

Original Article

YKL-40 is directly produced by tumor cells and is inversely linked to EGFR in glioblastomas

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Abstract: YKL-40 is a secreted chitinase-like molecule whose expression is associated with glioma grade. Expression is higher in astrocytomas than oligodendrogliomas and has been reported to predict shorter survival and radiation resistance in glioblastomas (GBMs). Whether YKL-40 is directly produced by glioma cells or other admixed nonneoplastic cells, and whether it correlates with 1p/19q status or other hallmark molecular abnormalities, are unclear. A rank-order list of YKL-40 expression was determined immunohistochemically in 79 untreated high-grade adult gliomas, including 28 anaplastic oligodendrogliomas (AOs) and 51 GBMs. Relative YKL-40 expression was compared with glioma class, key molecular alterations, and immunohistochemical markers via a series of Spearman rank correlations. YKL-40 mRNA in situ hybridization with colocalization assessment via confocal microscopy was also performed. YKL-40 mRNA was abundant in glioma cells as well as reactive astrocytes, but was low in admixed neurons and macrophages. YKL-40 expression was higher in GBMs than AOs ($P < 0.0001$) and among GBMs, YKL-40 expression was lower in tumors with either EGFR amplification ($P = 0.005$) or elevated EGFR expression ($P = 0.001$). Among AOs, no difference in YKL-40 expression was seen in tumors with 1p19q codeletion ($P = 0.3$), but loss of heterozygosity in 10q23 correlated with increased YKL-40 expression ($P = 0.03$). These data suggest that YKL-40 is predominantly expressed by neoplastic glial cells and is related to certain key molecular alterations.

Keywords: YKL-40, glioblastoma, oligodendroglioma, EGFR, 1p19q, 10q

Introduction

YKL-40, also known as human chitinase-like protein 1 (HC-gp39), is a secreted inflammatory molecule with no chitinolytic activity. Its gene, *CHI3L1*, is located on 1q32.1. YKL-40 has no known receptor but is capable of binding to N-acetylglucosamine oligomers and heparin, and appears to be upregulated in a variety of conditions that feature remodeling of the extracellular matrix. For example, elevated serum YKL-40 has been described as a robust biomarker of various inflammatory/fibrotic diseases, including sarcoidosis, rheumatoid arthritis, cirrhosis, and atherosclerotic plaques. It is secreted by microglia and astrocytes in SIV encephalitis and inhibits FGF-2 activity via displacement of ECM-bound FGF-2[1]. In degenerative joint disease YKL-40 is secreted by chondrocytes, inhibits collagen synthesis, and promotes proliferation

of synovial cells and chondrocytes through Ras/MAPK and Akt pathways [2]. Elevated serum YKL-40 expression is also associated with metastases, higher stage, and an overall worse outcome in a variety of neoplastic diseases [3-16].

Prior studies have suggested that YKL-40 is an important molecule in gliomas. YKL-40 expression increases with glioma grade and is stronger in astrocytic than oligodendroglial tumors [17-19]. High YKL-40 tumor expression has been shown to correlate with MAPK/Akt activation and 10q deletion, and predicts radiation resistance and shorter survival in GBMs [20-24]. Underscoring its association with behavior, it has recently been identified as part of a 9-gene paraffin tissue-based expression panel that most closely predicts GBM survival[25]. In glioma cell lines YKL-40 is produced in re-

sponse to hypoxia and radiation[26] and promotes radiation and apoptosis resistance, increased invasiveness, and increased 72 KDa metalloproteinase activity[22]. It also stimulates production of nicotinamide N-methyltransferase in glioma cells[22] which has been shown to facilitate invasiveness[27] and radioresistance [28] in urothelial carcinomas. Blocking VEGF production in glioma cell lines causes a large upregulation of YKL-40, perhaps explaining why anti-VEGF therapy can increase the malignancy of some cancers [23]. Finally, TNF has been shown to suppress YKL-40 via NF κ B in GBM but not other cancers[29].

In summary, YKL-40 is expressed in situations wherein extensive tissue remodeling and ECM turnover occurs, including inflammation, joint degeneration, and neoplasias, including gliomas. However, since YKL-40 might be expressed and secreted by reactive astrocytes and microglia which are often admixed within the tumor, it is unclear whether the majority of YKL-40 is directly produced by glioma cells *in vivo*. Aside from a link with 10q loss [22], it is unknown whether YKL-40 expression correlates with any other molecular abnormalities commonly seen in high grade gliomas. In particular, although anaplastic oligodendrogliomas (AOs) express less YKL-40 than GBMs, it is unknown whether expression in AOs varies according to 1p/19q codeletion status. AOs that carry this codeletion are more sensitive to chemotherapy and radiation, and have longer survival intervals [30].

