC, although the differences did not reach statistical significance (Fig. 1). A statistically significant positive correlation between the mRNA expression of the proinflammatory genes studied as well as with the gene expression levels of *HIF1A* was found (Table 3).

3.2.2. Association of gene expression levels and circulating concentrations of inflammatory markers

Moreover, *LCN2* expression levels were positively correlated with the circulating concentrations of TNF-α ($r=0.51$; $P=.010$) as well as with MCP-1 ($r=0.48$; $P=.016$) and negatively with circulating levels of adiponectin ($r=-0.51$; $P=.013$). Furthermore, *YKL40* expression levels were positively correlated with the circulating inflammatory markers CRP ($r=0.48$; $P=.019$) and IL-6 ($r=0.46$; $P=.017$), while a negative association was detected between the mRNA of *TNF* and adiponectin concentrations ($r=-0.61$; $P<.001$).

3.3. Gene expression levels of angiogenesis-related factors in visceral adipose tissue are up-regulated in patients with colon cancer

3.3.1 mRNA expression of angiogenesis-related factors

Real-time PCR analysis indicated that the mRNA expression of *HIF1A* ($P=.043$) and *VEGFA* ($P=.012$) in VAT were significantly higher in CC patients compared to control volunteers (Fig. 2). As expected, gene expression levels of *IGF1* followed the same trend, whereas the mRNA expression of *IGFBP*3, an IGF-I regulatory protein, was down-regulated in CC patients, although no statistically significant differences were detected (Fig. 2). Gene expression levels of the proangiogenic factors were positively associated between them (Table 3). In addition, mRNA expression of *HIF1A* ($r=0.52$; $P=0.026$) and *VEGFA* ($r=0.45$; $P=0.031$) were significantly correlated with circulating concentrations of CRP.

3.3.2 Gene expression levels and activity of MMPs

In order to assess the potential involvement of MMPs in VAT in CC, the gene expression levels and activity of MMP-2 and MMP-9 were determined in adipose tissue homogenates. Gene expression levels of *MMP2* were up-regulated ($P=.018$) in CC patients compared to

Table 2
Anthropometric and biochemical characteristics of the subjects included in the study

	Control	CC
n	18	11
Age (years)	44±3	66±3**
BMI (kg/m^2)	29.3±1.7	26.9±0.9
Fasting glucose (mg/dL)	99±4	109±8
Fasting insulin (μU/mL)	12.0±2.6	3.8±0.9*
HOMA	3.1±0.8	1.2±0.3*
QUICKI	0.33±0.01	0.41±0.02**
Leptin (ng/mL)	22.2±5.5	10.8±5.1
Adiponectin (μg/mL)	8.09±0.90	4.07±0.62**
C-reactive protein (mg/L)	4.31±0.90	11.96±6.94
IL-6 (pg/mL)	1.57±0.10	3.63±1.24**
IL-8 (pg/mL)	1.25±0.13	1.40±0.18
TNF-α (pg/mL)	2.08±0.11	2.70±0.24*
MCP-1 (pg/mL)	118.9±19.4	110.7±17.8
HGF (pg/mL)	95.6±7.7	150.0±26.7*
NGF (pg/mL)	4.52±0.70	3.90±1.11

Data are mean± S.E.M. Differences between groups were analyzed by Student's *t* tests.
*P<.05 and **P<.01 vs. controls.

control volunteers. A similar tendency was observed in *MMP9* mRNA expression, but the differences did not reach statistical significance (Fig. 3). On the contrary, the activity of MMP-9 in adipose tissue was significantly (P<.001) increased in CC patients compared to control subjects, whereas no significant differences were detected for the activity of MMP-2 (Fig. 3). Moreover, gene expression levels of *MMP-2* were significantly correlated (P<.001) with mRNA expression levels of *VEGFA* and *IGF1* with *MMP9* gene expression levels being also associated (P<.05) with *VEGFA* mRNA expression levels.

4. Discussion

Adipose tissue releases a variety of adipokines involved in the metabolic regulation and directly linked to obesity-associated comorbidities, including several types of cancer. The present study shows, for the first time, that the VAT of patients with CC exhibits increased gene expression levels of the proinflammatory adipokines *LCN2*, *YKL40*, *OPN* and *TNF* compared with patients without CC. In addition, we also detected an up-regulation of the angiogenic factors *HIF1A*, *VEGFA* and *MMP2* as well as an increased activity of MMP-9 in patients with CC in VAT. Furthermore, the circulating concentrations of pro-inflammatory markers such as IL-6, TNF-α and HGF were increased in CC patients in contrast to the reduced concentrations of the anti-inflammatory hormone adiponectin.

