Review

Serum YKL-40, A New Prognostic Biomarker in Cancer Patients?

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Abstract

YKL-40, a member of the "mammalian chitinase–like proteins," is expressed and secreted by several types of solid tumors. The exact function of YKL-40 in cancer diseases is unknown and is an important objective of future studies. YKL-40 exhibits growth factor activity for cells involved in tissue remodeling processes. YKL-40 may have a role in cancer cell proliferation, survival, and invasiveness, in the inflammatory process around the tumor, angiogenesis, and remodeling of the extracellular matrix. YKL-40 is neither organ- nor tumor-specific. However, the present retrospective clinical studies of patients with eight different types of primary or advanced solid tumors suggest that serum concentration of YKL-40 may be a new biomarker in cancer patients used as a "prognosticator." Elevated serum YKL-40 is found in a subgroup of patients with different types of solid tumors, including several types of adenocarcinomas, small cell lung carcinoma, glioblastoma, and melanoma. The highest serum YKL-40 is detected in patients with advanced cancer and with the poorest prognosis. In many cases, serum YKL-40 provides independent information of survival. Serum YKL-40 cannot be used as a single screening test for cancer. The use of serum YKL-40 has not received Food and Drug Administration approval for use as a biomarker for cancer or any other disease. Large multicenter retrospective and prospective studies of patients with different types of cancer are required to determine: (*a*) if serum YKL-40 is a useful prognostic cancer biomarker, (*b*) if serum YKL-40 can be of value in monitoring patients with cancer in order to provide information about metastases before these are detected by routine methods, and (*c*) if serum YKL-40 can be useful for screening of cancer together with a panel of other cancer biomarkers and imaging techniques. (Cancer Epidemiol Biomarkers Prev 2006;15(2):194–202)

YKL-40

In a search of new bone proteins, >10 years ago, we identified a protein secreted *in vitro* in large amounts by the human osteosarcoma cell line MG63 (1). We named the protein "YKL-40" based on its three NH$_2$-terminal amino acids tyrosine (Y), lysine (K), and leucine (L) and its molecular weight of 40 kDa (1). YKL-40 contains a single polypeptide chain of 383 amino acids and the complete amino acid and cDNA sequence of human YKL-40 was published in 1993 by Hakala et al. (2) (GenBank accession no. M80927). The sequence of YKL-40 from several other mammals is known: mouse (ref. 3, X93035), porcine (ref. 4, U19900), guinea pig (5), rat (AF062038), bovine (AF011373), and goat (ref. 6, AY081150). Based on its amino acid sequence, it has been found that YKL-40 belongs to the glycosyl hydrolase family 18 (7). This family consists of enzymes and proteins, and includes chitinases from various species, including mammalian, bacteria, fungi, nematodes, insects, and plants (8). YKL-40 shares significant amino acid sequence homology to bacterial chitinases (2, 9) and to six "mammalian chitinase–like proteins": oviduct-specific glyco-protein (10, 11), chitotriosidase (12), YKL-39 (13), TSA 1902 (14), inducible silicotic bronchoalveolar lavage protein-p^{58} (15) also named acidic mammalian chitinase (16), and mouse Ym1 (17) also named eosinophil chemotactic cytokine (18). Interestingly, *Drosophila melanogaster* secretes several proteins, DS47 and imaginal disc growth factors, with sequence homology to YKL-40 (19, 20).

The gene for YKL-40 (CHI3L1) is located on chromosome 1q31-q32 (21) and consists of 10 exons and spans about 8 kb of genomic DNA. The genes of all the other human mammalian chitinase–like proteins identified thus far are also located on chromosome 1. Recently, the transcriptional regulation of YKL-40 during human macrophage differentiation has been described (22). There are probably two independent transcription start sites and the promoter sequence contains binding sites for several known factors and specific binding of nuclear PU.1, Sp1, Sp3, USF, AML-1, and C/EBP proteins (22). The Sp1-family transcription factors seem to have a predominating role in controlling YKL-40 promoter activity.

The crystallographic three-dimensional structures of human YKL-40 (23, 24) and goat YKL-40 (6) display the typical fold of family 18 glycosyl hydrolases (25). The structure is divided into two globular domains: a big core domain which consists of a $(\beta/\alpha)_8$ domain structure with a triose-phosphate isomerase barrel fold, and a small α/β domain, composed of five antiparallel β-strands and one α-helix, inserted in the loop between strand $\beta7$ and helix $\alpha7$. This gives the active site of YKL-40 a groove-like character.

YKL-40 binds chitin of different lengths in a similar fashion as seen in family 18 chitinases (26), but has no chitinase activity (2, 26). The amino acids essential for the catalytic activity in chitinases are three acidic residues Asp, Glu, and Asp. The corresponding residues in human YKL-40 are Asp^{115}, Leu^{119},

and Asp[186]. The mutation of Glu to Leu in YKL-40 rules out its role as a glycolytic enzyme. YKL-40 is a glycoprotein in which most of the glucosamine is incorporated into N-linked complex oligosaccharides (5, 27). Two possible sites of glycosylation are found in YKL-40, but only the NH_2-terminal is glycosylated with two units of N-acetylglucosamine through β(1-4) linkage. Binding of either short or long oligosaccharides to human YKL-40 is possible, and the presence of two distinct binding sites with selective affinity for long and short oligosaccharides suggests that YKL-40 could function by cross-linking two targets. Glycosylation is a unique feature of the YKL-40 structure as the residue corresponding to Arg[84] does not exist in chitinases and is mutated to Pro in other mammalian chitinase–like proteins. YKL-40 also binds heparin (4) and amino acid sequence analysis reveals that YKL-40 contains one heparin binding motif (GRRDKQH at position 143-149) and two potential hyaluronan binding sites on the external face of the folded protein (23, 28). The putative heparin-binding site is located in a surface loop (23). It has been suggested that heparan sulfate is a more likely ligand of YKL-40, and unsulfated fragments of heparan sulfate can be accommodated in the binding groove of YKL-40 (23).