Herein we demonstrate via immunohistochemistry and *in situ* hybridization that YKL-40 mRNA is present within GBM cells and reactive astrocytes. We also show that YKL-40 expression is usually stronger in GBMs than AOs, is independent of 1p19q codeletion in AO, and is inversely correlated with EGFR expression and amplification in GBM.

Material and methods

Cohort organization

This cohort was a retrospective collection of institutional high grade gliomas, including 51 GBMs and 28 AOs. Only cases that were initial biopsies were included; previously treated gliomas were excluded. Formalin-fixed, paraffin-embedded (FFPE) tissues were acquired and de-identified according to an institutional review

board-approved protocol, conforming to the provisions of the Declaration of Helsinki. Histology was reviewed and the original diagnosis confirmed for each case. Survival from the time of initial biopsy was determined via the Social Security Death Index, but in only 42 of the 79 cases was definitive survival time available.

Dual mRNA in situ hybridization and immunofluorescence

Antisense YKL-40 DNA templates containing the T7 promoter were generated by PCR from the pUC57 vector (GenScript, Piscataway, NJ) containing the full length human YKL40 cDNA. 35S-labeled RNA probes were generated using MAXscript kit (Ambion, Austin, TX). After deparaffinization tissue sections were processed for *in situ* hybridization (ISH) and then for immunohistochemistry as described previously [1].

Fluorescence in situ hybridization (FISH)

Both FISH and PCR-based microsatellite analyses (see below) were done as part of the routine clinical workup of institutional gliomas. FISH method has been described previously[31]. Briefly, FISH was performed using probes for 1p36, 19q13, 9p21, and EGFR (7p12) (Abbott Molecular, Des Plaines, IL). For ploidy control, centromeric enumeration probes were used for chromosomes 7 (CEP7) and 9 (CEP9), while 1q25 and 19p13 were used as intrachromosomal controls for 1p and 19q. Approximately 60 cells were analyzed in the targeted region per case. Each tumor was assessed by the average and the maximum numbers of copies of gene per cell and the average ratio of gene to chromosome copy numbers. Amplification was defined as a ratio of gene signals to chromosome centromere signals of ≥ 2.0 . Deletion was defined if one or both 1p36, 19q13, and 9p21 signals were lost in at least 20% of nuclei. These cutoff points were derived using nonneoplastic autopsy brain tissue as controls.

PCR-based microsatellite analysis

Manual microdissection of the tissue sample was performed to include tumor tissue. Matched nonneoplastic tissue was available in some cases. Specimens with the minimum of 50% of tumor cells in a microdissection target were accepted for the analysis. DNA was isolated using standard laboratory procedures. Optical density readings were obtained. The

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assay utilized 7 microsatellite markers on chromosome 1p22-36 (D1S171, D1S162, D1S199, D1S1172, D1S1161, D1S407 and D1S226), 3 on 19q13 (D19S559, D19S112, and D19S206), 3 on 9p21-22 (*CDKN2A* gene, D9S1748, D9S1679, and D9S251), 2 on 10q23 (*PTEN* gene, D10S520 and D10S1173), and 3 on 17p13 (*TP53* gene, D17S516, D17S768, and D17S1844). PCR was performed and the PCR products were analyzed using capillary gel electrophoresis on GeneMapper ABI 3730 (Foster City, CA). Relative fluorescence was determined for individual alleles and the ratio of peaks was calculated. Neoplastic tissue was then analyzed to detect loss of heterozygosity. When normal tissue was not available, peak height ratios falling outside of 2 standard deviations beyond the mean of previously validated normal values for each polymorphic allele pairing were scored as showing loss of heterozygosity.

Immunohistochemistry

Immunohistochemical studies were performed on 4- μ m-thick sections obtained from paraffin-embedded material. The primary antibodies,

including manufacturer, clone, and dilution, were as follows: P53 (Dako, DO-7, 1:100); Ki67 (Dako M7240/ MIB-1/ 1:100); EGFR (Ventana 790-2988/ 3C6/ prediluted). The antibody labeling was performed using the avidin-biotin complex method and visualized using a horseradish peroxidase enzyme label and 2'-diaminobenzamide (DAB, Dako, Carpinteria, CA) as the substrate chromogen (brown).