The increasing prevalence of obesity is related to both increased energy intake and reduced energy expenditure. Disturbances in energy homeostasis lead to metabolic alterations, which are related to colorectal carcinogenesis [48]. Previous epidemiological studies have consistently shown associations between adiposity and increased risk of cancers of the colon, breast, endometrium, kidney and gallbladder [3]. Moreover, an association between obesity and cancer is consistent with data from animal models showing that caloric restriction decreases spontaneous and carcinogen-induced tumor incidence [49]. In this sense, it has been recently described that severe caloric restriction in humans may confer protection against invasive breast cancer [50]. Among the various pathophysiological mechanisms postulated to explain the link between obesity and cancer, the dysfunctional adipose tissue may be a unifying and underlying factor [51].

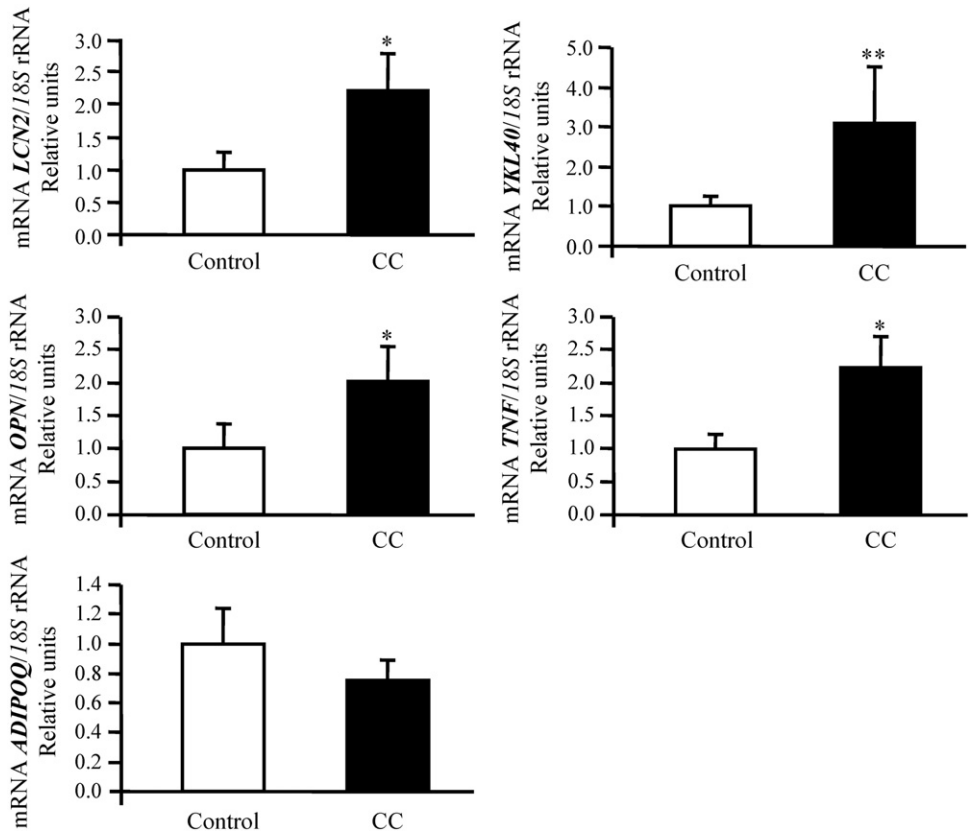

Fig. 1. Gene expression levels of *LCN2*, *YKL40*, *OPN*, *TNF* and *ADIPOQ* in VAT of controls and patients with CC. Bars represent the mean±S.E.M. of the ratio between gene expression to *18S* rRNA. The expression level in control subjects was assumed to be 1. Differences between groups were assessed by two-tailed unpaired Student's *t* tests (control: n=18; CC: n=11). *P<.05 and **P<.01 vs. control subjects.

Table 3
Univariate analysis of the correlation between pro-inflammatory gene expression levels and angiogenic-related factors in visceral adipose tissue

	LCN2		*YKL40*		*OPN*		*TNF*		*HIF1A*		*VEGFA*		*IGF1*	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
LCN2	–	–	0.43	**.025**	0.41	**.049**	0.63	**<.001**	0.61	**.004**	0.33	.103	.13	.521
YKL40	0.43	**.025**	–	–	0.40	**.041**	0.29	.136	0.50	**.015**	0.49	**.008**	0.21	.301
OPN	0.41	**.049**	0.40	**.041**	–	–	0.25	.206	0.59	**.006**	0.38	.059	0.17	.416
TNF	0.63	**<.001**	0.29	.136	0.25	.206	–	–	0.50	**.015**	0.32	.095	0.28	.243
HIF1A	0.61	**.004**	0.50	**.015**	0.59	**.006**	0.50	**.015**	–	–	0.44	**.042**	0.65	**<.001**
VEGFA	0.33	.103	0.49	**.008**	0.38	.059	0.32	.095	0.44	**.042**	–	–	0.55	**.003**
IGF1	0.13	.521	0.21	.301	0.17	.416	0.28	.243	0.65	**<.001**	0.55	**.003**	–	–
MMP2	0.58	**.006**	0.50	**.012**	0.07	.753	0.13	.559	0.32	.149	0.73	**<.001**	0.68	**<.001**
MMP9	0.03	.989	0.38	**.049**	0.27	.203	0.19	.344	0.16	.554	0.39	**.048**	0.23	.261

