YKL-40 Expression is Associated with Poorer Response to Radiation and Shorter Overall Survival in Glioblastoma

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Abstract

Purpose: YKL-40 is a secreted protein that has been reported to be overexpressed in epithelial cancers and gliomas, although its function is unknown. Previous data in a smaller sample set suggested that YKL-40 was a marker associated with a poorer clinical outcome and a genetically defined subgroup of glioblastoma. Here we test these findings in a larger series of patients with glioblastoma, and in particular, determine if tumor YKL-40 expression is associated with radiation response.

Experimental Design: Patients (n = 147) with subtotal resections were studied for imaging-assessed changes in tumor size in serial studies following radiation therapy. An additional set (n = 140) of glioblastoma patients who underwent a gross-total resection was tested to validate the survival association and extend them to patients with minimal residual disease.

Results: In the subtotal resection group, higher YKL-40 expression was significantly associated with poorer radiation response, shorter time to progression and shorter overall survival. The association of higher YKL-40 expression with poorer survival was validated in the gross-total resection group. In multivariate analysis with both groups combined (n = 287), YKL-40 was an independent predictor of survival after adjusting for patient age, performance status, and extent of resection. YKL-40 expression was also compared with genetically defined subsets of glioblastoma by assessing epidermal growth factor receptor amplification and loss at chromosome 10q, two of the common recurring aberrations in these tumors, using fluorescent in situ hybridization. YKL-40 was significantly associated with 10q loss.

Conclusions: The findings implicate YKL-40 as an important marker of therapeutic response and genetic subtype in glioblastomas and suggest that it may play an oncogenic role in these tumors.

Glioblastoma is an aggressive disease with median overall survival of 10 to 12 months after diagnosis. Despite advances in surgical techniques, postoperative supportive care, radiation delivery, and adjuvant systemic therapy, the life expectancy of patients with glioblastoma has remained essentially unchanged over the last several decades. Radiation therapy, given after primary surgical resection, is the standard adjuvant treatment with proven efficacy for glioblastoma (1–3). However, this tumor is regarded as clinically radioresistant, as a relatively large proportion of patients experience tumor progression during radiotherapy (4, 5). Most lesions recur/progress within 1 to 2 cm from the primary surgical margin, well within radiotherapy fields (6–8). Although factors that underlie radiation resistance/sensitivity in glioblastoma are not well-understood, older patients have been reported to exhibit a poorer response to radiation (9). With respect to molecular correlates of radiation response, gain in chromosome 7 and losses in chromosomes 9p and 13q copy number have been reported to be associated with a poorer response (10). We have previously found that overexpression of epidermal growth factor receptor (EGFR) correlates with a less favorable response (10). A better understanding of the molecular factors that confer radioresistance in glioblastoma may lead to new approaches to improve the radiation sensitivity of these tumors.

YKL-40 (also known as CHI3L1 or human cartilage glycoprotein-39) is located on chromosome 1q32.1 and is a secreted protein whose function is poorly understood and has homology with glycosyl hydrolases. YKL-40 may have a role in cell migration (12) and connective tissue modeling (13–15) and is involved in the inflammatory response (16, 17). Increased YKL-40 levels have been associated with disease activity in rheumatoid arthritis and other autoimmune disorders (18–24). Additionally, it has been implicated as a serum marker for aggressive disease in colon (25), ovarian (26, 27), and breast carcinoma (28, 29). Elevated YKL-40 levels were
identified in a gene expression profiling study of glioblastoma, as was the presence of YKL-40 in the serum of glioblastoma patients (30). Preliminary data from our laboratory showed the existence of an association between higher YKL-40 expression levels and worse overall survival in glioblastoma. Because radiotherapy is a major treatment modality for glioblastoma following surgery, we hypothesized that YKL-40 might be associated with response to radiation. To test this hypothesis, we identified a group of glioblastoma patients who had undergone subtotal resections, selected in order that measurable residual disease could be followed on serial imaging studies. We examined the relationship between YKL-40 expression, radiation response, and survival in this set. In addition, we tested the prognostic association between YKL-40 expression and glioblastoma in an independent sample of gross totally resected patients with glioblastoma. Finally, we examine relationships of YKL-40 expression with EGFR amplification and chromosome 10 status to test whether YKL-40 is associated with this genetic subset.