YKL-40 staining was performed using goat anti-human chitinase 3-like antibody (1:50, R&D Systems, Minneapolis, MN) immunohistochemistry was performed as described previously [1]. A rank-order list of all cases was generated by sorting the relative YKL-40 staining intensity from strongest to weakest while blinded to diagnosis and molecular results. Only tumor cell staining was counted during the ranking.

P53 expression was scored based on a modified protocol to that reported previously [32]. Briefly, only cases wherein at least 50% of tumor nuclei had dense staining were considered positive because those are the cases most likely to harbor p53 mutations [33].

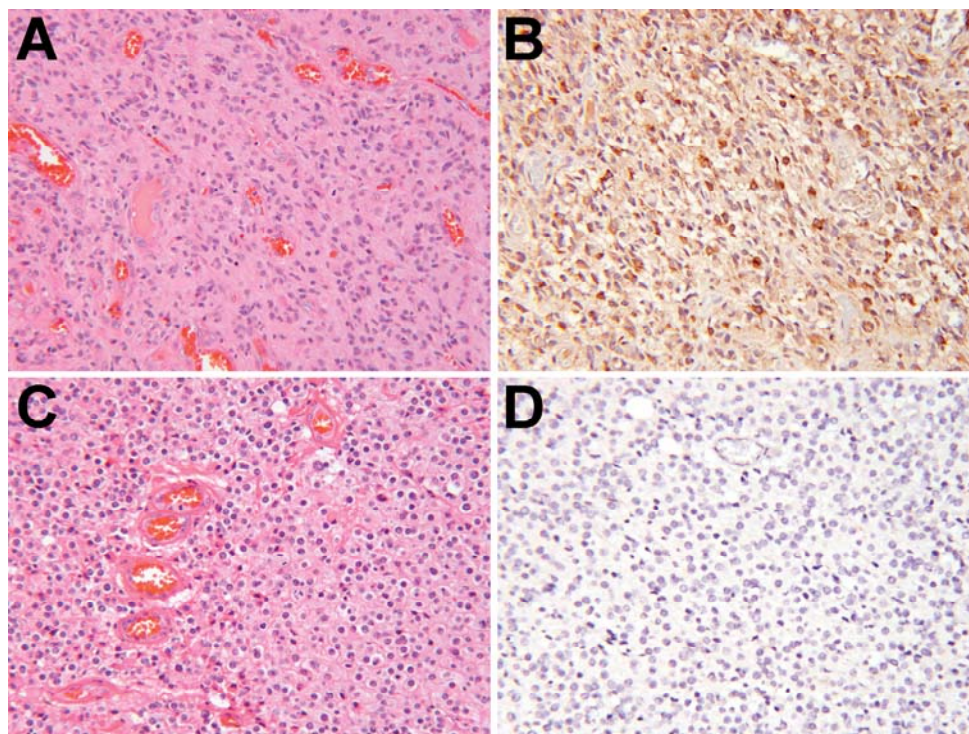


Figure 1. YKL-40 expression is stronger in GBM than AO. 58 GBMs and 21 AOs were immunostained for YKL-40 and ranked according to staining intensity (see Materials and Methods). On average, GBMs (A) showed much stronger YKL-40 expression (B) than did AOs (C & D). $P < 0.0001$ via Spearman rank correlation. All images are 200x magnification.