Bold values indicate statistically significant *P* values.

Inflammation is independently linked with VAT expansion [52]. In this sense, a large body of evidence shows that visceral obesity is associated with a higher risk of CC development with waist circumference and waist-to-hip ratio (markers of intra-abdominal fat) showing a higher association with the development of CC than BMI [53–55]. We detected an increased mRNA expression of recently identified adipokines in human VAT in patients with CC. In this context, recent reports have described LCN2 as an adipokine closely related to obesity and insulin resistance [47,56]. Gene expression levels of LCN2 were significantly up-regulated in VAT of patients with CC, which is in accordance with previous studies where LCN2 is induced in epithelial cells in inflammatory and neoplastic colorectal diseases [57], suggesting that LCN2 may play a role in regulating cellular growth [58]. Moreover, it has been also described that the overexpression of LCN2 in human breast cancer cells up-regulates mesenchymal markers while down-regulating E-cadherin, thereby increasing cell motility and invasiveness [24]. Two functions of LCN2 related to tumorigenesis, one involving the induction of apoptosis in normal hematopoietic cells and the other consisting in tisular invasion by neoplastic cells have been recently proposed [59]. LCN2 is reportedly induced by TNF-α, a well-known and relevant factor in inflammatory processes [56]. In line with this functional relation, we have established a significant positive association between *LCN2* mRNA and gene expression levels of *TNF* in VAT as well as with its circulating concentrations. *LCN2* mRNA levels were also positively correlated with MCP-1 levels, a well-established marker of chronic inflammation. However, other results suggest that LCN2 could be a potential metastasis suppressor gene in CC cells [60] and, in this regard, it has been suggested that LCN2 may act as a negative regulator of the effect of inflammatory molecules [61].

YKL-40 is a growth factor with functions in inflammation, cancer cell proliferation angiogenesis and remodelling of the extracellular matrix [20]. Increased *YKL40* gene expression levels in VAT of CC patients were observed in the present study. It has been shown that elevated postoperative serum YKL-40 levels in patients undergoing curative resection of colorectal carcinoma was associated with a high risk of tumour recurrence and poor survival [62,63]. Moreover, it has been shown that YKL-40 acts synergistically with IGF-I in stimulating the growth of fibroblasts and in a concentration range similar to that of IGF-I. The inflammatory factor NF-κB is essential for the induction of YKL-40 [64] and, in this sense, we found a significant positive association with CRP and IL-6, which is in line with previous results in animal models where YKL-40 is regulated by IL-6 in both wild-type and IL-6 knockout mice [20].

Recent findings have shown that OPN expression correlates with colon tumour progression [65] and that OPN plasma concentrations of