Which cells secrete YKL-40?

Based on a search of the YKL-40 protein sequence against the dbEST database at the National Center for Biotechnology Information, it was found that YKL-40 is expressed by several types of solid tumors (breast, colon, lung, kidney, ovary, prostate, uterine, pancreas, osteosarcoma, thyroid, oligoden-droglioma, glioblastoma, and germ cell tumors). Microarray gene analyses have identified the human YKL-40 gene to be one of the most overexpressed genes in glioblastoma multi-forme (29-33), in papillary thyroid carcinoma (34), and in extracellular myxoid chondrosarcoma (35). In murine mam-mary tumors, YKL-40 mRNA is expressed selectively by neu/ras oncogenes (3). YKL-40 is secreted in vitro by the following human cancer cell lines (obtained from American Type Culture Collection, Manassas, VA): osteosarcoma (MG63; ref. 1), glioblastoma (U87; ref. 36), colon cancer (DLD-1, SW1417), ovarian cancer (SW626), prostate cancer (DV-145), and malignant melanoma (SK-MEL-28).[4] Immunohistochemi-cal analyses have shown YKL-40 protein expression in the cancer cells in biopsies of glioblastomas (37, 38), breast cancer (ref. 39; Fig. 1), and colon cancer (40). Furthermore, high YKL-40 protein expression in glioblastoma was associated with loss of chromosome 10q but not with epidermal growth factor receptor amplification status (38). YKL-40 mRNA and protein is not expressed by 20 human small cell lung cancer cell lines in vitro or in vivo, but is strongly expressed in macrophages in the peritumoral stroma in human small cell lung cancer biopsies (41). Treatment with phorbol 12-myristate 13-acetate of human tumor cell lines that originate from immature cells of the monocytic differentiation lineage corresponding to mono-blasts (U937, THP-1) and myeloblasts (HL-60) induce differen-tiation of monocytes into an adherent macrophage-like cell type and an increase in YKL-40 expression (22, 42, 43).

YKL-40 is also expressed by nonmalignant human cells. In normal bone marrow, the myelocyte-metamyelocyte expresses YKL-40 protein, and it is stored in the specific granules of neutrophil granulocytes and released from fully activated cells (44). Serial analysis of gene expression has shown 288-fold increased YKL-40 transcripts in monocytes stimulated with granulocyte-macrophage colony-stimulating factor, 182-fold in macrophage colony-stimulating factor–stimulated monocytes and 31-fold increased YKL-40 transcripts
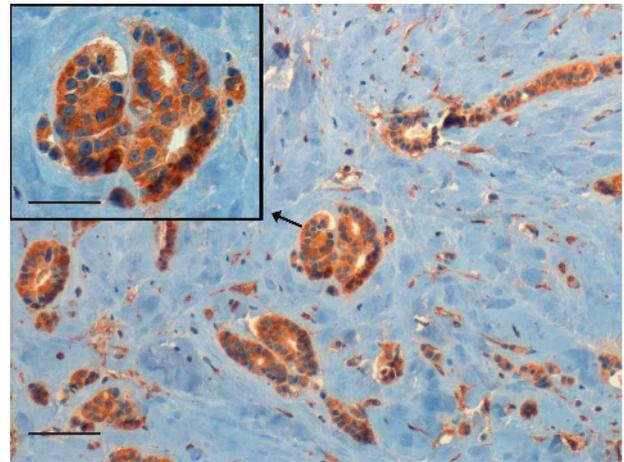

Figure 1. Immunohistochemical staining for YKL-40 in invasive ductal breast cancer cells. Original magnification, ×10; bar, 100 μm. Inset, original magnification, ×20; bar, 50 μm.

in lipopolysaccharide-stimulated monocytes (45, 46). YKL-40 is secreted by macrophages during late stages of differentia-tion (21, 22, 26, 47), by arthritic or differentiating fetal chondrocytes (2, 48-50), by differentiated vascular smooth muscle cells (4, 51, 52) and by fibroblast-like synovial cells (2, 53, 54). In vivo YKL-40 mRNA and protein are expressed by macrophages in inflamed synovial membrane (19, 55, 56), atheromatous plaques (57), arteritic vessels from patients with giant cell arteritis (58), and by arthritic chondrocytes (56). RT-PCR has shown that YKL-40 is one of the most differentially expressed genes in liver tissue from endstage cirrhosis due to hepatitis C virus compared with non-diseased liver tissue (59), and immunohistochemical analysis of liver biopsies from patients with different liver diseases have found YKL-40 protein in areas with fibrosis (60).

What is the function of YKL-40 in cancer diseases?

The biological function of YKL-40 in cancer is not known. It has been suggested that YKL-40 may play a role in the proliferation and differentiation of malignant cells, protects the cancer cells from undergoing apoptosis, stimulates angiogen-esis, has an effect on extracellular tissue remodeling, and stimulates fibroblasts surrounding the tumor, although in vivo proof of these hypotheses are yet to be obtained.

