

# Allergy and inflammatory transcriptome is predominantly negatively correlated with CD133 expression in glioblastoma

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Allergies and the use of anti-inflammatory medication appear to be associated with reduced glioblastoma risk. However, these observations may merely reflect systemic immunosuppression induced by the tumor. To better understand the effect of this tumor on allergies and inflammation, we used CD133 mRNA expression as an indicator of tumor aggressiveness and systematically examined its relation to mRNA expression levels of 919 allergy- and inflammation-related genes in 142 glioblastoma tissue samples. We found that 69% of these genes are negatively correlated with CD133 expression including allergy-related (eg, interleukin [IL]-4R- $\alpha$ ; Pearson correlation coefficient  $[r] = -0.40$ ; 95% confidence interval [CI] =  $-0.53, -0.25$ ) and immunoregulatory genes (eg, TGF- $\beta$ 1;  $r = -0.35$ ; 95% CI =  $-0.49, -0.20$ ). Exceptions to this negative trend include the proinflammatory cytokine IL-17- $\beta$  ( $r = 0.22$ ; 95% CI =  $0.06, 0.37$ ) and 2 IL-17 receptors. Also positively related to CD133 expression are NCAM-1 ( $r = 0.45$ ; 95% CI =  $0.31, 0.57$ ) and PDGFR- $\alpha$  ( $r = 0.45$ ; 95% CI =  $0.30, 0.57$ ). Previous literature suggests that NCAM-1<sup>+</sup> T cells infiltrate glioblastoma and may cause suppression of antitumor immunity, whereas PDGFR- $\alpha$  is involved in neurogenesis and amplified in glioblastoma. Ours is the first study to document down-regulation of the majority of allergy- and inflammation-related genes with glioblastoma progression. However, IL-17 and NCAM-1 may play proinflammatory and immunosuppressive roles, respectively, during the late

stage of glioblastoma progression. Our findings suggest that immune function continues to change as the tumor progresses.

**Keywords:** allergy, CD133, glioblastoma, inflammation

Glioma and its microenvironment employ a variety of strategies to promote tumor tolerance and suppress the immune system.<sup>1</sup> For example, glioma cells may secrete cytokines that recruit immunosuppressive CD4<sup>+</sup> CD25<sup>+</sup> regulatory T cells into the tumor microenvironment.<sup>2</sup> Of the major immunosuppressive cytokines that are present both in the glioma microenvironment and peripheral blood of glioma patients, IL-10 and TGF- $\beta$ <sup>3,4</sup> also induce immune tolerance, a state that inhibits allergy and asthma.<sup>5</sup>

Epidemiological studies<sup>6</sup> provide strong and consistent evidence for an inverse relationship between the occurrence of allergies and the diagnosis of malignant glial tumors. However, it is not known whether allergies reduce glioma risk or their relative absence in glioma patients is merely a reflection of the immunosuppressive effects of the tumor. Yet, the effects of tumor progression on IL-4 and IL-13 cytokines that play central roles in IgE synthesis and allergic conditions,<sup>7</sup> have not been documented.

Similarly, numerous epidemiological studies show people who use COX-1 or COX-2 blocking nonsteroidal anti-inflammatory drugs (NSAIDs) have a lower risk of glioma than people who do not.<sup>8,9</sup> In addition, there is extensive evidence showing that chronic inflammatory disease and other sources of inflammation increase cancer risk in several different tissues and organs.<sup>10</sup> However, there are no known systemic studies of the effects of glioblastoma progression on inflammation-related genes.