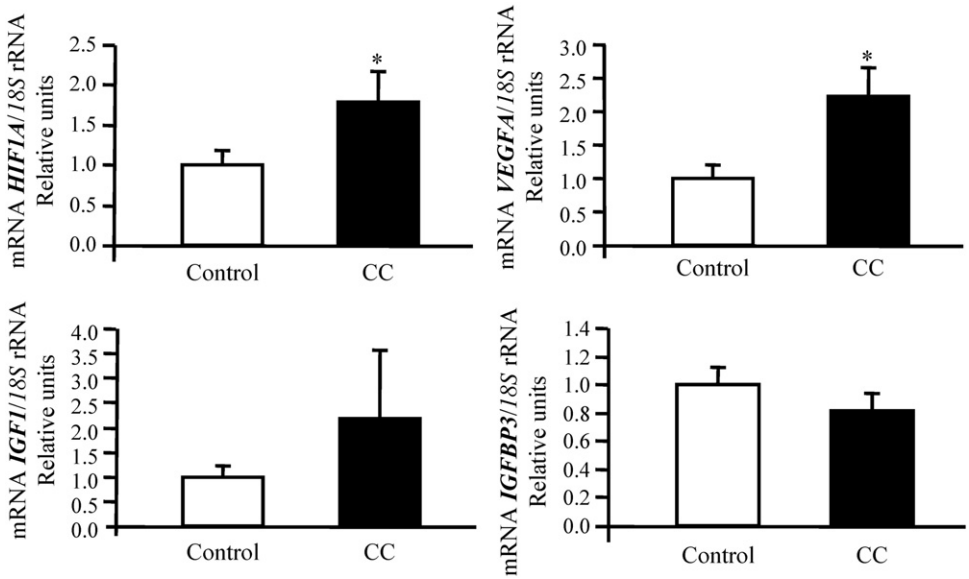

Fig. 2. Gene expression levels of *HIF1A*, *VEGF*, *IGF1* and *IGFBP3* in VAT of controls and patients with CC. Bars represent the mean± S.E.M. of the ratio between gene expression to *18S* rRNA. The expression level in control subjects was assumed to be 1. Differences between groups were assessed by two-tailed unpaired Student's *t* tests (control: *n*=18; CC: n=11). **P*<.05 vs. control subjects.

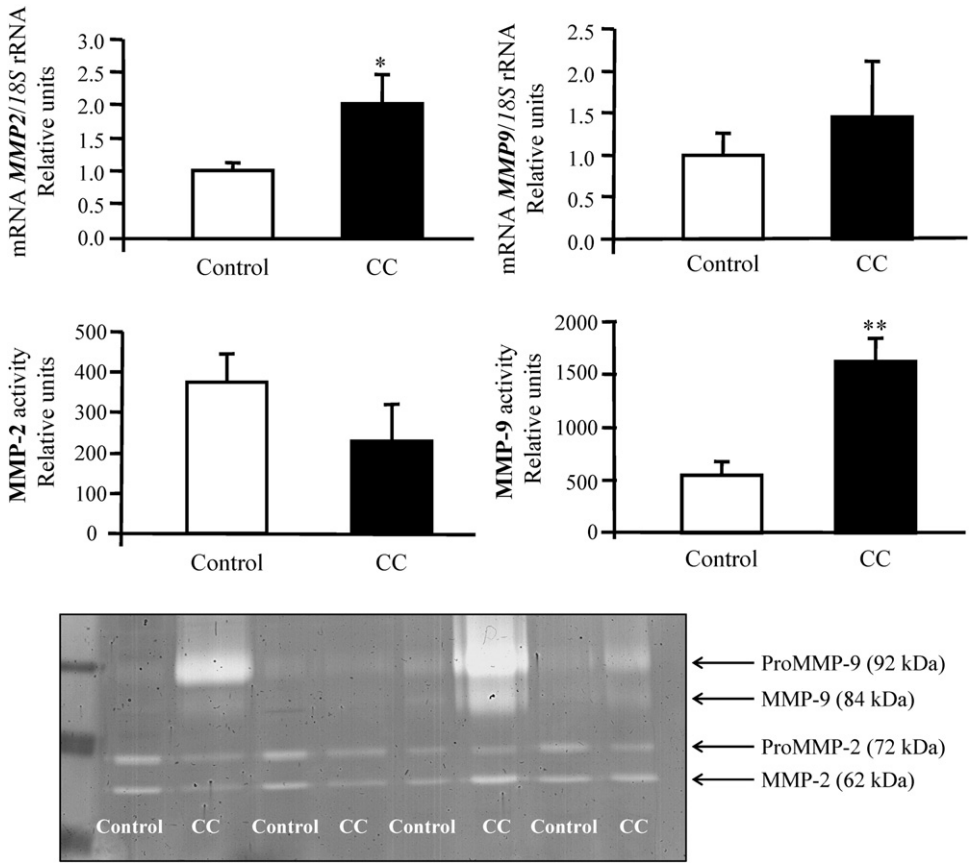

Fig. 3. Real-time PCR and zymography analysis of MMP-2 and MMP-9 in VAT of control volunteers and patients with colon cancer (CC). Bars represent the mean± S.E.M.. The expression level in control subjects was assumed to be 1. Differences between groups were assessed by two-tailed unpaired Student's *t* test (control: $n=18$; CC: $n=11$). *$P<.05$ vs. control subjects. A representative gelatin zymography is shown at the bottom of the figure.

patients with metastatic disease are significantly higher compared to those of control volunteers [66]. The link between OPN, HIF-1α and the PI3-K/Akt signalling pathway has been recently described and may provide a molecular explanation for the prosurvival activity of OPN in the context of tumour progression [67]. Interestingly, we have shown a positive association of all the pro-inflammatory genes studied with HIF-1α. In this regard, we also detected an up-regulation of the gene expression levels of *HIF1A* paralleling the increased *VEGFA* levels in patients with CC. Hypoxia is common in the microenvironment of solid tumours [68,69]. Several studies have associated *HIF1A* expression with human cancer progression and with cell proliferation [70]. It has been also suggested that hypoxia exerts an important effect on adipocyte metabolism, underpinning the inflammatory response in adipose tissue in obesity and the subsequent development of obesity-associated diseases such as cancer [36]. The increased mRNA expression levels of *VEGFA* detected in our study are in agreement with previous results where elevated levels of vascular growth factors were detected in overweight and obese subjects suggesting their contribution to the increased risk of metastatic disease in obese subjects with cancer [34,71]. Gene expression levels of both *HIF1A* and *VEGFA* were positively associated with CRP, indicating an association with inflammation.