Studies of macrophages (21, 22, 26, 47) and fetal chon-drocytes (49, 50) indicate that YKL-40 is a differentiation marker. YKL-40 is not produced by fibroblasts, but it is a growth factor for fibroblasts, synovial cells, and chondrocytes (61-63). YKL-40 acts synergistically with insulin-like growth factor-I in stimulating the growth of fibroblasts and in a concentration range similar to insulin-like growth factor-I (62). YKL-40 initiates mitogen-activated protein kinase and phosphoinositide-3-kinase signaling cascades in fibroblasts, leading to the phosphorylation of both the extracellular signal-regulated kinase-1/2 mitogen-activated protein kinase and protein kinase B (AKT)-mediated signaling cascades (62, 63), which are associated with the control of mitogenesis. This indicates a role of YKL-40 as an antiapoptotic protein. The phosphoinositide-3-kinase pathway, and in particular, the phosphorylation of AKT, is strongly associated with cell survival. It has also been suggested that YKL-40 plays a role in the malignant phenotype as a cellular survival factor in an adverse microenvironment because up-regulated YKL-40 expression is found in a human glioblastoma cell line by genotoxic and microenvironmental stress (i.e., hypoxia,

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[4] Personal observation.

ionizing radiation, etoposide, ceramide, p53 inhibition, antioxidant treatment, confluence, serum depletion; ref. 36). The response in YKL-40 expression was late, 24 to 72 hours after stimuli, indicating that YKL-40 is a secondary response downstream of other mechanisms. It has also recently been shown that immortalized human astrocytes transfected with YKL-40 had increased resistance to radiation and increased invasion capacity *in vitro* (33). Furthermore, YKL-40 modulates vascular endothelial cell morphology by promoting the formation of branching tubules, indicating that YKL-40 may play a role in angiogenesis by stimulating the migration and reorganization of vascular endothelial cells (28). YKL-40 is also an adhesion and migration factor for vascular smooth muscle cells (52). It has recently been shown in interleukin (IL)-6 wild-type and IL-6 knockout mice that YKL-40 is regulated by IL-6.[5] IL-6 is a multifunctional cytokine with varied system functions and plays a role in inflammatory processes, induces cell differentiation, and is involved in the regulation of solid tumor growth in both a paracrine and autocrine manner (64).

The identity of cellular receptors mediating the biological effects of YKL-40 are currently not known, but the activation of cytoplasmic signal-transduction pathways suggests that YKL-40 interacts with one or several signaling components on the plasma membrane. Ling and Recklies (63) showed that stimulation of human articular chondrocytes or skin fibroblasts with IL-1 or tumor necrosis factor-α in the presence of YKL-40 results in the reduction of both p38 and stress-activated protein kinase/c-*Jun*-NH$_2$-kinase phosphorylation, and that YKL-40 suppresses the cytokine-induced secretion of matrix metalloproteinases-1, matrix metalloproteinase-3, and matrix metalloproteinase-13, and the chemokine IL-8. The suppressive effect of YKL-40 is dependent on kinase activity, and treatment of articular chondrocytes and skin fibroblasts with YKL-40 results in AKT-mediated serine/threonine phosphorylation of the apoptosis signal-regulator kinase, ASK1. It was suggested that YKL-40 elicits an anticatabolic effect preserving the extracellular matrix during tissue remodeling/destruction (63).

YKL-40 in mice is called the "breast regression protein" (Brp-39; ref. 3) because it is induced in mammary epithelial cells a few days after weaning. Mammary involution involves programmed cell death, and Mohanty et al. (6) hypothesized that YKL-40 uses a chitin oligosaccharide binding ability while participating in the various signal transduction pathways that lead to apoptosis of the regressing cells and that YKL-40 is a protective signaling factor that determines which cells are to survive the drastic tissue remodeling that occurs during involution.

Vertebrates in an embryonic stage use short chito-oligosaccharides as primers for the synthesis of hyaluronan (65-67), and Fusetti et al. (23) suggested that YKL-40 may recognize hyaluronan (or its precursor) as a substrate in the extracellular matrix and interfere with the synthesis and local concentrations of hyaluronan. If this is true, then YKL-40 could consequently influence the extent of cell adhesion and migration during the tissue remodeling processes that take place during embryogenesis, metastasis, inflammation, fibrosis, and atherogenesis.

Detection of circulating YKL-40

YKL-40 concentrations in human serum or EDTA plasma can be determined by an in-house RIA (9) or by a commercial two-site, sandwich-type ELISA (Quidel, Santa Clara, CA; ref. 68) using streptavidin-coated microplate wells, a biotinylated-Fab monoclonal mouse antibody against human

YKL-40 (capture antibody), and an alkaline phosphatase–labeled polyclonal rabbit antibody against human YKL-40 (detection antibody). Bound enzyme activity is detected with *p*-nitrophenyl phosphate as substrate. The detection limit of the ELISA is 20 ng/mL and the intraassay coefficient of variation is <3.6% (68). The short-term interassay coefficient of variation (during an 11-day period) is <3.7% (68), and the long-term interassay coefficient of variation (during a 5-year period) is <8.6%.[4] The YKL-40 ELISA is useful for the measurement of serum (or EDTA plasma) concentrations of YKL-40 in humans (68), baboons (69), and cynomolgus macaques (70), but not in other species like bovine, swine, rabbit, mouse, and rat. The YKL-40 ELISA could also measure YKL-40 levels in conditioned medium from human cell cultures (36, 48).