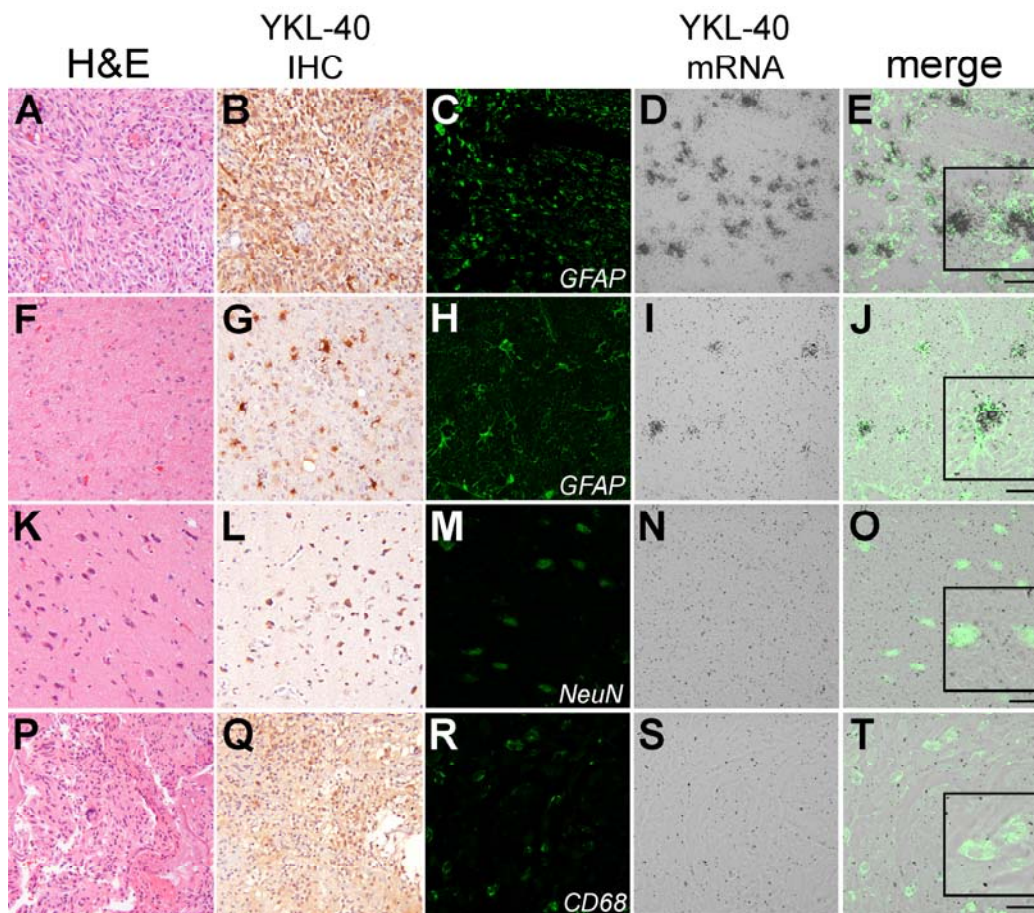


Figure 2. YKL-40 is directly produced by glioma cells and reactive astrocytes. Confocal microscopy showed that YKL-40 mRNA colocalizes with GFAP in both glioblastoma (A-E) and tumor-induced non-neoplastic reactive astrocytosis (F-J). In contrast, although neurons adjacent to glioma are immunopositive for YKL-40, little mRNA is present (K-O). Admixed CD68-positive macrophages and microglia likewise do not appear to produce YKL-40 mRNA in the neoplastic setting (P-T). Scale bars: A-E=50um; F-J, K-O and P-T=20um. Insets are higher-magnification images of selected cells within each merged field. The first and second column images (A & B, F & G, K & L, P & Q) are 200x magnification.

EGFR expression was semiquantified by assigning tumor cell staining intensity into 1 of 4 numerical groups: 1—negative, 2—weak, 3—moderate, and 4—strong. Distribution was scored as 1—focal or 2—diffuse. The intensity score was then multiplied by the distribution score to produce an EGFR score, from 0 (negative) to 6 (strong and diffuse).

Statistical analysis

The strength of association between YKL-40 and other variables, including demographic, histologic, and molecular characteristics, was determined via a series of nonparametric Spearman rank correlations using GraphPad software (La Jolla, CA). Associations between 2 variables were considered significant when $P <$

0.05. Linear regression was employed to determine strength of association between genetic variables apart from YKL-40 rank.

Results

YKL-40 expression has previously been shown to be stronger in astrocytomas than oligodendrogliomas [18, 19]. To verify this, a rank-order list of YKL-40 immunostaining intensity was compiled using 79 high grade gliomas (see Materials and Methods). Via Spearman rank correlation, GBMs were significantly stronger for YKL-40 expression than AOs ($P < 0.0001$, **Figure 1**, **Table 1**).

The precise source of YKL-40 mRNA in gliomas was shown to be predominantly in glioma cells

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Table 1. Rank-order list of high grade gliomas according to relative YKL-40 immunohistochemical intensity.