MMPs, including MMP-9 and MMP-2, play a key role in cancer progression as well as in adipose tissue remodelling [38]. Although an up-regulation of VAT *MMP2* from the CC group was shown in the present study, no changes in *MMP9* gene expression were detected. On the contrary, higher MMP-9 activity was observed in CC patients but no differences were evident in MMP-2 activity. A constant increase in *MMP2* mRNA expression during adipose differentiation has been described, whereas *MMP9* expression reportedly presents a different profile [39]. This different behaviour may underlie the inverse profile between activity and gene expression levels of both MMPs observed in our study. Moreover, an interrelationship between MMPs and LCN2, YKL-40 and OPN had been previously reported [72–74].

Adiponectin is an anti-inflammatory protein produced exclusively by adipose tissue [75]. Lower circulating concentrations and a tendency towards decreased gene expression levels of adiponectin in patients with CC compared to the control group were observed. In line with these results, hypoadiponectinaemia has been significantly correlated with a higher risk of colorectal cancer [76] and recently it has been demonstrated that adiponectin inhibits colorectal cancer cell growth via activation of AMPK [77].

These findings represent the first observation that VAT of patients with CC overexpress the inflammatory adipokines *LCN2*, *YKL-40* and *OPN*. We also found higher VAT mRNA levels of *TNF*, *HIF1A*, *VEGFA* and *MMP2* as well as an increased activity of MMP-9 in patients with CC. Moreover, a tendency towards increased *IGF1* mRNA levels as well as the opposite tendency for *IGFBP3* was also observed. On the contrary, circulating concentrations of adiponectin were lower in patients with CC with its gene expression levels in VAT showing a trend to be decreased. The supply of adipose tissue-derived angiogenic factors as well as the production of inflammatory cytokines by adipocytes or infiltrating macrophages that take place in adipose tissue may promote a paracrine stimulation at the same time as maintaining a microenvironment that is favourable for tumorigenesis. Further studies in larger cohorts to better understand the implication and pathways of these adipokines in tumour

development are warranted in order to establish the specific contribution of recently identified and emerging adipokines to the CC development.

Acknowledgments

The authors gratefully acknowledge the valuable collaboration of the all the members of the Multidisciplinary Obesity Team. CIBER de Fisiopatología de la Obesidad y Nutrición (CIBEROBN) is an initiative of the Instituto de Salud Carlos III, Spain.