Several factors must be considered when handling blood samples for the measurement of YKL-40. The time interval between drawing of blood and centrifugation of blood stored at room temperature must be <3 hours for serum and 8 hours for EDTA plasma samples. Otherwise significant and non-disease–related elevations of YKL-40 are found in the serum and EDTA plasma samples left on the clot for a longer time when compared with YKL-40 concentrations in serum and EDTA plasma samples centrifuged within 1 hour after venipuncture. If the blood is stored at 4°C before centrifugation, YKL-40 concentration is stable in serum for 24 hours and in EDTA plasma for 72 hours (71). Degranulation of neutrophils with release of YKL-40 from the specific granules is the most likely explanation for this time-dependent increase in YKL-40 concentrations in serum and EDTA plasma. Repetitive freezing and thawing of serum samples up to nine times has no effect on serum YKL-40 (9, 68, 71). YKL-40 concentration in serum is stable in samples stored up to 5 days at room temperature (9), up to 9 days at 4°C (71), and at −80°C for at least 8 years.[4] YKL-40 concentrations in corresponding serum and EDTA plasma samples are correlated ($\rho = 0.98$, $P < 0.001$), but YKL-40 is significantly higher in serum compared with EDTA plasma with a YKL-40 serum/EDTA plasma ratio of 1.4 (9, 71). This is probably caused by a small release of YKL-40 from activated neutrophils during the coagulation process.

The median serum concentration of YKL-40 in healthy adults is 43 μg/L (90% percentile = 95 μg/L; 95% percentile = 124 μg/L; ref. 68). There is no difference in serum YKL-40 between genders (68, 72), but serum YKL-40 increases in elderly people (72). There is no circadian variability in serum YKL-40 in healthy subjects and the long time coefficient of variation in serum YKL-40 levels is <5% during a 3-year period.[4] We suggest that any changes in serum YKL-40 levels >20% should be considered to be indicative of a significant change.

Serum YKL-40 levels in patients with nonmalignant diseases

Elevated serum YKL-40 levels, compared with age-matched healthy subjects, are found in some patients with nonmalignant diseases characterized by inflammation or tissue remodeling such as rheumatoid arthritis (~40% with active disease and 13% with inactive disease have elevated serum YKL-40; refs. 9, 56, 68, 73, 74), severe osteoarthritis (16-30% have elevated serum YKL-40; refs. 56, 72), severe bacterial infections (~80% have elevated serum YKL-40; refs. 75, 76), inflammatory bowel disease (29-38% with active disease and 11-24% with inactive disease have elevated serum YKL-40; refs. 74, 77, 78), and liver disease (75-90% with cirrhosis, 60% with slight fibrosis, and 25% with fatty liver have elevated serum YKL-40; refs. 60, 79, 80). Dupont et al. (81) found that 33 patients with benign gynecologic disease all had normal serum YKL-40,

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[5] Unpublished data, manuscript in preparation.

Table 1. Serum levels of YKL-40 (μg/L) in patients with cancer and the percentage of patients with elevated serum YKL-40

Diagnosis	n	Serum YKL-40	High YKL-40 (%)*	Reference
Primary breast cancer [†]	271	57*** (22-688)	19	Johansen et al. (91)
Metastatic breast cancer, all [‡]	54	80*** (20-560)	41	Johansen et al. (83)
Soft tissue	10	59 (29-433)	20	
Bone	25	75*** (21-560)	35	
Viscera	19	157*** (20-468)	61	
Metastatic breast cancer, all [‡]	100	65*** (20-430)	31	Jensen et al. (90)
Nodes and skin only	36	51 (20-267)	9	
Bone	28	61*** (24-310)	24	
Viscera	36	110*** (21-430)	57	
Colorectal cancer, [‡] all [†]	603	86*** (27-1,298)	26	Cintin et al. (84)
Dukes A	58	73** (27-295)	16	
Dukes B	223	86*** (27-604)	26	
Dukes C	175	77*** (27-582)	19	
Dukes D	147	119*** (27-1,298)	39	
Glioblastoma multiforme	45	130*** (38-654)	72	Tanwar et al. (31)
Lower grade gliomas	20	101*** (50-225)	57	
Ovarian cancer, all [†]	50	94*** (20-517)	72	Dupont et al. (81)
Ovarian cancer, stages I-II [†]	31	75*** (20-517)	65	Dupont et al. (81)
Ovarian cancer, stage III [†]	47	168*** (32-1,808)	74	Høgdall et al. (89)
Ovarian cancer, relapse	73	94*** (20-1,970)	55	Dehn et al. (87)
Small cell lung cancer, all [‡]	131	82*** (23-1,188)	32	Johansen et al. (92)
Local disease	59	71* (23-417)	22	
Extensive disease	72	101*** (27-1,188)	40	
Metastatic renal cell cancer	58	235*** (45-1,896)	83	Geertsen et al. (88)
Metastatic prostate cancer	153	112*** (20-2,080)	43	Brasso et al. (86)
Metastatic malignant melanoma	110	95*** (20-1,262)	45	Schmidt et al. (93)

NOTE: Values are median (range). *, $P < 0.02$; **, $P < 0.01$, and ***, $P < 0.001$ compared with controls (Mann-Whitney test).
*The percentage of patients with elevated serum YKL-40 levels in relation to the serum YKL-40 level in healthy subjects adjusted for age. The normal reference region was calculated on the log-transformed serum or plasma YKL-40 levels obtained from healthy subjects (ages 18-79 years; $n = 260$ for RIA values and $n = 245$ for ELISA values). The upper 95th percent confidence limit was chosen for the limit and adjusted for age (103).
[†]Preoperative levels.
[‡]RIA analysis but the data were corrected to ELISA values (YKL-40^{ELISA} = YKL-40^{RIA} × 0.479). All the other studies used the ELISA method (68).

except for two patients with atypical endometrial hyperplasia and slightly elevated serum YKL-40. Nineteen out of 20 patients with benign prostatic hyperplasia had normal serum YKL-40.[4] There are no studies on serum YKL-40 levels in patients with benign diseases of the intestine, breast, and kidney.