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In 2004, Singh et al.<sup>11</sup> observed that CD133 (prominin-1) could be characterized as a marker for glioma initiating or a “stem-like” cell subpopulation. These authors found that CD133 positive cells derived from patient glioblastomas were tumorigenic in nude mice, whereas cells lacking CD133 failed to form tumors even when implanted at high numbers. Since this report was published, there is conflicting evidence related to the significance of this marker.<sup>12–14</sup> Nonetheless, Gunther et al.<sup>15</sup> report that CD133 expression characterizes an invasive tumor phenotype. Whether CD133 expression is causal in this context or merely an indicator of tumor aggression, a number of recent studies show a poor clinical outcome for glioma and glioblastoma patients with elevated CD133 expression. In a study of 95 glioma patients, Zeppernick et al.<sup>16</sup> found that patients whose tumor consists of at least 1% CD133<sup>+</sup> cells have a 95% chance of dying before patients whose tumors contain 1% or fewer CD133<sup>+</sup> cells. More evidence for an association between CD133 expression and glioma progression comes from Beier et al.<sup>17</sup> who report, in a sample of 36 glioblastoma patients, that patients with tumors that do not express CD133 survive longer than patients with tumors that do (176 vs 83.6 weeks,  $P < .05$ ). These investigators also observed, in the same case series, that patients with CD133 expression had a 73% chance of dying before patients who do not express CD133 mRNA ( $P = 0.002$ ). Consistent with these findings, in a study of 80 glioblastoma patients, Murat et al.<sup>18</sup> observed the same 73% probability ( $P = .004$ ) associated with an expression signature dominated by HOX genes (genes involved in development), which includes CD133. Rebetz et al.<sup>19</sup> also report results from a study of 23 glioma patients showing that CD133 expression is correlated with shorter survival time (94.6 vs 21.5 weeks,  $P = .025$ ). Finally, in a study of 44 glioblastoma patients, Pallini et al.<sup>20</sup> saw that the presence of at least 2% CD133<sup>+</sup> cells in patient tumor samples predict poor overall (hazard ratio [HR] = 2.22; 95% CI = 1.09, 4.52) and progression-free (HR = 2.10; 95% CI = 1.05, 4.21) survival. Thus, even though evidence for the use of CD133 as a stem cell marker remains conflicting, a body of consistent evidence from multiple studies demonstrates a clear link between CD133 positivity or expression and poor prognosis in glioma patients.

On the basis of the reported inverse association between allergy and glioma and the immunosuppressive character of glioblastoma, we determined the relationship between the expression of CD133 and a set of allergy- and inflammation-related genes in microarray data sets from glioblastoma tissue samples collected from 142 patients. We report, for the first time, that a tumor allergy and inflammation transcriptome is predominantly inversely correlated with glioblastoma aggressiveness or progression. In particular, several genes on the IgE pathway show decreased expression, providing genetic evidence of an inverse correlation between allergies and glioblastoma previously based only on self-reported medical history. We also unexpectedly found

down-regulation of the COX-1, COX-2, and several major immunosuppressive genes (eg, IL-10, TGF- $\beta$ ). In addition to negative trends, we also observed that the proinflammatory cytokine gene, IL-17- $\beta$ , and 2 IL-17 receptors are up-regulated as is the NCAM-1 gene that may play a role in tumor immunosuppression<sup>21</sup> and the PDGFR- $\alpha$  gene that is involved in neural cell development.<sup>22</sup>

## Materials and Methods

Using the National Cancer Institute’s (NCI) Cancer Genome Atlas (TCGA) web site, we identified 6 publicly available mRNA expression data sets based on the analysis of tissue samples from 142 glioblastoma patients. All 6 data sets were constructed using the Affymetrix HT\_HG-U133A microarray platform. The expression values were then standardized using the BRBArray Tool developed by NCI. Next, we calculated Pearson’s product moment correlation coefficients ( $r$ ) between the natural log of CD133 expression and the natural log of expression levels of a subset of 919 allergy- and inflammation-related genes identified by Loza et al.<sup>23</sup> (Only 919 of the 1026 inflammation-related genes identified by Loza et al. were included on the Affymetrix panel.) Associations between log-transformed genes are more likely to be linear, an assumption on which  $r$  is based.

Next, we constructed a “volcano plot” (Fig. 1) where correlation coefficients for the association between CD133 and each of the 919 genes were plotted against the negative log of the  $P$  values (based on Fisher’s  $z$ -transformation) associated with the test of hypotheses of the equality of the correlation coefficients to zero. The logs of  $P$  values are used to avoid scientific notation or large numbers of zeroes and their negative value transforms the smallest  $P$  values (ie, those associated with strongest evidence against the null hypothesis) into the largest numbers (which has intuitive appeal given the meaning of  $P$  values). The regression line in the graph represents an average and is not meant to model the association between correlation coefficients and their  $P$  values.

We then ranked correlation coefficients by their absolute values. Genes with the highest absolute correlation coefficients were included in Fig. 3 (the cut point being an absolute value of  $r \geq 0.40$ ). To gain further insight into interrelationships among the genes most strongly associated with CD133 expression ( $r \geq 0.34$ ), we plotted their expression values on a heat map (Fig. 3) and used unsupervised hierarchical cluster analysis with Manhattan distance as the distance metric.