References

[1] Ogden CL, Carroll MD, Curtin LR, McDowell MA, Tabak CJ, Flegal KM. Prevalence of overweight and obesity in the United States, 1999-2004. JAMA 2006;295:1549–55.
[2] International Agency for Reasearch on Cancer. IARC Handbooks of Cancer Prevention. Handbooks of Cancer Prevention. Lyon: Weight control and physical activity; 2002.
[3] Calle EE, Kaaks R. Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. Nat Rev Cancer 2004;4:579–91.
[4] Frezza EE, Wachtel MS, Chiriva-Internati M. Influence of obesity on the risk of developing colon cancer. Gut 2006;55:285–91.
[5] Renehan AG, Tyson M, Egger M, Heller RF, Zwahlen M. Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. Lancet 2008;371:569–78.
[6] Giovannucci E, Colditz GA, Stampfer MJ, Willett WC. Physical activity, obesity, and risk of colorectal adenoma in women (United States). Cancer Causes Control 1996;7:253–63.
[7] Russo A, Franceschi S, La Vecchia C, Dal Maso L, Montella M, Conti E, et al. Body size and colorectal-cancer risk. Int J Cancer 1998;78:161–5.
[8] Schoen RE, Tangen CM, Kuller LH, Burke GL, Cushman M, Tracy RP, et al. Increased blood glucose and insulin, body size, and incident colorectal cancer. J Natl Cancer Inst 1999;91:1147–54.
[9] Giovannucci E, Michaud D. The role of obesity and related metabolic disturbances in cancers of the colon, prostate, and pancreas. Gastroenterology 2007;132:2208–25.
[10] Pischon T, Lahmann PH, Boeing H, Friedenreich C, Norat T, Tjonneland A, et al. Body size and risk of colon and rectal cancer in the European Prospective Investigation Into Cancer and Nutrition (EPIC). J Natl Cancer Inst 2006;98:920–31.
[11] Kim Y, Lee S. An association between colonic adenoma and abdominal obesity: a cross-sectional study. BMC Gastroenterol 2009, doi:10.1186/1471-230X-9-4 [in press].
[12] Coussens LM, Werb Z. Inflammatory cells and cancer: think different! J Exp Med 2001;193:F23–6.
[13] Coussens LM, Werb Z. Inflammation and cancer. Nature 2002;420:860–7.
[14] Balkwill F, Coussens LM. Cancer: an inflammatory link. Nature 2004;431:405–6.
[15] Khasawneh J, Schulz MD, Walch A, Rozman J, Hrabe de Angelis M, Klingenspor M, et al. Inflammation and mitochondrial fatty acid β-oxidation link obesity to early tumor promotion. Proc Natl Acad Sci USA 2009;106:3354–9.
[16] Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. J Clin Endocrinol Metab 2004;89:2548–56.
[17] Frühbeck G. Overview of adipose tissue and its role in obesity and metabolic disorders. Methods Mol Biol 2008;456:1–22.
[18] Tilg H, Moschen AR. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. Nat Rev Immunol 2006;6:772–83.
[19] Rangaswami H, Bulbule A, Kundu GC. Osteopontin: role in cell signaling and cancer progression. Trends Cell Biol 2006;16:79–87.
[20] Johansen JS, Jensen BV, Roslind A, Nielsen D, Price PA. Serum YKL-40, a new prognostic biomarker in cancer patients? Cancer Epidemiol Biomarkers Prev 2006;15:194–202.
[21] Yang J, Moses MA. Lipocalin 2: a multifaceted modulator of human cancer. Cell Cycle 2009;8:15.
[22] Lim R, Ahmed N, Borregaard N, Riley C, Wafai R, Thompson EW, et al. Neutrophil gelatinase-associated lipocalin (NGAL) an early-screening biomarker for ovarian cancer: NGAL is associated with epidermal growth factor-induced epithelio-mesenchymal transition. Int J Cancer 2007;120:2426–34.
[23] Zhang H, Xu L, Xiao D, Xie J, Zeng H, Wang Z, et al. Upregulation of neutrophil gelatinase-associated lipocalin in oesophageal squamous cell carcinoma: significant correlation with cell differentiation and tumour invasion. J Clin Pathol 2007;60:555–61.
[24] Yang J, Bielenberg DR, Rodig SJ, Doiron R, Clifton MC, Kung AL, et al. Lipocalin 2 promotes breast cancer progression. Proc Natl Acad Sci USA 2009;106:3913–8.
[25] Rodrigues LR, Teixeira JA, Schmitt FL, Paulsson M, Lindmark-Mansson H. The role of osteopontin in tumor progression and metastasis in breast cancer. Cancer Epidemiol Biomarkers Prev 2007;16:1087–97.
[26] Johansen JS, Bojesen SE, Mylin AK, Frikke-Schmidt R, Price PA, Nordestgaard BG. Elevated plasma YKL-40 predicts increased risk of gastrointestinal cancer and decreased survival after any cancer diagnosis in the general population. J Clin Oncol 2009;27:572–8.
[27] Kelesidis I, Kelesidis T, Mantzoros CS. Adiponectin and cancer: a systematic review. Br J Cancer 2006;94:1221–5.
[28] Otani K, Kitayama J, Yasuda K, Nio Y, Iwabu M, Okudaira S, et al. Adiponectin suppresses tumorigenesis in Apc(Min)(/+) mice. Cancer Lett 2009, doi:10.1016/j.canlet.2009.06.037 (in press).
[29] Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell 1996;86:353–64.
[30] Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. Nature 2000;407:249–57.
[31] Cao Y. Angiogenesis modulates adipogenesis and obesity. J Clin Invest 2007;117:2362–8.