**Materials and Methods**

**Patient characteristics.** One hundred and forty-seven cases of subtotally resected glioblastoma were identified at the University of Texas M.D. Anderson Cancer Center from January 1993 until June 2003. Inclusion criteria required that the patient (a) had not received any prior therapy for the tumor; (b) underwent a preoperative and immediate postoperative (within 48 hours) magnetic resonance imaging (MRI) of the brain to assess the extent of resection; (c) received radiation therapy; (d) underwent at least one post-radiotherapy MRI (within 10 weeks of completion); and (e) had archival paraffin-embedded tissue available for immunohistochemical staining. Cases were re-reviewed by a neuropathologist (K.D. Aldape) to ensure that they fulfilled histologic criteria for glioblastoma using current WHO guidelines, which include a high-grade astrocytic tumor with microvascular proliferation and/or necrosis. One hundred and thirteen of the 135 patients with reported radiation therapy doses (84%) received a radiation tumor dose of 5,400 cGy. Most of the patients who received lower doses either underwent hypofractionated regimens, or deteriorated during their treatment course and could not complete radiation therapy. One hundred and nine (78%) received systemic chemotherapy. At least one cycle of 39 unique treatment regimens (single agent or in combination), composed of 28 different agents were used. Of these various chemotherapeutic regimens, most (98 of 109) were either procarbazine, lomustine, and vincristine-based (n = 20) or temozolamide-based (n = 68). Institutional Review Board approval was obtained for these studies.

The radiation therapy response was determined by comparing the change in enhancing tumor size between the post-surgical assessment and first post-radiation therapy MRI in a manner previously defined by Barker and colleagues (31). The magnitude of radiation therapy response was assessed using a five-tiered scoring system, which ranged from +2 (≥50% size reduction; Fig. 1A and B) to −2 (≥50% tumor growth; Fig. 1C and D). The +1/−1 scores represented a change (reduction and increase, respectively) of <50% magnitude in size in the enhancing cross-sectional area. Post-radiation therapy MRI films were unavailable for seven patients and although these seven patients were not included in the response analyses, they were included in overall survival analyses.

A second cohort of 140 patients with gross total resections during the same time interval represented a group for whom associations between YKL-40 and overall survival could be tested and used to validate the findings in the first group. Inclusion was based on criteria a and b above, with a verified gross total (>50%) resection, along with archival paraffin tissue available for YKL-40 staining. This patient group had characteristics similar to that of the subtotal resection group (Table 1). Sixty out of 78 patients with reported doses (77%) received a radiation tumor dose of ≥5,400 cGy. In the patients with unreported doses (received radiation therapy at outside facilities), the radiation therapy was described as "conventional" in most cases. Six patients did not receive adjuvant radiation therapy.

Overall survival was determined from the date of diagnosis to the date of death or last follow-up. Time to progression was determined from the date of the initial therapeutic surgery to the date of radiographically detected (MRI) enhancing tumor progression in subtotal resection patients or the date of last radiographic follow-up. The Radiation Therapy Oncology Group (RTOG) recursive partitioning analysis (RPA) classification system for malignant glioma (33, 34), recently modified by Shaw and colleagues (32), was used. The modified RTOG RPA classification for glioblastoma takes into account age, Karnofsky performance status (KPS), extent of resection, and the ability of patients to perform activities of daily living (described as working [W+]/[−]).

**Immunohistochemistry and tissue array construction.** Paraffin blocks were obtained from the Department of Pathology archives at University of Texas M.D. Anderson Cancer Center. Each case was reviewed by a neuropathologist (K.D. Aldape) to identify blocks with sufficient tumor availability for analysis. A polyclonal antibody to YKL-40 was obtained from Quidel Corporation (San Diego, CA). Immunohistochemistry was done as previously described (33) and slides were incubated in primary antibody overnight at 4 °C at an antibody dilution of 1:1,500. Staining was scored using a three-tiered system: 2+, strongly positive staining in the majority of tumor cells at least 1 medium power (100×) microscopic field (2+); 1+, weak/patchy staining in tumor cells; and 0, no staining (Fig. 2A-E). Staining was scored while blinded to clinical data. Cases known to be positive and negative were used as controls for each batch of tumor samples.