## Results

Correlations between CD133 and allergy-related genes are shown in Table 1. In the first column are genes central to allergic conditions: Th2 cytokines, their transcription factors, and IgE binding genes. Also important in both allergy and tumors are the regulatory or

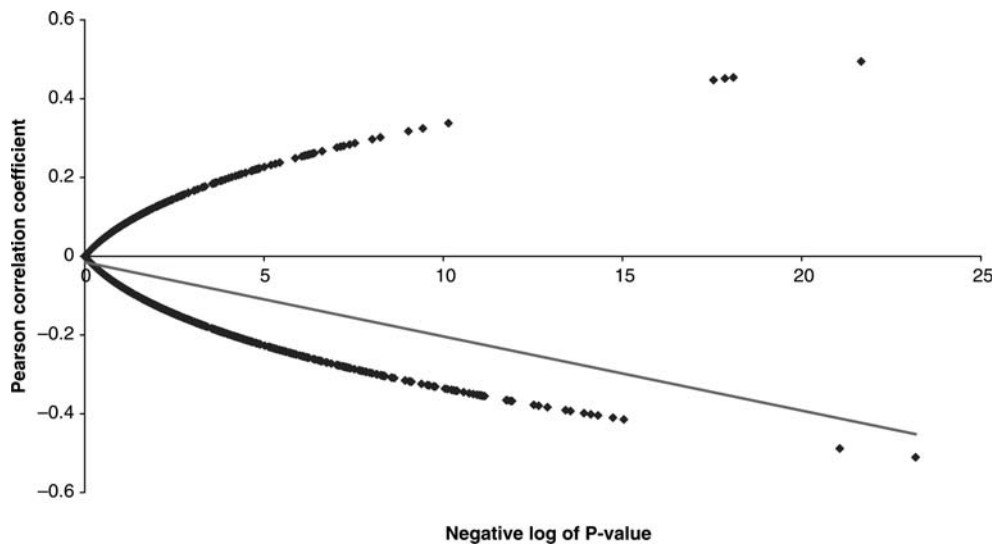


Fig. 1. Plot of negative logs of  $P$  values against correlation coefficients representing association between CD133 expression and expression of 919 allergy and inflammation-related genes ( $n = 142$  glioblastoma patients). Regression line shows average of all points.