[32] Lijnen HR. Angiogenesis and obesity. Cardiovasc Res 2008;78:286–93.
[33] Gómez-Ambrosi J, Catalán V, Diez-Caballero A, Martínez-Cruz LA, Gil MJ, García-Foncillas J, et al. Gene expression profile of omental adipose tissue in human obesity. FASEB J 2004;18:215–7.
[34] Gómez-Ambrosi J, Catalán V, Rodríguez A, Ramírez B, Silva C, Gil MJ, et al. Involvement of serum vascular endothelial growth factor family members in the development of obesity in mice and humans. J Nutr Biochem 2009, doi:10.1016/j.nutbio.2009.05.004 (in press).
[35] Trayhurn P, Wood IS. Adipokines: inflammation and the pleiotropic role of white adipose tissue. Br J Nutr 2004;92:347–55.
[36] Trayhurn P, Wang B, Wood IS. Hypoxia in adipose tissue: a basis for the dysregulation of tissue function in obesity? Br J Nutr 2008;100:227–35.
[37] Hosogai N, Fukuhara A, Oshima K, Miyata Y, Tanaka S, Segawa K, et al. Adipose tissue hypoxia in obesity and its impact on adipocytokine dysregulation. Diabetes 2007;56:901–11.
[38] Egeblad M, Werb Z. New functions for the matrix metalloproteinases in cancer progression. Nat Rev Cancer 2002;2:161–74.
[39] Bouloumie A, Sengenes C, Portolan G, Galitzky J, Lafontan M. Adipocyte produces matrix metalloproteinases 2 and 9: involvement in adipose differentiation. Diabetes 2001;50:2080–6.
[40] Renehan AG, Frystyk J, Flyvbjerg A. Obesity and cancer risk: the role of the insulin-IGF axis. Trends Endocrinol Metab 2006;17:328–36.
[41] Pollak MN, Schernhammer ES, Hankinson SE. Insulin-like growth factors and neoplasia. Nat Rev Cancer 2004;4:505–18.
[42] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF. Turner RC. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–9.
[43] Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, et al. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab 2000;85:2402–10.
[44] Catalán V, Gómez-Ambrosi J, Rotellar F, Silva C, Rodríguez A, Salvador J, et al. Validation of endogenous control genes in human adipose tissue: relevance to obesity and obesity-associated type 2 diabetes mellitus. Horm Metab Res 2007;39:495–500.
[45] Catalán V, Gómez-Ambrosi J, Rodríguez A, Silva C, Rotellar F, Gil MJ, et al. Expression of caveolin-1 in human adipose tissue is upregulated in obesity and obesity-associated type 2 diabetes mellitus and related to inflammation. Clin Endocrinol 2008;68:213–9.
[46] Sier CF, Kubben FJ, Ganesh S, Heerding MM, Griffioen G, Hanemaaijer R, et al. Tissue levels of matrix metalloproteinases MMP-2 and MMP-9 are related to the overall survival of patients with gastric carcinoma. Br J Cancer 1996;74:413–7.
[47] Catalán V, Gómez-Ambrosi J, Rodríguez A, Ramírez B, Silva C, Rotellar F, et al. Increased adipose tissue expression of lipocalin-2 in obesity is related to inflammation and matrix metalloproteinase-2 and metalloproteinase-9 activities in humans. J Mol Med 2009;87:803–13.
[48] Gunter MJ, Leitzmann MF. Obesity and colorectal cancer: epidemiology, mechanisms and candidate genes. J Nutr Biochem 2006;17:145–56.
[49] Dunn SE, Kari FW, French J, Leininger JR, Travlos G, Wilson R, et al. Dietary restriction reduces insulin-like growth factor I levels, which modulates apoptosis, cell proliferation, and tumor progression in p53-deficient mice. Cancer Res 1997;57:4667–72.
[50] Michels KB, Ekbom A. Caloric restriction and incidence of breast cancer. JAMA 2004;291:1226–30.
[51] van Kruijsdijk RC, van der Wall E, Visseren FL. Obesity and cancer: the role of dysfunctional adipose tissue. Cancer Epidemiol Biomarkers Prev 2009;18:2569–78.
[52] Despres JP, Lemieux I. Abdominal obesity and metabolic syndrome. Nature 2006;444:881–7.
[53] Giovannucci E, Ascherio A, Rimm EB, Colditz GA, Stampfer MJ, Willett WC. Physical activity, obesity, and risk for colon cancer and adenoma in men. Ann Intern Med 1995;122:327–34.
[54] Moore LL, Bradlee ML, Singer MR, Splansky GL, Proctor MH, Ellison RC, et al. BMI and waist circumference as predictors of lifetime colon cancer risk in Framingham Study adults. Int J Obes Relat Metab Disord 2004;28:559–67.
[55] Oh TH, Byeon JS, Myung SJ, Yang SK, Choi KS, Chung JW, et al. Visceral obesity as a risk factor for colorectal neoplasm. J Gastroenterol Hepatol 2008;23:411–7.
[56] Yan QW, Yang Q, Mody N, Graham TE, Hsu CH, Xu Z, et al. The adipokine lipocalin 2 is regulated by obesity and promotes insulin resistance. Diabetes 2007;56:2533–40.
[57] Nielsen BS, Borregaard N, Bundgaard JR, Timshel S, Sehested M, Kjeldsen L. Induction of NGAL synthesis in epithelial cells of human colorectal neoplasia and inflammatory bowel diseases. Gut 1996;38:414–20.
[58] Hraba-Renevey S, Turler H, Kress M, Salomon C, Weil R. SV40-induced expression of mouse gene 24p3 involves a post-transcriptional mechanism. Oncogene 1989;4:601–8.