Cases (n = 140) were randomly selected from the subtotal resection group (n = 94) and gross-total resection group (n = 52) from which tissue arrays were constructed. A Beecher (Sun Prairie, WI) manual tissue arrayer was used to generate tissue arrays. A minimum of two cores were used for each case, and most had three cores or greater.

**Fluorescence in situ hybridization.** EGFR amplification and chromosome 10 loss was assessed using fluorescence in situ hybridization analysis of glioma specimens distributed on tissue microarrays. A dual-probe dual-color probe set for EGFR (red fluorophore) and the centromere of chromosome 7 (green fluorophore) was used to assess EGFR amplification. A dual-color probe set for PTEN (red) and the centromere of chromosome 10 (green) was used to assess chromosome 10 loss. Both probe sets were obtained from Vysis, Inc. (Downer's Grove, IL). Hybridization methods and criteria for EGFR amplification and chromosome 10 loss have been previously reported (34).

**Statistical analysis.** Spearman's Rho correlation was used to determine associations between clinicopathologic variables. Kaplan-Meier (35) survival analysis was used to compare overall survival and time to progression between subgroups. Patients who were alive at last follow-up (for overall survival) or who had no documented time to progression at last follow-up were considered to be censored. Cox-regression multivariate analysis was used for determining independent prognostic factors.

**Results**

Subtotal resection cases (n = 147) were accrued to evaluate the effect of radiotherapy by observing changes in residual tumor size in serial imaging studies. With respect to therapeutic regimen, 113 of the 135 patients with reported radiation therapy doses (84%) received a radiation tumor dose of ≥5,400 cGy. One hundred and nine (78%) received systemic chemotherapy at some time during the disease course. Radiation response, time to progression, and overall survival were
evaluable end points in this cohort. Seven patients did not have available MRI or adjuvant treatment data, but are included in the overall survival analysis. Gross total resection glioblastoma cases (gross-total resection group) were used in a validation study with overall survival as the only clinical end point. The clinical characteristics of both subtotal resection and gross-total resection groups are summarized in Table 1.

Radiation response and survival in the subtotal resection group. Table 1 (left), shows patient characteristics of the subtotal resection group. Approximately half (52%) of the patients progressed after radiation (scores of −1 or −2), whereas the remainder had either no change or a positive response. There was no significant difference in the interval of the time of radiation therapy completion to the time of MRI used for response scoring between the patients who responded versus patients who progressed. Specifically, the median time to MRI after completion of radiation therapy was 18 days for responders and 21 days for those who progressed (P = 0.7). Clinical factors associated with poorer response to radiation included older age (P = 0.04) and worse RPA classification (P = 0.02, Spearman’s rank correlation).

To evaluate the relationship between imaging-assessed changes in size of enhancing tumor and overall survival, response scores were compared with survival in the subtotal resection group. Response to radiation therapy was a strong predictor of overall survival in univariate analysis (Fig. 3A). The median overall survival of patients with radiation therapy response scores of +1 or +2 was 90 weeks versus 42 weeks for those with progression (scores of −1 or −2). Patients with stable disease (score 0) had an intermediate median overall survival at 55 weeks (P < 0.0001). When stratified by modified RTOG RPA class, a positive radiation therapy response continued to show a favorable impact on overall survival across all RPA classes (P < 0.0001; Fig. 3B). Poorer radiation response was associated with older age (<50 versus ≥50; P = 0.04, Spearman’s rank correlation). In Cox multivariate analysis, older age group (HR, 2.0), lower radiation response score (HR, 3.3) and lower KPS (HR, 3.3) were independent adverse predictors of survival (all P < 0.01).

A meaningful survival analysis regarding the use of chemo- therapeutic agents in the subtotal resection group could not be done. The 31 patients who did not receive chemotherapy typically had a rapid clinical deterioration and received only supportive care after radiation therapy; subjecting any comparison to a profound selection bias. Of the 98 patients who received either temozolomide or procarbazine-, lomustine-, and vincristine-based chemotherapy, there was no survival difference between these two groups (P = 0.9).