Age (yrs)	Gender	Location	Class	Survival (days)	1p/19q codeletion	9p21 LOH	CDKN2A deletion	10q23 LOH	17p13 LOH	MIB-1 PI (%)	p53 IHC	EGFR IHC score	EGFR amplification	YKL-40 rank
72	M	left frontal lobe	GBM	176	no	yes	no	yes	no	19	neg	6	no	1
58	M	left temporal	GBM	na	no	no	yes	yes	yes	25	pos	1	no	2
50	M	right frontal lobe	GBM	na	no	yes	yes	yes	no	25	neg	3	no	3
79	F	left internal capsule	GBM	na	no	no	yes	yes	no	15	neg	0	no	4
60	M	na	GBM	na	no	no	yes	yes	yes	25	neg	2	no	5
83	F	right temporal	GBM	12	no	no	yes	yes	no	32	neg	2	no	6
60	M	right parietal	GBM	517	no	no	no	yes	yes	25	pos	2	no	7
81	M	left parietal	GBM	77	no	no	yes	no	no	30	neg	2	no	8
72	M	right frontal lobe	GBM	154	no	no	no	yes	no	25	neg	4	no	9
83	M	left parietal	GBM	32	no	no	no	no	yes	20	neg	3	no	10
58	F	na	AO	na	yes	no	yes	yes	no	20	neg	1	no	11
33	M	midbrain	GBM	361	no	no	no	yes	no	10	neg	2	no	12
37	F	left hemispehre	AO	na	no	na	na	na	na	5	na	4	no	13
59	M	na	GBM	na	no	yes	yes	yes	yes	30	neg	6	yes	14
80	M	left temporal	GBM	348	no	no	no	yes	yes	15	neg	4	no	15
80	M	right parietal	GBM	285	no	yes	yes	yes	yes	50	pos	2	no	16
53	M	frontal	GBM	519	no	no	yes	yes	no	25	neg	6	yes	17
68	M	right frontal lobe	GBM	64	no	no	no	no	no	8	neg	1	no	18
76	F	left temporal	GBM	60	no	no	no	yes	yes	25	pos	4	no	19
62	M	right frontal lobe	GBM	128	no	yes	yes	yes	yes	15	neg	3	no	20
60	F	corpus callosum	GBM	371	no	yes	yes	yes	yes	10	neg	6	yes	21
74	M	left temporal	GBM	na	no	no	yes	yes	no	20	neg	6	yes	22
70	M	thalamus	AO	na	no	yes	yes	no	yes	20	neg	1	no	23
85	M	left temporal	GBM	112	no	yes	no	yes	no	55	neg	2	no	24
57	F	occipital	GBM	230	no	yes	yes	yes	no	60	neg	4	no	25
64	M	left frontal lobe	GBM	255	no	yes	yes	yes	no	40	neg	6	yes	26

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77	M	left frontal lobe	GBM	112	no	yes	yes	yes	no	15	neg	6	yes	27
82	F	left temporal	GBM	30	no	no	no	no	yes	35	pos	4	no	28
16	F	left thalamus	GBM	na	no	yes	yes	no	no	10	neg	0	no	29
59	F	right parietal	AO	na	yes	no	no	yes	no	5	neg	6	no	30
57	M	rght hemisphere	GBM	482	no	no	yes	yes	no	30	neg	6	yes	31
61	M	na	GBM	407	no	no	no	yes	no	12	neg	4	No	32
55	M	na	GBM	143	no	yes	yes	yes	no	20	neg	6	yes	33
70	M	right temporal	GBM	190	no	no	yes	yes	no	12	neg	6	yes	34
80	M	left hemisphere	GBM	na	no	no	yes	yes	no	10	neg	6	yes	35
61	M	right temporo-parietal	GBM	na	no	no	no	yes	no	30	neg	6	yes	36
55	M	left temporal	GBM	na	no	yes	no	yes	no	20	neg	2	no	37
58	M	right hemisphere	GBM	na	no	no	yes	no	yes	25	pos	1	no	38
55	M	right temporal	GBM	na	no	no	no	yes	no	20	pos	2	no	39
63	M	right thalamus	GBM	221	no	yes	yes	yes	no	30	neg	6	yes	40
27	F	na	GBM	na	no	no	no	no	yes	20	neg	2	no	41
85	M	na	GBM	62	no	yes	yes	yes	no	25	neg	6	yes	42
49	M	right occipital	AO	na	yes	yes	no	no	no	30	neg	4	no	43
50	M	right frontal	AO	na	yes	no	na	yes	no	10	na	4	na	44
51	F	left frontal	AO	na	yes	yes	na	no	no	30	neg	6	na	45
49	M	left temporal	AO	na	yes	na	na	na	na	15	neg	2	no	46
86	M	left temporal	GBM	25	no	yes	yes	yes	no	10	neg	6	yes	47
75	M	left parietal	AO	168	no	yes	no	yes	no	20	pos	4	no	48
43	F	right thalamus	GBM	395	no	no	no	yes	no	40	pos	1	no	49
68	F	na	GBM	na	no	yes	yes	yes	no	20	neg	6	yes	50
55	M	right basal ganglia	GBM	125	no	yes	no	yes	no	25	neg	6	yes	51
48	M	left frontal	AO	960	yes	yes	na	no	no	50	na	3	na	52
61	M	right temporal	GBM	166	no	yes	yes	yes	no	20	neg	6	yes	53
41	M	left frontal	AO	na	yes	na	na	na	na	na	na	6	no	54
75	F	right parietal	GBM	na	no	yes	yes	yes	no	20	neg	6	yes	55