Is serum YKL-40 useful as a new biomarker for screening of cancer?

Under the auspices of the Office of the Director, NIH, the ''Biomarkers and Surrogate Endpoint Working Group'' agreed on a classification system and definitions for biomarkers (82). A ''biomarker'' (biological marker) is defined as ''a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention'' (82). YKL-40 in serum can be objectively measured by a commercial ELISA and the serum concentration of YKL-40 is stable at a low level in healthy subjects. In the following sections, we will review the present literature regarding serum YKL-40 levels in cancer patients.

In 1995, we reported increased serum levels of YKL-40 in some patients with metastatic breast cancer (83). Recent studies have found elevated serum levels of YKL-40 in patients with several types of localized or advanced solid cancer (Table 1; refs. 31, 81, 84-93). These cancer patients were scored as having elevated serum YKL-40 if it was higher than the upper 95th percentile confidence limit of serum YKL-40 in healthy subjects adjusted for age (68, 72). Preoperative serum levels of YKL-40 were elevated in 19% of patients with primary breast cancer, and patients with metastases to axillary lymph nodes had higher serum YKL-40 compared with lymph node–negative patients (91). In patients with first recurrence of breast cancer, serum YKL-40 was elevated in 31% to 41% of the patients (83, 90) and high serum YKL-40 was associated with metastatic sites and large tumor load: 9% to 20% of patients with recurrence to lymph nodes or skin only had elevated serum YKL-40, 24% to 35% of patients with bone metastases, and 57% to 61% of patients with visceral metastases had elevated serum YKL-40 (83, 90). The highest serum YKL-40 levels were found in patients with more than two different metastatic sites (90). Preoperative serum levels of YKL-40 from patients with colorectal cancer were elevated in 26%, and there was an association between serum YKL-40 and Dukes' stage: 16% of the patients with Dukes' A, 26% with Dukes' B, 19% with Dukes' C, and 39% with Dukes' D had elevated preoperative serum YKL-40 (84). Serum YKL-40 decreased significantly after curative operation for colorectal cancer in patients with high preoperative YKL-40 (85) indicating that serum YKL-40 reflects tumor burden. Preoperative serum YKL-40 was elevated in 65% of stage I and II ovarian cancer (81), in 74% to 91% of patients with ovarian cancer stage III and IV (81, 89), and in 55% of ovarian cancer patients at time of first recurrence (87). In patients with small cell lung cancer, 22% with local disease and 40% of the patients with extended disease had elevated serum YKL-40 (92). Forty-three percent of patients with metastatic prostate cancer (86), 83% of patients with metastatic renal cell cancer (88), and 45% of patients with metastatic malignant melanoma (93) had elevated serum YKL-40. In patients with glioblastoma, the serum YKL-40 level was related to tumor grade and burden: 72% of patients with glioblastoma multiforme and 57% of patients with lower grade gliomas had high serum YKL-40 (31). It needs to be determined if YKL-40 is elevated in the serum of patients with hematopoietic malignancies.

Not all patients with cancer had elevated serum YKL-40 levels compared with healthy age-matched controls, suggesting that not all tumors secrete YKL-40 or that the protein is secreted at a low level. Cancer cells that secrete YKL-40 may

have a different phenotype than cancer cells that do not express and secrete YKL-40, and the protein may therefore reflect differences in the biology of various cancer cells. Serum concentrations of YKL-40 were independent of serum carcinoembryonic antigen in patients with colorectal cancer (84, 85), of serum CA-125 and CA15-3 in patients with ovarian cancer (81, 87, 89), of serum HER2 in patients with metastatic breast cancer (90), of serum prostate-specific antigen in patients with metastatic prostate cancer (86), and of serum lactate dehydrogenase in patients with small cell lung cancer (92) or metastatic malignant melanoma (93), indicating that serum YKL-40 reflects other aspects of tumor growth and metastasis than these tumor markers.

The present studies show that serum concentrations of YKL-40 do not have a high sensitivity for primary cancer, and that determination of serum YKL-40 cannot be used as a single screening test for cancer. At time of first cancer diagnosis, 16% to 74% of the patients had elevated serum YKL-40, and only 16% to 65% of patients with primary localized cancer had elevated serum YKL-40. However, in patients with advanced cancer at the time of diagnosis, serum YKL-40 levels were elevated in 39% to 91%. Large studies are needed to evaluate if serum YKL-40 can be useful as a biomarker for screening of cancer together with a panel of other tumor markers and imaging techniques.

Does serum YKL-40 reflect prognosis in cancer patients?

Several studies have shown that elevated serum concentrations of YKL-40 in patients with breast, colorectal, ovarian, kidney, prostate, small cell lung cancers, and malignant melanoma was an independent prognostic variable of short recurrence-free interval and short overall survival with hazard ratios between 1.3 and 4.1. This observation was found in patients with local or advanced cancer at the time of first cancer diagnosis and at the time of relapse (Table 2; refs. 81, 83-93).