YKL-40 expression and outcome in the subtotal resection group. Positive staining for YKL-40 was found in the cytoplasm of glioblastoma tumor cells (Fig. 2), a finding in contrast to a previous report suggesting that it is expressed in
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YKL-40 and outcome in the gross-total resection group. To validate an association between YKL-40 and overall survival, we studied an independent sample of 140 glioblastoma patients who underwent gross-total resection for newly diagnosed glioblastoma. The characteristics of these patients are described in Table 1 (right). With respect to therapy, the reported doses of radiation were available for 78 patients. Sixty of the 78 patients with reported doses (77%) received a radiation tumor dose of ≥5,400 cGy. In the patients with unreported doses (received radiation therapy at outside facilities), the radiation therapy was described as “conventional” in most cases. Six patients did not receive adjuvant radiation therapy. The distribution of YKL-40 expression in this group was similar to the subtotal resection group. Of the eighty (57%) cases that were strongly stained for YKL-40, 37 (26%) had an intermediate level of staining, and 23 (16%) were negative. In this group, YKL-40 was also significantly associated with overall survival. Patients with YKL-40 scores of 0 in this group had a median overall survival of 116 weeks, compared to a median survival of 53 weeks for cases with 1+ staining, and 41 weeks in patients with scores of 2+ (P = 0.0008; Fig. 5B; Table 3). As in the subtotal resection group, univariate analysis revealed a higher expression of YKL-40, older age and lower KPS to have a significant association with a decreased overall survival (Table 3).

Association between YKL-40 and established genetic markers in glioblastoma. Previous studies of glioblastoma have indicated that discrete molecular genetic subtypes exist on the basis of the presence or absence of signature aberrations, including amplification of the EGFR gene and loss of chromosome 10 (37, 38). To determine if YKL-40 expression was associated with either of these two genetic lesions, a subset of the glioblastoma cases from each group were subjected to fluorescence in situ hybridization for EGFR and chromosome 10 using tissue arrays constructed from a subset of the patients from the subtotal resection and gross-total resection groups. One hundred and thirty-four cases were evaluable for EGFR status and amplification was found in 59 of them (44%). One hundred and nineteen cases were evaluable for chromosome 10 status and loss was identified in 52 cases (44%). There was no association between the expression of YKL-40 expression and EGFR amplification (P = 0.78). However, a higher YKL-40 staining score was significantly associated with loss of chromosome 10 (Spearman’s rank correlation 0.26, P = 0.004), consistent with a concurrent study from our laboratory. That study suggests that loss of chromosome 10 defines subsets of glioblastoma with differential expression patterns across the genome, including differences in the expression of YKL-40 (elevated average YKL-40 mRNA levels in cases with chromosome 10 loss; ref. 39).

Multivariate and subset analysis in the combined groups. The two patient groups (subtotal resection and gross-total resection) were combined (n = 287) to identify associations between the expression of YKL-40 and clinical factors to identify independent prognostic factors. A higher level of YKL-40 expression was positively associated with older age group and lower KPS (both P < 0.01, Spearman’s rank correlation). Cox survival analysis, including variables that were significant in univariate analyses (YKL-40, KPS, age, and extent of resection), revealed that YKL-40 positivity (HR, 1.4; P = 0.04), lower KPS score (HR, 1.4; P = 0.016), and age ≥50 years (HR, 1.7; P = 0.002) were independent adverse prognostic factors. Extent of resection in this multivariate model (biopsy versus subtotal resection) was only significant in the univariate model (data not shown). Table 3 provides the results of the multivariate analysis using the Poisson model, showing that the overall survival was significantly associated with KPS (HR, 1.7; P = 0.04), lower KPS (HR, 1.4; P = 0.016), age ≥50 years (HR, 1.7; P = 0.002) and YKL-40 expression (HR, 1.4; P = 0.016).

Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Factor</th>
<th>Total no. of patients</th>
<th>Subtotally resected group (147)</th>
<th>Gross totally resected group (140)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>98 (68%)</td>
<td>78 (56%)</td>
<td>49 (33%)</td>
</tr>
<tr>
<td>Female</td>
<td>49 (33%)</td>
<td>62 (44%)</td>
<td></td>
</tr>
<tr>
<td>RTOG-RPA Class</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>25 (17%)</td>
<td>22 (16%)</td>
<td>3 (20%)</td>
</tr>
<tr>
<td>IV</td>
<td>91 (62%)</td>
<td>91 (65%)</td>
<td></td>
</tr>
<tr>
<td>V+VI</td>
<td>31 (21%)</td>
<td>27 (19%)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (± SD)</td>
<td>57 (±14 years)</td>
<td>60 (±13 years)</td>
<td></td>
</tr>
<tr>
<td>&lt;50 years</td>
<td>45 (31%)</td>
<td>32 (23%)</td>
<td></td>
</tr>
<tr>
<td>≥50 years</td>
<td>102 (69%)</td>
<td>108 (77%)</td>
<td></td>
</tr>
<tr>
<td>Median survival (weeks)</td>
<td>49.6</td>
<td>51.4</td>
<td></td>
</tr>
<tr>
<td>Vital status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deceased</td>
<td>127 (86%)</td>
<td>100 (72%)</td>
<td></td>
</tr>
<tr>
<td>Alive at last contact</td>
<td>20 (14%)</td>
<td>40 (28%)</td>
<td></td>
</tr>
<tr>
<td>KPS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>90-100</td>
<td>67 (46%)</td>
<td>92 (66%)</td>
<td></td>
</tr>
<tr>
<td>70-80</td>
<td>70 (48%)</td>
<td>44 (31%)</td>
<td></td>
</tr>
<tr>
<td>&lt;70</td>
<td>10 (7%)</td>
<td>4 (3%)</td>
<td></td>
</tr>
<tr>
<td>Surgery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gross-total resection</td>
<td>—</td>
<td>140 (100%)</td>
<td></td>
</tr>
<tr>
<td>Subtotal resection</td>
<td>134 (91%)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Biopsy</td>
<td>13 (9%)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Radiation therapy response score</td>
<td>+2, +1 37 (25%)</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>26 (18%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>−1, −2</td>
<td>77 (52%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No data</td>
<td>7 (5%)</td>
<td></td>
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</tr>
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</table>
resection versus gross-total resection) was not statistically significant ($P = 0.8$). When the patients were stratified by the modified RTOG RPA classification, a higher expression of YKL-40 was associated with poorer overall survival across all groups ($P = 0.009$; Fig. 5C).

**Discussion**

In this study, YKL-40 expression was a strong predictor of aggressive clinical behavior in glioblastoma. Increased YKL-40 expression was associated with increased resistance to radiotherapy, shorter time to progression, and worse overall survival. To evaluate radiation response, we initially focused on subtotal resection cases, in which imaging-identified residual disease could be evaluated for response after radiation therapy. Radiation response, time to progression, and overall survival were measurable end points in this group. Although response to radiation was not quantifiable in the second cohort of gross-total resection glioblastoma cases, the observation of an association between YKL-40 expression and poor survival in...
ovary, as well as glioblastoma (26, 28, 43, 44), our finding that 
the blood of patients with carcinomas of the breast, colon, and
apoptosis signal-regulating kinase 1, were also observed. 
of nuclear factor-
-3, and -13, as well as secretion of the chemokine interleukin-
activation, cytokine-induced secretion of matrix metalloproteinases-1, 
activated protein kinase/Jun NH2-terminal kinase phosphoryla-
a reduction in p38 mitogen-activated protein kinase and stress-
fibroblasts and chondrocytes in the presence of YKL-40 showed 
astrocytomas and low-grade glioma (41). A study examining 
that microvascularization is entirely absent in anaplastic 
ref. 30), a molecular finding that may parallel the observation 
overexpression was seen in most glioblastoma tumors when 
facilitate invasion, migration, and/or angiogenesis. YKL-40 
extends to patients with minimal residual disease.