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58	M	left frontal lobe	GBM	285	no	no	yes	yes	no	5	neg	6	yes	56
59	M	left frontal lobe	GBM	na	no	no	yes	no	no	5	neg	4	no	57
35	M	right parietal	AO	na	yes	no	yes	no	na	75	neg	1	no	58
49	F	right frontal lobe	GBM	323	no	yes	yes	yes	no	30	neg	6	yes	59
43	M	left temporal	GBM	317	no	no	no	yes	no	20	pos	4	no	60
55	F	bifrontal	AO	na	yes	yes	na	na	na	20	na	4	na	61
62	F	right frontal lobe	GBM	17	no	yes	yes	yes	yes	20	neg	6	no	62
30	F	na	AO	254	no	no	na	no	yes	30	neg	6	na	63
42	F	right frontal	AO	na	yes	yes	na	no	yes	10	na	3	na	64
29	F	left temporal	AO	na	no	no	na	no	yes	41	na	6	na	65
41	M	right frontal	AO	1683	no	yes	na	yes	no	30	na	6	na	66
74	M	left occipital	AO	999	yes	yes	na	no	yes	25	neg	3	no	67
52	M	na	AO	1757	yes	no	na	no	yes	na	na	6	na	68
46	M	left frontal	AO	124	yes	no	na	no	yes	40	neg	6	no	69
55	F	na	GBM	436	no	yes	yes	yes	no	10	neg	6	no	70
50	F	right thalamus	GBM	109	no	yes	no	yes	no	50	neg	6	yes	71
38	M	left frontal	AO	na	no	yes	na	no	no	10	pos	3	na	72
52	F	right frontal	AO	na	yes	no	na	no	no	20	neg	4	na	73
63	F	left temporal	AO	na	yes	na	na	na	na	30	na	0	no	74
53	M	na	AO	na	yes	yes	no	no	no	25	neg	3	no	75
52	M	right temporal	AO	na	yes	na	na	na	na	25	neg	6	no	76
37	M	right frontal	AO	na	yes	na	na	na	na	25	neg	6	no	77
47	F	left temporal	AO	na	yes	yes	no	no	yes	50	neg	4	no	78
42	F	right frontal	AO	na	yes	no	no	no	no	20	neg	6	no	79

79 high grade gliomas were ranked according to YKL-40 expression via immunohistochemistry while blinded to all other clinical and pathologic variables (see Materials and Methods). Spearman rank correlations with key molecular and immunohistochemical features were then performed. AO = anaplastic oligodendroglioma; GBM = glioblastoma; IHC = immunohistochemistry; LOH = loss of heterozygosity; PI = proliferation index; na = not available.