High preoperative serum YKL-40 in patients with primary breast cancer was an independent prognostic variable of short recurrence-free interval and short overall survival when axillary lymph node and estrogen receptor status, age, tumor size and histology, menopausal status, and serum YKL-40 were included in the multivariate Cox analysis (91). There are no longitudinal studies of the changes in serum YKL-40 in patients with breast cancer after operation and adjuvant chemotherapy, antiestrogen therapy, or radiotherapy. However, elevated levels of serum YKL-40 in patients with breast cancer at the time of first recurrence predicted shorter time to progression and shorter overall survival (90). Multivariate Cox analysis (including estrogen receptor status and axillary lymph node status at primary diagnosis, liver metastases, more than two metastatic sites, symptomatic disease at recurrence, and serum HER2 and YKL-40 levels) showed that high serum YKL-40 and HER2 were independent prognostic variables of short time to disease progression and death (90). Figure 2 illustrates survival curves in patients with metastatic breast cancer according to elevated or normal serum concentrations of YKL-40 and HER2 at the time of first relapse (90). Patients with both high serum YKL-40 and HER2 had the poorest median survival of only 9 months as opposed to 32 months for patients with normal serum YKL-40 and HER2.

High preoperative serum concentrations of YKL-40 in patients with colorectal cancer was also a prognostic variable of short recurrence-free interval and short overall survival. The serum YKL-40 level in these patients was an independent prognostic variable when Dukes' stage, age, gender, and serum carcinoembryonic antigen were included in the multivariate Cox analysis (84). In patients with stage III ovarian cancer, a high preoperative plasma concentration of YKL-40 was an independent prognostic variable of short survival (the multivariate Cox analysis included plasma YKL-40, serum CA125, optimal versus suboptimal results from primary surgery, age, and histologic type of tumor; ref. 89) and patients with early stage ovarian cancer and high serum YKL-40 had a poor prognosis (81). Similar results were found in patients with a recurrence of ovarian cancer (the multivariate Cox analysis included serum YKL-40 and CA125, age, localization of tumor and its size, performance status, primary, and second-line treatment; ref. 87). An elevated serum YKL-40 level was also an independent prognostic variable of short survival in patients with metastatic prostate cancer (multivariate Cox analysis included age, performance status, tumor grade, serum prostate-specific antigen, total and bone alkaline phosphatase, PINP, CTX-I, and YKL-40; ref. 86), in patients with metastatic renal cell carcinoma (multivariate Cox analysis included serum YKL-40, serum lactate dehydrogenase, performance status, number of organ disease sites, organ site involvement, prior nephrectomy, and time from diagnosis to metastases; ref. 88), and in patients with metastatic malignant melanoma (multivariate Cox analysis included serum YKL-40, serum lactate dehydrogenase, performance status, number of metastatic sites, treatment, and location of metastases; ref. 93). In patients with small cell lung cancer, a high serum YKL-40 at the time of diagnosis and before chemotherapy was a variable for death within the following 6 months and independent of age, performance status, and serum lactate dehydrogenase (92). There are no studies of serum YKL-40 levels in patients with glioblastoma and prognosis, but high YKL-40 gene (33) or protein (38) expressions in glioblastoma tumor samples were related to short survival. Furthermore, high YKL-40 protein expression in glioblastoma tumor samples was associated with poorer radiation response and shorter time to progression (38).

Table 2. Serum level of YKL-40 is an independent prognostic variable of overall survival in cancer patients

Diagnosis	Hazards ratio (95% confidence intervals)	P	Reference
Primary breast cancer ($n = 271$)	1.8 (1.0-3.1)	0.04	Johansen et al. (91)
Metastatic breast cancer ($n = 100$)	2.6 (1.6-4.1)	0.0002	Jensen et al. (90)
Colorectal cancer ($n = 603$)	1.4 (1.1-1.8)	0.007	Cintin et al. (84)
Ovarian cancer stage III ($n = 47$)	4.0 (1.5-10.3)	0.005	Høgdall et al. (89)
Recurrent ovarian cancer ($n = 73$)	2.3 (1.3-4.1)	0.006	Dehn et al. (87)
Small cell lung cancer ($n = 131$)	1.9 (1.1-3.4)	0.02	Johansen et al. (92)
Metastatic prostate cancer ($n = 129$)	1.3 (1.0-1.7)	0.02	Brasso et al. (86)
Metastatic renal cell cancer ($n = 58$)	4.1 (1.9-8.8)	0.001	Geertsen et al. (88)
Metastatic melanoma ($n = 110$)	1.9 (1.2-2.8)	0.004	Schmidt et al. (93)

NOTE: These results are from multivariate Cox regression analyses applying routinely used prognostic variables. These cancer patients were scored as having elevated serum YKL-40 if it was higher than the upper 95th percentile confidence limit of serum YKL-40 in healthy subjects adjusted for age (se also footnotes to Table 1).