Although the exact function of YKL-40 is unclear, based on 
the limited knowledge of this protein, it seems to be a secreted 
protein, which is involved in extracellular matrix remodeling 
and cellular mitogenesis. Exposure of chondrocytes to YKL-40, 
has been reported to result in an increase in proteoglycan 
synthesis (14). It has been shown to increase the proliferation 
rates of various cell lines (chondrocytes, squamous, fetal lung 
fibroblasts, and synovial) via simultaneous stimulation of 
the mitogen-activated protein kinase and the phosphoinositide 
3-kinase activity pathways (40). Through the alteration of the 
extracellular matrix and its proliferative properties, YKL-40 may 
facilitate invasion, migration, and/or angiogenesis. YKL-40 
overexpression was seen in most glioblastoma tumors when 
compared with gliomas of lower WHO grades (grades III and II; 
ref. 30), a molecular finding that may parallel the observation that 
microvascularization is entirely absent in anaplastic astrocytomas and low-grade glioma (41). A study examining 
interleukin-1 and tumor necrosis factor-α stimulation of 
fibroblasts and chondrocytes in the presence of YKL-40 showed a 
reduction in p38 mitogen-activated protein kinase and stress-
activated protein kinase/Jun NH2-terminal kinase phosphoryla-
tion, cytokine-induced secretion of matrix metalloproteinases-1, 
-3, and -13, as well as secretion of the chemokine interleukin-
8 (42). Antiapoptotic events, including nuclear translocation 
of nuclear factor-κB and AKT-mediated phosphorylation of 
apoptosis signal-regulating kinase 1, were also observed.

As YKL-40 has been reported to be present at high levels in 
the blood of patients with carcinomas of the breast, colon, and 
avary, as well as glioblastoma (26, 28, 43, 44), our finding that it is associated with both response and survival provides a potential opportunity to establish a minimally invasive means to obtain prognostic information prior to surgery. In addition, this observation raises the question of whether serum YKL-40 levels can be used as a surrogate marker for disease activity or response to treatment for those patients with YKL-40-overexpressing tumors. In particular, changes in blood YKL-40 levels during therapy might provide an indication as to the effectiveness of therapy. The inability of current imaging modalities to accurately distinguish radiation necrosis from tumor progression is an ever-present conundrum in clinical practice, and the ability to identify a minimally invasive marker would be a significant advance in this area. Archived preoperative blood specimens of patients in this study were not available for analysis. These issues will be investigated in planned clinical trials to test for a relationship of tumor YKL-40 expression with the presence of YKL-40 in the blood. Although YKL-40 is a secreted extracellular matrix protein, we found the staining to be most pronounced in the cytoplasm of glioblastoma tumor cells. This likely reflects a high concentration of the protein in the compartment in which it is synthesized, as has been observed with other extracellular matrix proteins in astrocytic tumors (45). Previous studies employing immuno-
histochemistry for YKL-40 in other tissues also indicate a predominant cytoplasmic localization (46, 47). Whether YKL-
40 has additional functions related to its intracellular localiza-
tion remains to be elucidated in future studies.

We found that a higher expression of YKL-40 was significantly associated with older age. Despite this association, YKL-40 remained an independent prognostic factor in multivariate analysis after adjustment for age, suggesting that it is not merely a surrogate marker for the tumors of older patients. As its expression seems to be associated with age, as well as being a prognostic marker independent of age, it potentially could add additional information in patient evaluation and could also in part account for the well-known association between older age and a worse prognosis in gliomas (4, 5). It is tempting to speculate that although high YKL-40 levels can be considered a marker of the presence of tumor in an older patient, a YKL-40-overexpressing tumor in a younger patient may be expected to exhibit a more aggressive