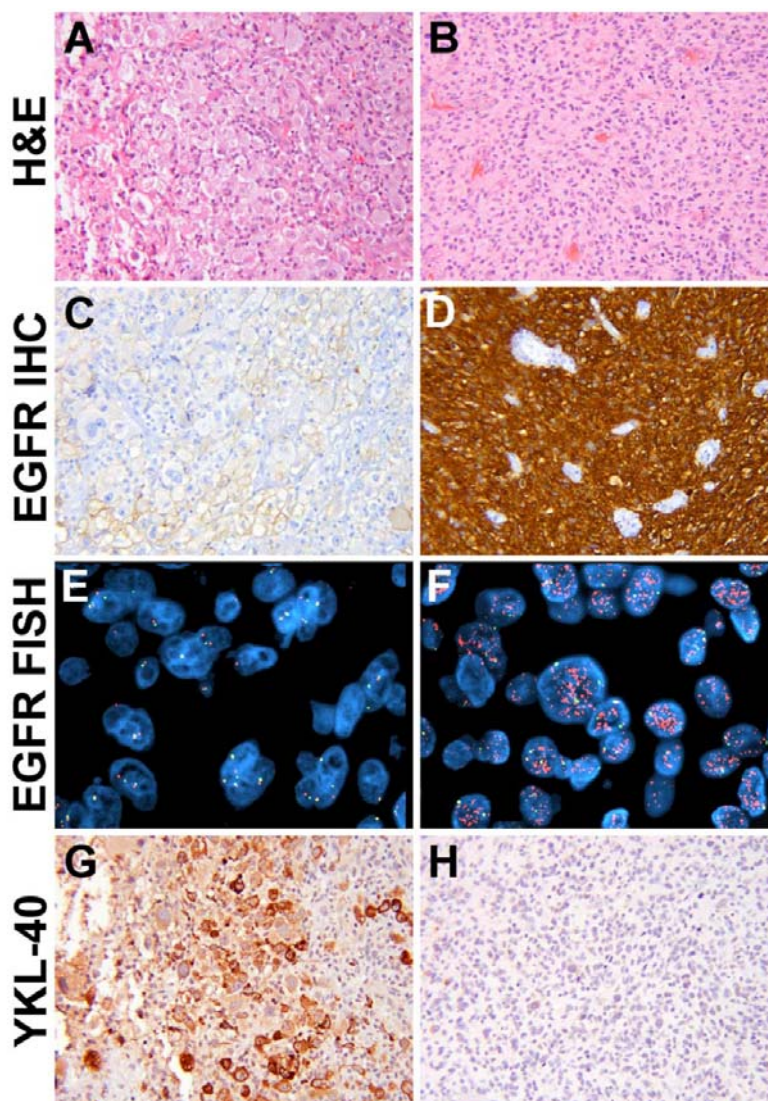


Figure 3. YKL-40 expression is reduced in GBMs with EGFR amplification. Tumors with weak EGFR expression (A, C) also tended to lack EGFR amplification (E) but produced YKL-40 (G). In contrast, tumors strong for EGFR (B, D) were likely to show EGFR amplification (F) but not YKL-40 (H). ($P = 0.003$ via FISH and $= 0.001$ via EGFR IHC). Orange signal = 7q34, green = CEP7. All H & E and immunohistochemical images are 200x magnification; both FISH images are 1000x magnification.

(Figure 2A-E), although scattered reactive non-neoplastic astrocytes also produced appreciable amounts of YKL-40 mRNA (Figure 2F-J). Neurons and macrophages/microglia, on the other hand, did not show significant YKL-40 mRNA (Figure 2K-O and P-T, respectively), although neurons (2L) and macrophages (2Q) did show varying degrees of immunopositivity. Thus, YKL-40 is directly produced by glioma tumor cells and reactive astrocytes, with less contribution from other nonneoplastic elements.

EGFR signaling, including gene amplification, is well-known to be a key component of many

GBMs [34] and YKL-40 has been shown to correlate with MAPK activation [20, 21]. While the strength of EGFR immunoreactivity positively correlated with EGFR amplification ($P < 0.0001$), both EGFR immunostaining ($P = 0.0012$) and gene amplification ($P = 0.0054$) negatively correlated with YKL-40 rank (Figure 3, Table 2). 17p LOH ($P = 0.0298$), and increased patient age ($P = 0.0356$) positively correlated with higher YKL-40 IHC rank. Trends toward positive associations with YKL-40 were identified for 9p21 LOH ($P = 0.0558$) and male gender ($P = 0.0684$). No links were identified between GBM YKL-40 and 10q LOH, CDKN2A/

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Table 2. Strength of associations with YKL-40 immunohistochemical intensity.

Parameter	Association with YKL-40 IHC rank (<i>P</i>)		
	All gliomas	GBM only	AO only
Patient age	< 0.0001	0.0356	0.3953
Gender	0.0882	0.0684	0.9281
Glioma type	< 0.0001	NA	NA
Survival	0.1612	0.8630	0.7131
1p19q codeletion	< 0.0001	NA	0.3097
9p LOH	0.0602	0.0558	0.9493
Homozygous CDKN2A deletion	0.4536	0.7296	0.1328
10q23 LOH	0.0006	0.8783	0.0276
17p13 LOH	0.5380	0.0298	0.5023
Ki67 (MIB-1) PI	0.3723	0.3795	0.1141
P53 accumulation	0.6380	0.7702	> 0.9999
EGFR positive	0.0034	0.0012	0.2361
EGFR amplification	0.3671	0.0054	NA

79 high grade gliomas were ranked according to YKL-40 expression via immunohistochemistry while blinded to all other clinical and pathologic variables (see Materials and Methods). Spearman rank correlations with key clinical and molecular features were then performed. AO = anaplastic oligodendroglioma; GBM = glioblastoma; LOH = loss of heterozygosity; PI = proliferation index; NA = not applicable (none of the GBMs showed 1p19q codeletion and none of the AOs showed EGFR amplification).

p16 deletion, p53 accumulation, Ki67 proliferation index (PI), or survival (**Table 2**), although shorter survival was significantly correlated with increased age ($P < 0.0001$ via linear regression). Additional significant associations in GBMs were identified between 17p13 LOH and p53 accumulation ($P = 0.0031$); 9p21 LOH and CDKN2A homozygous deletion ($P = 0.022$); 10q23 LOH and EGFR amplification ($P = 0.0159$); and 10q23 LOH and increased EGFR immunoreactivity ($P = 0.002$).