In all eight types of solid cancer tested until now, a high serum YKL-40 level was related to poor prognosis, and serum YKL-40 was independent of other known prognosticators when tested in multivariate Cox analysis. These results suggest that serum YKL-40 may be a useful "prognosticator" identifying a subgroup of cancer patients with a poor prognosis. The function of YKL-40 in cancer diseases is unknown, but these clinical studies indicate that the elevated serum YKL-40 level found in some cancer patients reflects YKL-40 secretion from a subset of tumors with a more aggressive phenotype. It is of major importance to evaluate if YKL-40 has a role in promoting the growth, invasion, and metastatic potential of cancer cells. It has been hypothesized that YKL-40 is a growth factor of cancer cells or protects them from undergoing apoptosis. Furthermore, YKL-40 may have a role in angiogenesis (see "What is the function of YKL-40 in cancer diseases?"). The stroma around the periphery of solid tumors has several similarities with granulation tissue, such as that found in wound-healing or inflammation (94, 95), and tumor-associated macrophages play a major role in cancer progression (96-98). YKL-40 is a growth factor of fibroblasts (62), and one could speculate that YKL-40 secreted by cancer cells and inflammatory cells (macrophages and neutrophils) surrounding the tumor also has a role in proliferation, activation, and differentiation of the fibroblasts/myofibroblasts surrounding the tumor. These tumor-activated fibroblasts/myofibroblasts play a major role in cancer development and spread, affecting the proliferation, differentiation, invasion, or regression of cancer cells and in particular, cancers of epithelial origin (95, 99).
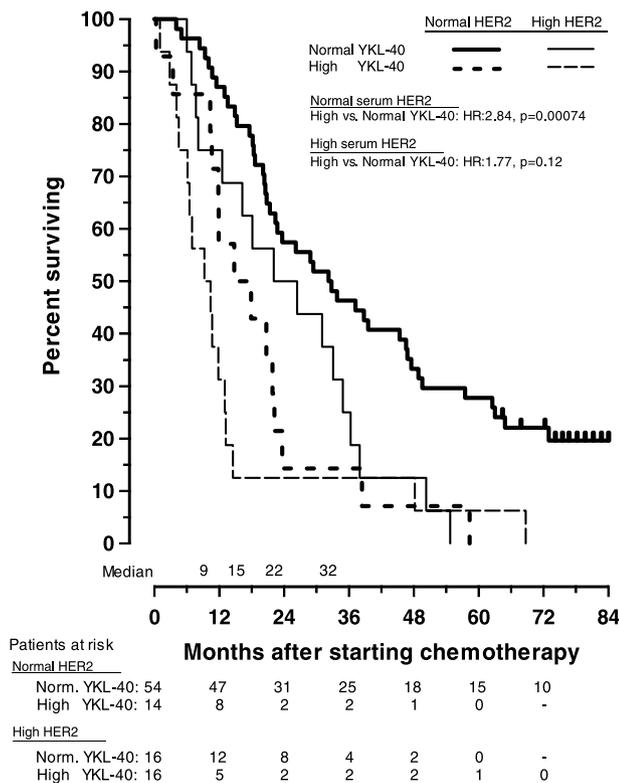

Figure 2. Survival curves in relation to serum concentrations of HER2 and YKL-40 in 100 patients with the first metastatic manifestations of breast cancer before first-line anthracycline-based chemotherapy. With a normal serum HER2 level (*fat lines*), the serum YKL-40 level separated the patients into those with a good prognosis (normal serum YKL-40, *straight fat line*) and those with a bad prognosis (high serum YKL-40, *dotted fat line*). This figure has previously been published (90) and is reprinted with permission from Clinical Cancer Research.

Is serum YKL-40 useful for monitoring cancer patients?

One study of curatively operated colorectal cancer patients has evaluated serum YKL-40 levels during the follow-up after surgery (85). It was found that patients with elevated serum YKL-40 6 months after the operation had significantly shorter recurrence-free intervals and overall survival than patients with normal serum YKL-40 at 6 months postoperative. This result was independent of serum carcinoembryonic antigen levels at 6 months postoperative. Multivariate Cox analysis scoring serum YKL-40 as a time-dependent covariant and including age, Dukes' stage, gender, and tumor localization showed that a high serum YKL-40 postoperatively in curatively operated colorectal cancer patients increased the risk of recurrence within the following 6 months by 6.9-fold, and the risk of death within the following 6 months by 8.5-fold (85).

The result of this study indicate that serum concentrations of YKL-40 may be useful for the monitoring of cancer patients. However, large longitudinal studies of patients with other types of cancer are needed to evaluate if determination of serum YKL-40, in combination with other prognostic tumor biomarkers, could be useful in monitoring cancer patients after primary operation, adjuvant chemotherapy, antihormonal therapy, and radiotherapy in order to detect first recurrence early. It is unknown if pretreatment serum YKL-40 levels could help to identify patients who will or won't respond to a given therapy. Longitudinal studies are also needed to evaluate if serum YKL-40 could provide clinical information about disease progression in patients with metastatic cancer before this is detected by routine methods. The present studies found elevated serum YKL-40 levels, particularly in patients with advanced disease with metastases occurring in the liver and lung (81, 83-85, 87-90, 92, 93).

Could YKL-40 be a target of cancer therapy?

Unfortunately, the biological function of YKL-40 in cancer development and metastasis is unknown and the elucidation of a possible function of YKL-40 in cancer diseases is an important objective of future studies. It has been shown that YKL-40 exhibits growth factor activity for cell types involved in tissue remodeling processes, and it has been suggested that YKL-40 has a role in cell growth and survival, the inflammatory process around the tumor, angiogenesis, and remodeling of the extracellular matrix. Based on the present clinical studies of serum YKL-40 levels in cancer patients, one could hypothesize that YKL-40 will prove to have a role in cancer cell proliferation, survival, and invasiveness or a regulating role in cell-matrix interactions and in the production of the altered extracellular matrix surrounding the cancer cells. If future studies show that YKL-40 has a role in the ability of cancer cells to proliferate, invade, and metastasize, YKL-40 could be an attractive target in the design of anticancer therapy. Any approach that would inhibit the function of YKL-40 (e.g., inhibition of YKL-40 gene expression, protein synthesis and secretion, neutralization of YKL-40 activity, blocking YKL-40 conversion from a latent to an active form, interruption of YKL-40 affinity, or reaction with its receptor) might limit cancer growth and metastases and improve the survival of cancer patients with YKL-40–expressing tumor cells. Potential inhibitors of YKL-40 activity include methods to inhibit YKL-40 production (e.g., small interfering RNA), monoclonal antibodies specific for YKL-40 or its receptor, YKL-40 receptor antagonists, or substrate molecules that competitively bind to YKL-40. Such potential inhibitors of YKL-40 could be expected to have therapeutic efficacy in cancer patients with tumors that produce YKL-40. It is therefore of major importance to explore if YKL-40 could become a major target for the development of new cancer therapeutics.