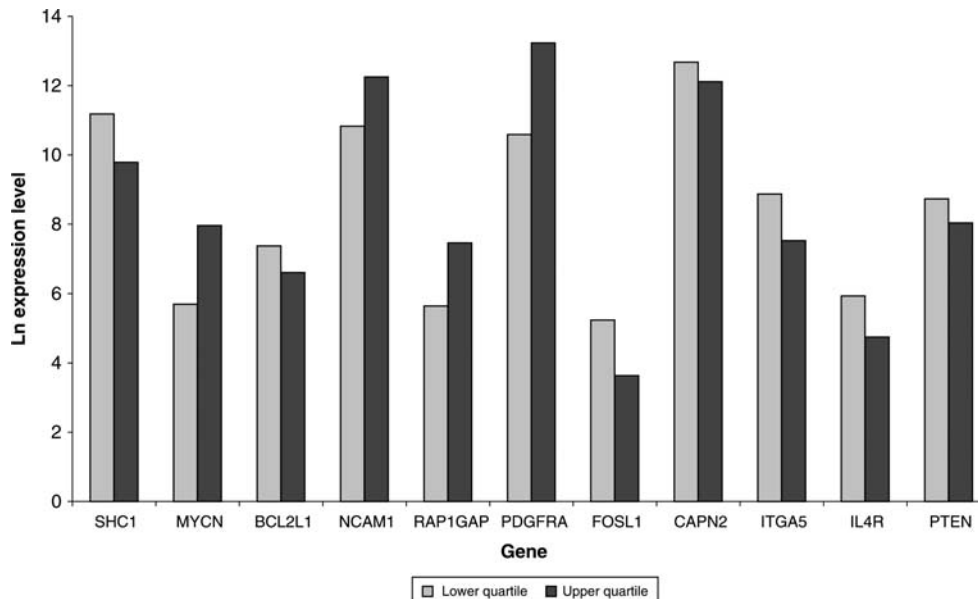


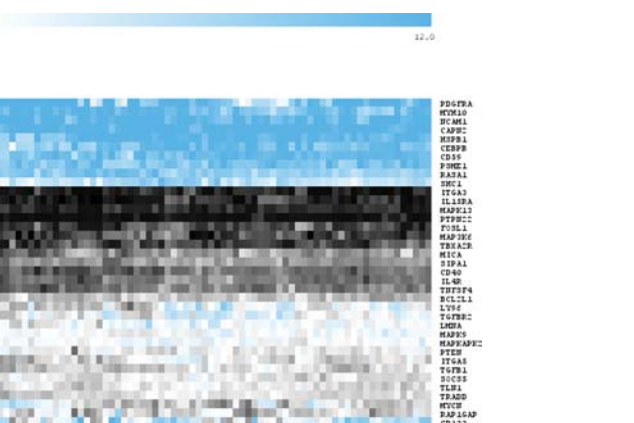
Fig. 2. Expression levels of 11 allergy and inflammation-related genes with absolute value of correlation coefficients (representing association between CD133 and these genes) of at least 0.40.

immunosuppressive genes (column 5) that may inhibit both allergy and tumor immunity. Additional genes in Table 1 are on the Th1 and Th17 pathways that interact with or regulate the Th2 allergy pathway.

There is no association between CD133 expression and allergy-related IL-13, IL-4, and IL-5 (which are expressed at relatively low levels), and there is even a small positive association between CD133 and IL-13R- $\alpha$ 2 (consistent with previous literature<sup>24</sup>). However, IL-4R- $\alpha$  and 2 allergy-related transcription factors, GATA3 and STAT6, are strongly inversely related to CD133 as are several immunosuppressive cytokines that regulate allergies (eg, TGFB1, TGFB3,

IL-10). Overall, approximately 80% of the genes in Table 1 are inversely related to CD133. However, the proinflammatory cytokines: IL-17- $\beta$ , IL-17R- $\alpha$ , and IL-17R- $\beta$  receptors (in bold italics in Table 1) are positively correlated with CD133 and are therefore exceptions to the overall pattern.

To further explore the inverse association between the use of NSAIDs and glioblastoma risk, we examined the association between inflammatory genes on the prostanoïd pathway (eg, COX-1, COX-2, also called PTGS1, PTGS2) and CD133 expression. Again as in Table 1, most genes in Table 2 (72%) are negatively correlated with CD133 including COX-1 and to a lesser extent



the value of  $r \geq 0.34$ ) and of CD133 (bottom row) for each of 142

Th1, and Th17 cytokines and related genes

| Genes                           | Correlation with CD133 | P value | Expression level in upper quartile of CD133 |
|---------------------------------|------------------------|---------|---------------------------------------------|
| Immunosuppressive genes         |                        |         |                                             |
| TGF- $\beta$ 1                  | -0.35                  | 0.00    | 6.94                                        |
| TGF- $\beta$ 2                  | 0.03                   | 0.71    | 7.36                                        |
| TGF- $\beta$ 3                  | -0.32                  | 0.00    | 5.42                                        |
| TGF- $\beta$ R1                 | -0.17                  | 0.04    | 2.39                                        |
| TGF- $\beta$ R2                 | -0.39                  | 0.00    | 8.39                                        |
| IL-10                           | -0.23                  | 0.01    | 2.45                                        |
| IL-10R- $\alpha$                | -0.20                  | 0.02    | 6.89                                        |
| IL-10R- $\beta$                 | -0.17                  | 0.04    | 8.23                                        |
| FoxP3                           | 0.00                   | 0.99    | 2.21                                        |
| CTLA-4                          | -0.07                  | 0.44    | 1.98                                        |
| CCL2                            | -0.12                  | 0.17    | 10.07                                       |
| CCR4                            | -0.13                  | 0.13    | 1.90                                        |
| Th1 cytokines and related genes |                        |         |                                             |
| IFN- $\gamma$                   | 0.13                   | 0.14    | 2.20                                        |
| IFN- $\gamma$ R1                | -0.10                  | 0.26    | 11.26                                       |
| IFN- $\gamma$ R2                | -0.15                  | 0.08    | 10.09                                       |
| IL-12- $\alpha$                 | -0.09                  | 0.29    | 2.36                                        |
| IL-12- $\beta$                  | -0.06                  | 0.45    | 2.38                                        |
| IL-12R- $\beta$ 1               | -0.13                  | 0.13    | 2.19                                        |
| IL-12R- $\beta$ 2               | -0.09                  | 0.31    | 2.15                                        |
| STAT4                           | -0.25                  | 0.00    | 2.30                                        |

een these genes and CD133.