Twenty-one of 28 AOs (75%) in this cohort had 1p/19q codeletion but showed no correlation with YKL-40 expression ($P = 0.3097$, **Table 2**). On the other hand, 10q23 LOH correlated with stronger YKL-40 staining in AOs ($P = 0.0276$). No associations were found between YKL-40 and age, 9p21 LOH, 17p13 LOH, CDKN2A deletion, p53 accumulation, EGFR expression, Ki67 PI, or survival in AOs (**Table 2**).

Discussion

YKL-40 has recently attracted attention as a biomarker of metastatic cancers and chronic inflammatory conditions, as well as a possible effector molecule contributing to specific features that are characteristic of neoplastic glial cells (e.g. invasiveness, radioresistance). Our

data suggest that YKL-40 expression is stronger in GBMs compared to AOs and is chiefly produced by neoplastic glial cells and reactive astrocytes. YKL-40 expression is inversely associated with EGFR in GBMs. In contrast, YKL-40 tends to be higher in AOs with 10q23 LOH.

There appears to be a wide variety of cells that secrete YKL-40, including stromal vascular fraction cells in adipose tissue[35], chondrocytes[2], carcinoma cells, tumor associated macrophages, neutrophils, and mast cells[36]. Herein we demonstrate that the YKL-40 in gliomas is actively being transcribed and translated within the tumor cells, with reactive astrocytes also producing YKL-40. Macrophages, microglia, and neurons, while sometimes showing YKL-40 immunopositivity, do not appear to actively produce the molecule. However, given that macrophages and microglia have already been shown to produce and secrete YKL-40 in viral encephalitis [1], it is possible that these cells contribute to the YKL-40 pool in gliomas in a more temporally limited manner.

The actions of YKL-40, while still mostly unknown, are becoming clearer. As a secreted molecule it can displace FGF-2 from the extracellular matrix and inhibit its actions [1], link membrane-bound syndecan-1 with integrins

[37], and inhibit collagen degradation via inhibition of matrix metalloproteinases[35, 38], though other work has shown upregulation of metalloproteinase activity[22]. It may also promote collagen synthesis[35], but another model found opposing results[39]. Recent work has identified YKL-40 as a promoter of angiogenesis in neoplasms, including activating the MAPK/ERK pathway in endothelial cells[37]. Interestingly, blocking VEGF sharply upregulates YKL-40 expression [23]. These findings are intriguing in light of the fact that microvascular proliferation is used as a diagnostic criterion for GBM and, to a lesser extent, AO.

Prior work has indicated a correlation between YKL-40 expression and activation of MAPK and Akt pathways [2, 20, 37, 38]. Because EGFR can signal through both pathways, initially it was postulated that tumors with strong EGFR expression and *EGFR* amplification might also exhibit higher YKL-40 expression. Finding the opposite result (**Figure 3, Table 2**) suggests that, while YKL-40 may activate MAPK and/or Akt, it may itself be negatively regulated by EGFR. Further mechanistic studies to address YKL-40 regulation will be of interest.

The association between YKL-40 expression and 10q23 LOH in high grade gliomas, specifically AOs, is similar to what has been reported in GBMs [22], strengthening the association between 10q and YKL-40. Similar association in this GBM subgroup was not seen, perhaps because the vast majority (84%) had 10q23 LOH, making correlation with YKL-40 rank difficult. In AOs, given that 10q deletion is more common in GBMs than AOs, and AOs with 10q deletion often show more aggressive behavior with shorter survival [40-43], it is possible that AOs with 10q deletion are more like GBMs in terms of genetics and biology, including YKL-40 expression.

In summary, YKL-40 is more abundant in GBMs than AOs, is directly produced by neoplastic cells, and accounts for the majority of YKL-40 in high-grade gliomas. An inverse relationship exists between EGFR and YKL-40 in GBMs, while a direct correlation exists with 10q23 LOH and YKL-40 in AOs. This molecule appears to be an important factor in many gliomas and, as the mechanisms of YKL40 action and regulation become more precisely defined, the importance of this molecule in understanding of glioma biology will be better understood.

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