Table 3. A tumor marker can be classified according to six different clinical criteria such as biochemical characteristics, organ specificity, or clinical usefulness in order to assess the value of tumor markers in clinical practice

Is serum YKL-40 a tumor marker?	
1. "The marker is produced exclusively by specific tumor cells (tumor specific)"	No
2. "The marker is absent in healthy or benign disease (high specificity)"	No
3. "The marker is present frequently in the targeted malignancy (high sensitivity)"	No
4. "The marker is detectable in early stage subclinical disease (useful for screening)"	+/−
5. "The marker's concentration reflects prognosis for an individual patient (prognosticator)"	+
6. "The marker's degree of expression correlates with therapeutic results (useful for monitoring)"	+/−

NOTE: Tumor markers classified according to Werner et al. (100). (+/−), utility scale defined by Hayes et al. (101, 102): data are suggestive that the marker may correlate with biological process and/or biological end point, and preliminary data suggest that use of the marker may contribute to favorable clinical outcome, but more definite studies are required. Thus, the marker is still considered highly investigational and should not be used for standard clinical practice. (+), utility scale defined by Hayes et al. (101, 102): sufficient data are available to show that the marker correlates with the biological process and/or end point related to the use, and that the marker results might affect favorable clinical outcome for that use. However, the marker is still considered investigational and should not be used in the standard clinical setting for one of three reasons: (*a*) the marker correlates with another marker or test that has been established to have clinical utility, but the new marker has not been shown to clearly provide any advantage, (*b*) the marker may contribute independent information, but it is unclear whether that information provides clinical utility because treatment options have not been shown to change outcome, and (*c*) preliminary data for the marker is quite encouraging, but the level of evidence is lacking to document clinical utility.

Is serum YKL-40 a new "tumor marker" in cancer patients?

The term "tumor marker" embraces a spectrum of molecules of widely divergent characteristics, but sharing an association with malignancy that facilitates their application in the clinical detection (diagnosis and screening) and management (monitoring and prognosis) of cancer patients. Werner et al. (100) have suggested that tumor markers are classified according to six different clinical criteria such as biochemical characteristics, organ specificity, or clinical usefulness in order to assess the value of tumor markers in clinical practice (Table 3). The acceptance of novel tumor markers in clinical settings requires thorough validation before being implemented into routine clinical use. In order to propose guidelines on how promising tumor markers progress from the laboratory into the clinic, Hayes and colleagues have introduced the "tumor marker utility grading system" (101, 102). According to this system, YKL-40 is on the "utility scale +" and "utility scale +/−." YKL-40 is neither organ- nor tumor-specific, but the present 12 retrospective clinical studies of 1,712 patients with eight different types of solid cancer indicate that serum concentrations of YKL-40 may be useful as a "prognosticator" and may have a role in screening and monitoring of cancer patients. Elevated serum YKL-40 levels are found in a subgroup of patients with eight different types of solid tumors (including several types of adenocarcinomas, small cell carcinoma, glioblastoma, and malignant melanoma). The highest serum YKL-40 levels are found in patients with advanced cancer and with the poorest prognosis and serum YKL-40 provided independent information of survival.

According to the tumor marker utility grading system, a number of validation requirements are suggested which have to be fulfilled before the marker can be considered to have reached level of evidence I ("LOE I"), whereupon clinical implementation is feasible. Most tumor marker studies are "LOE III," defined as retrospective studies in which samples were not originally collected with the intent of testing the value (e.g., prognostic value) of the marker of interest. The intermediate level "LOE II" is constituted by companion studies with prospectively collected specimens as part of therapeutic trial with pre-established end points and evaluation of both the marker and the therapeutic intervention. Finally, "LOE I" studies are either (*a*) highly powered prospective studies specifically addressing the issue of the utility of the marker or (*b*) an overview or metaanalysis of studies, each of which has a lower level of evidence.

The use of serum YKL-40 has not yet received Food and Drug Administration approval for use as a biomarker in patients with cancer or any other disease. It is unknown if knowledge of the serum YKL-40 level in an individual patient could be reliably used to make clinical decisions that will improve the outcome for the patient. All the present studies regarding serum YKL-40 as a tumor marker in cancer patients are retrospective in design and include a fairly small number of patients, and are of a lower level of evidence "LOE III." There are therefore limitations to the conclusions that can be made from the present studies. It is unknown if serum YKL-40 can be used in screening for cancer and in monitoring of cancer patients. Hopefully, the diagnostic value of serum YKL-40 could be tested in the large Prostate, Lung, Colorectal, and Ovarian cancer study in the U.S. regarding new biomarkers to diagnose patients with prostate, lung, colorectal, and ovarian cancer. The prognostic value of serum YKL-40 levels in patients with different types of primary and advanced cancer should be confirmed in additional large retrospective studies from different departments of oncology. If a sufficient number of large retrospective studies of high quality confirm the association between serum YKL-40 and poor prognosis, a summarizing metaanalysis could bring the generated data to "LOE I." According to the tumor marker utility grading system guidelines, the next step would be to launch an appropriate prospective study in which the benefit of using serum YKL-40 levels in the clinical decision-making process is assessed. Endpoints should include overall survival, disease-free survival, quality of life, and cost-effectiveness. The study could be designed either as a single, highly powered, prospective, controlled study with the primary objective of testing serum YKL-40 levels as a "prognosticator" or a similar prospective study in which the primary goal could be the testing of a therapeutic hypothesis, and secondly, for testing serum YKL-40 as a biomarker.

Acknowledgments

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