**Table 2.** Correlation between CD133 and COX-1, COX-2, and other members of inflammatory prostanoid pathway

| Gene          | Correlation with CD133 | P value | Expression level in upper quartile of CD133 |
|---------------|------------------------|---------|---------------------------------------------|
| COX-1 (PTGS1) | −0.21                  | 0.01    | 7.05                                        |
| COX-2 (PTGS2) | −0.08                  | 0.36    | 5.80                                        |
| PTGDR         | −0.08                  | 0.36    | 2.30                                        |
| PTGDS         | 0.15                   | 0.07    | 12.02                                       |
| PTGER1        | 0.13                   | 0.13    | 2.47                                        |
| PTGER2        | −0.28                  | 0.00    | 2.93                                        |
| PTGER3        | −0.19                  | 0.03    | 2.93                                        |
| PTGER4        | −0.32                  | 0.00    | 6.39                                        |
| PTGES         | −0.26                  | 0.00    | 2.64                                        |
| PTGES2        | −0.07                  | 0.41    | 5.32                                        |
| PTGES3        | 0.13                   | 0.13    | 13.48                                       |
| PTGFR         | −0.16                  | 0.05    | 2.34                                        |
| PTGIR         | −0.00                  | 0.97    | 2.40                                        |
| PTGIS         | 0.02                   | 0.83    | 5.42                                        |

COX-2. However, prostaglandin D2 synthase (PTGDS) and prostaglandin E synthase 3 (PTGES3) are positively associated with CD133 and expressed at high levels.

In Tables 1 and 2, we focused on associations between CD133 and allergy, between selected immunoregulatory and inflammation-related genes for which previous literature provides hypotheses. In Fig. 1, we show correlations between CD133 and all 919 allergy- and inflammation-related genes. Note that negative correlation coefficients predominate as indicated by the negative slope of the regression line even among genes with small correlation coefficients (absolute value of  $r \leq 0.10$ ). Specifically, 69% of the correlation coefficients are negative.

To empirically identify genes that are most strongly associated with CD133, we ranked all 919 genes by the absolute value of their correlation coefficients. The 11 genes shown in Fig. 2 and Table 3 are those whose correlation coefficients reached an absolute value of at least 0.40. Each of these correlation coefficients is associated with a  $P$  value of  $<1 \times 10^{-6}$ . Figure 2 shows average expression values for each gene at the lower (light blue) and upper (dark blue) quartiles of CD133 expression. If the light blue bar is higher than the dark blue bar then the gene is inversely related to CD133 and vice versa. In addition to the size of its correlation coefficient, the magnitude of expression levels may indicate the relative importance of a gene.

Expression levels for the 36 genes with correlation coefficients of at least 0.36 are shown in the heat map (Fig. 3; see bottom row for CD133 expression levels). Low expression levels are represented in black and high in blue. Although this graph illustrates individual expression values that are therefore more variable than correlation coefficients, patterns are broadly consistent with those shown in Fig. 1 and Table 3. In Figure 3

**Table 3.** Genes with absolute correlations with CD133 of at least 0.40

| Gene    | Correlation with CD133 | Ln P value |
|---------|------------------------|------------|
| SHC1    | −0.51                  | 23.18      |
| MYCN    | 0.49                   | 21.66      |
| BCL2L1  | −0.49                  | 21.06      |
| NCAM1   | 0.45                   | 18.10      |
| RAP1GAP | 0.45                   | 17.86      |
| PDGFRA  | 0.45                   | 17.54      |
| FOSL1   | −0.41                  | 15.04      |
| CAPN2   | −0.41                  | 14.74      |
| ITGA5   | −0.40                  | 14.32      |
| IL4R    | −0.40                  | 14.12      |
| PTEN    | −0.40                  | 13.93      |