| Table 2. Univariate time to progression and overall survival analysis of the subtotally resected group |
|-----------------|-----------------|-----------------|-----------------|
| Factor                        | Time to progression | Overall survival |
|                              | Median (weeks) | P     | Median (weeks) | P     |
| YKL-40 Score                |                 |       |                 |       |
| 0                            | 21              |       | 68              |       |
| 1+                           | 18              | 0.04  | 47              | 0.02  |
| 2+                           | 13              |       | 50              |       |
| Age                          |                 |       |                 |       |
| ≥50 years                    | 16              | 0.03  | 68              | 0.0001|
| ≥50 years                    | 13              |       | 47              |       |
| KPS                          |                 |       |                 |       |
| 90-100                       | 15              |       | 62              |       |
| 70-80                        | 14              | 0.004 | 46              | 0.002 |
| ≤70                          | 11              |       | 33              |       |
| Surgery                      |                 |       |                 |       |
| Subtotal resection           | 14              | 0.6   | 50              | 0.05  |
| Biopsy                       | 15              |       | 39              |       |
| Radiation therapy response score |          |       |                 |       |
| +2, +1                       | 47              |       | 90              |       |
| 0                            | 26              | <0.0001| 55              | <0.0001|
| −1, −2                       | 11              |       | 42              |       |

Fig. 4. Average radiation response score following stratification by YKL-40 expression. YKL-40 expression score was used to calculate the average radiation response score for the subtotally resected glioblastoma cases (as described in Materials and Methods). Results are plotted along with SE. Two-sided t test (P < 0.001).
clinical behavior (including radioresistance), which is typical in older patients. The modified RTOG RPA glioblastoma classification takes into account the KPS, the extent of resection and working status, in addition to age, for risk stratification. Our finding that YKL-40 adds prognostic information in addition to these clinically relevant factors supports the hypothesis that it directly contributes to aggressive behavior in glioblastoma.

A better understanding of the factors that underlie the relative lack of radiosensitivity exhibited by most glioblastoma tumors is needed. It is well established that adjuvant radiation therapy significantly improves overall survival (2, 3, 31, 48, 49). The radiation therapy response data from this study show a clear advantage in overall survival when patients have radiosensitive lesions, as has been reported previously (50). Although only 25% of the patients had a positive response to radiation therapy, and an additional 18% had stable disease in the first post-radiation scan, these patients showed improved survival compared with the 52% of patients with tumor progression. Similar to a previous report (9), tumors from patients in an older age group tended to be more radioresistant than those in the younger patients. This relationship may account, in part, for the known association between older age and poorer survival in gliomas (5). In multivariate analysis, both response and age were independent predictors of survival, suggesting that although radiation response is an important factor, additional as yet uncharacterized age-related factors are also pertinent.

Because this was a retrospective study based on patients who were not treated on uniform protocols, a potential exists for differences in adjuvant treatment to confound the survival data. We believe this is unlikely in our dataset. A detailed review of chemotherapeutic regimens was performed on the subtotal resection group. Whereas the specifics of administration differed, most of the regimens could be classified as either procarbazine-, lomustine-, vincristine-, or temozolamide-based chemotherapy. No difference in survival ($P = 0.9$) was seen in these two groups, suggesting that the treatment differences did not have a significant impact on the associations of YKL-40 with survival.

Finally, to place YKL-40 expression in the context of previously established recurring genetic lesions, we compared YKL-40 expression with two of the commonly described genetic aberrations in glioblastoma: amplification of EGFR and loss of chromosome 10. Whereas there was no significant association between YKL-40 and EGFR amplification status, advantage in overall survival when patients have radiosensitive lesions, as has been reported previously (50). Although only 25% of the patients had a positive response to radiation therapy, and an additional 18% had stable disease in the first post-radiation scan, these patients showed improved survival compared with the 52% of patients with tumor progression. Similar to a previous report (9), tumors from patients in an older age group tended to be more radioresistant than those in the younger patients. This relationship may account, in part, for the known association between older age and poorer survival in gliomas (5). In multivariate analysis, both response and age were independent predictors of survival, suggesting that although radiation response is an important factor, additional as yet uncharacterized age-related factors are also pertinent.

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Finally, to place YKL-40 expression in the context of previously established recurring genetic lesions, we compared YKL-40 expression with two of the commonly described genetic aberrations in glioblastoma: amplification of EGFR and loss of chromosome 10. Whereas there was no significant association between YKL-40 and EGFR amplification status,
References


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Christopher E. Pelloski, Anita Mahajan, Moshe Maor, et al.


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