[59] Leng X, Lin H, Ding T, Wang Y, Wu Y, Klumpp S, et al. Lipocalin 2 is required for BCR-ABL-induced tumorigenesis. Oncogene 2008;27:6110–9.
[60] Lee HJ, Lee EK, Lee KJ, Hong SW, Yoon Y, Kim JS. Ectopic expression of neutrophil gelatinase-associated lipocalin suppresses the invasion and liver metastasis of colon cancer cells. Int J Cancer 2006;118:2490–7.
[61] Zhang J, Wu Y, Zhang Y, Leroith D, Bernlohr DA, Chen X. The role of lipocalin 2 in the regulation of inflammation in adipocytes and macrophages. Mol Endocrinol 2008;22:1416–26.
[62] Cintin C, Johansen JS, Christensen IJ, Price PA, Sorensen S, Nielsen HJ. Serum YKL-40 and colorectal cancer. Br J Cancer 1999;79:1494–9.
[63] Cintin C, Johansen JS, Christensen IJ, Price PA, Sorensen S, Nielsen HJ. High serum YKL-40 level after surgery for colorectal carcinoma is related to short survival. Cancer 2002;95:267–74.
[64] Recklies AD, Ling H, White C, Bernier SM. Inflammatory cytokines induce production of CHI3L1 by articular chondrocytes. J Biol Chem 2005;280:41213–21.
[65] Agrawal D, Chen T, Irby R, Quackenbush J, Chambers AF, Szabo M, et al. Osteopontin identified as lead marker of colon cancer progression, using pooled sample expression profiling. J Natl Cancer Inst 2002;94:513–21.
[66] Fedarko NS, Jain A, Karadag A, Van Eman MR, Fisher LW. Elevated serum bone sialoprotein and osteopontin in colon, breast, prostate, and lung cancer. Clin Cancer Res 2001;7:4060–6.
[67] Song G, Ouyang G, Mao Y, Ming Y, Bao S, Hu T. Osteopontin promotes gastric cancer metastasis by augmenting cell survival and invasion through Akt-mediated HIF-1α up-regulation and MMP9 activation. J Cell Mol Med 2008, doi: 10.1111/j.1582-4934.2008.00540.x (in press).
[68] Harris AL. Hypoxia-a key regulatory factor in tumour growth. Nat Rev Cancer 2002;2:38–47.
[69] Yang MH, Wu MZ, Chiou SH, Chen PM, Chang SY, Liu CJ, et al. Direct regulation of TWIST by HIF-1α promotes metastasis. Nat Cell Biol 2008;10:295–305.
[70] Zhong H, De Marzo AM, Laughner E, Lim M, Hilton DA, Zagzag D, et al. Overexpression of hypoxia-inducible factor 1α in common human cancers and their metastases. Cancer Res 1999;59:5830–5.
[71] Silha JV, Krsek M, Sucharda P, Murphy LJ. Angiogenic factors are elevated in overweight and obese individuals. Int J Obes 2005;29:1308–14.
[72] Hormigo A, Gu B, Karimi S, Riedel E, Panageas KS, Edgar MA, et al. YKL-40 and matrix metalloproteinase-9 as potential serum biomarkers for patients with high-grade gliomas. Clin Cancer Res 2006;12:5698–704.
[73] Kubben FJ, Sier CF, Hawinkels LJ, Tschesche H, van Duijn W, Zuidwijk K, et al. Clinical evidence for a protective role of lipocalin-2 against MMP-9 autodegradation and the impact for gastric cancer. Eur J Cancer 2007;43:1869–76.
[74] Chen YJ, Wei YY, Chen HT, Fong YC, Hsu CJ, Tsai CH, et al. Osteopontin increases migration and MMP-9 up-regulation via αvβ3 integrin, FAK, ERK, and NF-kappaB-dependent pathway in human chondrosarcoma cells. J Cell Physiol 2009;221(1):98–108.
[75] Kadowaki T, Yamauchi T, Kubota N, Hara K, Ueki K, Tobe K. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. J Clin Invest 2006;116:1784–92.
[76] Wei EK, Giovannucci E, Fuchs CS, Willett WC, Mantzoros CS. Low plasma adiponectin levels and risk of colorectal cancer in men: a prospective study. J Natl Cancer Inst 2005;97:1688–94.
[77] Sugiyama M, Takahashi H, Hosono K, Endo H, Kato S, Yoneda K, et al. Adiponectin inhibits colorectal cancer cell growth through the AMPK/mTOR pathway. Int J Oncol 2009;34:339–44.