patients are vertically clustered into 3 major groups that roughly correspond to low, medium, and high values of CD133 expression. Note that the sample cluster to the extreme left (first 16 columns) is characterized by relatively low expression of CD133 (shown in black and grey) and relatively high expression of the majority of genes (blue). Consistent with Fig. 1, higher levels of CD133 in the largest cluster to the extreme right (including about 60% of the area of the graph) are associated with lower levels of the majority of genes. Further support for the results based on correlation coefficients is shown by the SHC1 gene, which is most strongly correlated with CD133 ( $r = 0.51$ , Table 3) and most closely inversely mirrors CD133 on this graph. There are only 5 genes on the graph that are positively related to CD133: PDGFR- $\alpha$ , NCAM-1, MYH10, MYCN, and RAP1GAP. However, the first 3 genes included in this list are characterized by the highest expression levels in the array and may therefore affect glioblastoma progression.

## Discussion

Our central finding, the predominance of down-regulation of the majority of allergy and inflammation genes during late glioblastoma progression, indicates that immunosuppression is widespread and not restricted to a functional subset of genes. Among the down-regulated genes are several central to IgE production (Table 1). This observation is consistent with the reported inverse association between allergies and glioblastoma.<sup>6</sup> We also found decreased expression of the COX-1 and COX-2 genes (Table 2), although the latter gene is reported to be up-regulated during the progression of tumors at several sites.<sup>10</sup> Furthermore, during late stage tumor progression, immunosuppression does not appear to be orchestrated by either IL-10 or any of the TGF- $\beta$  genes that we evaluated (Table 1). This latter result is consistent with the reported decline, in the peripheral circulation, of the proportion of IL-10 expressing regulatory T cells late in a mouse model of late glioma progression.<sup>25</sup> Nonetheless,

during tumor progression expression of IL-17- $\beta$ , NCAM-1, and PDGFR- $\alpha$  is up-regulated and the latter 2 genes are expressed at high levels perhaps indicating their importance in this environment.

Strictly speaking, our cross-sectional results do not allow longitudinal inferences. That is, we cannot legitimately talk about tumor progression because we have expression data from only one time point. However, we base our inferences on a consistent and growing body of literature that suggests that CD133 is an indicator of tumor aggressiveness or poor patient survival.<sup>20</sup> We refer to glioblastoma “progression” or “aggressiveness” throughout the present manuscript rather than to tumor growth because we do not know whether tumors in individuals with high CD133 expression are larger than those in individuals with low expression. We do know, however, that individuals with high CD133 expression levels have a higher probability of dying, and it is in that sense that their tumors have progressed or are aggressive. It is, however, possible that differences between tumors with low and high CD133 expression are differences between 2 subtypes of glioblastoma.<sup>15</sup> In this case, differences in immune function genes that we found are not associated with progression but rather are applicable to these two subtypes.

Another point concerning inferences based on the present study is that it is descriptive and expression levels of immune function genes are correlated with each other; therefore, definitive conclusions concerning the effects of individual genes await experimental confirmation. Nonetheless, our descriptive results provide a context for further experimental research. Focus on the effects of individual cytokines, for example, without an appreciation of the broader environment in which they are found, may lead to a misunderstanding of their influence on the tumor.

Our results for IgE-related genes are consistent with suppression of allergies by glioblastoma; however, they do not exclude a role of allergy in tumor prevention. Butovsky et al.<sup>26</sup> report that microglia activated by IL-4 (an allergy-related cytokine) induce neurogenesis possibly making stem cells less susceptible to neoplastic transformation. Only studies of longitudinal cohorts or genetic variants that alter the allergy risk<sup>27–29</sup> can definitively determine whether allergies reduce glioblastoma risk or are suppressed by the preclinical tumor or both.

In our data set (Table 1), IL-10 ( $r = -0.23$ ) and its receptors are down-regulated with tumor progression. Although these findings appear to contradict the known mechanisms of local and systemic immunosuppressive effects characteristic of glioblastoma,<sup>1</sup> they may be consistent with observations by Kennedy et al.<sup>25</sup> who found that the proportion of IL-10 secreting regulatory T cells in peripheral circulation of glioma mouse models increased by 26 days post-tumor implantation, but decreased at 40 days postimplantation. And although these authors found that the proportion of CD4<sup>+</sup> tumor infiltrating lymphocytes (TILs) decreased over time, they did not assess IL-10 expression of regulatory T cells in TILs, so that a direct comparison with our findings is not possible. In contrast to their findings

for regulatory T cells and our results, they observed no change in the proportion of tumor-associated macrophages or microglia that expressed IL-10 in the late stages of glioma growth. However, they found decreased TNF- $\alpha$  production during this period, which is consistent with our observations ( $r = -0.08$ ).

In contrast to the overall negative trend, we found positive associations between glioblastoma and several genes including IL-17- $\beta$ , 2 IL-17 receptors (IL-17- $\alpha$  and IL-17- $\beta$ ), NCAM-1, and PDGFR- $\alpha$ . Previous literature, discussed below, confirming positive associations of these genes with glioma provides indirect support for the validity of our negative findings to the extent that it supports the plausibility of results based on our data.

Kehlen et al.<sup>30</sup> report that in glioblastoma cell lines, the proinflammatory cytokine IL-17 can degrade I $\kappa$ B- $\alpha$ , which is an inhibitor of NF- $\kappa$ B, a transcription factor present in the brain that responds to initiators of inflammatory response. Another study<sup>31</sup> found evidence for a role of IL-17 in rodent astrocytes. In these astrocytes, IL-17, together with IFN- $\gamma$  and other cytokines, stimulates inducible nitric acid synthase during central nervous system inflammation. There are also reports of elevated expression of this cytokine in several tumors including prostate cancer<sup>32</sup> and osteosarcoma.<sup>33</sup> Nam et al.<sup>34</sup> found that TGF- $\beta$  induces IL-17 secretion, which subsequently promotes tumor progression. At the same time, Ma et al.<sup>35</sup> report that expression of the IL-17R- $\beta$  receptor is a favorable prognostic indicator for breast cancer and Muranski et al.<sup>36</sup> show that under specific experimental conditions, tumor-specific Th17 polarized cells can eradicate melanoma in mice. Finally, Koenen et al.<sup>37</sup> found that regulatory T cells may differentiate into IL-17 producing cells, perhaps explaining the absence of indicators in our data of the presence of regulatory T cells (Table 1). As is the case with many cytokines, the context in which they are expressed determines their function, yet our data indicate that IL-17 may be involved in glioblastoma progression.

Also positively associated with CD133, NCAM-1<sup>+</sup> NK cells may play immunosuppressive or immunoregulatory roles.<sup>38</sup> Waziri et al.<sup>21</sup> observed that nearly half of all T cells infiltrating glioblastoma specimens are NCAM-1<sup>+</sup> T cells. It is therefore possible that, with tumor progression, NCAM-1<sup>+</sup> T cells rather than regulatory T cells are responsible for directing tumor immunosuppression.<sup>21</sup>

PDGFR- $\alpha$ , positively related to CD133 in our study, plays a role in both inflammation and neurogenesis. In asthma, PDGFR- $\alpha$  contributes to bronchial smooth muscle hyperplasia.<sup>39</sup> Glial and neural progenitors express PDGFR- $\alpha$  during embryogenesis,<sup>22</sup> but in the adult brain PDGFR- $\alpha$  expression is down-regulated and restricted to neural progenitor cells in the ventricular and subventricular areas of the brain. Exposure to the PDGFR- $\alpha$  ligand by infusion into murine lateral ventricles leads to hyperplasia similar to that found in the early stages of glioma.<sup>40</sup> In addition to this experimental evidence, PDGFR- $\alpha$  haplotypes are related to

glioblastoma risk<sup>41</sup> and a subset of glioblastomas is characterized by PDGFR- $\alpha$  amplification.<sup>42</sup> The literature on the effects of PDGFR- $\alpha$  on glioma survival is contradictory and sparse; however, in a study of 87 gliomas Puputti et al.<sup>43</sup> found that PDGFR- $\alpha$  amplification is directly related to survival time in a univariate (5-year survival with amplification present = 33%, without amplification = 63%,  $P = 0.047$ ) but not in a multivariate model. Yet, Haberler et al.<sup>44</sup> saw no association between PDGFR- $\alpha$  expression and survival time in a cohort of 101 glioblastoma patients.

Our findings suggest alterations of immune function with glioblastoma progression. This result has practical

implications for immunotherapy in that it suggests that different approaches may be required depending on the stage of glioblastoma development.

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*Erratum* for “Allergy and inflammatory transcriptome is predominantly negatively correlated with CD133 expression in glioblastoma” by Schwartzbaum et al. *Neuro Oncol.* (2010) 12(4):320-277, doi:10.1093/neuonc/nop035

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