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Immune Signatures Predict Prognosis in Localized Cancer

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Abstract

The host immune response can impact cancer growth, prognosis, and response to therapy. In colorectal cancer, the presence of cells involved with T-cell-mediated adaptive immunity predicts survival better than the current staging method. We used the expression of genes recently associated with host immune responses (T_{H1}-mediated adaptive immunity, inflammation, and immune suppression) to perform hierarchical clustering of multiple large cohorts of cancer specimens to determine if immune-related gene expression resulted in clinical significant groupings of tumors. Microarray data from prostate cancer (n = 79), breast cancer (n = 132), lung cancer (n = 84), glioblastoma multiforme (n = 120), and lymphoma (n = 127) were analyzed. Among adenocarcinomas, the T_{H1}-mediated adaptive immunity genes were consistently associated with better prognosis, while genes associated with inflammation and immune suppression were variably associated with outcome. Specifically, increased expression of the T_{H1}-mediated adaptive immunity genes was associated with good prognosis in breast cancer patients under 45 years of age (p = .04, hazard ratio [HR] = 0.42) and in prostate cancer patients (p = .03, HR = 0.36) but not in lung cancer patients (p = 0.45, HR = 1.37). In lymphoma, patients with increased expression of inflammation and immune suppression genes had better prognosis than those expressing the T_{H1}-mediated adaptive immunity genes (p = .01, HR = 0.43) and in

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glioblastoma multiforme, the expression of inflammation genes conferred improved prognosis than those expressing immune suppression genes ($p = 0.05$, HR = 0.62). In aggregate, the gene expression signatures implicating specific components of the immune response hold prognostic import across solid tumors.

Keywords

Gene expression; Cancer; Immunology

INTRODUCTION

The mammalian immune system consists of multiple cell types and mediators that interact within a complex network to ensure protection against foreign pathogens while maintaining tolerance toward the self-antigen. It is also evident that host immune response can impact cancer growth, prognosis, and response to therapy. The association between specific immune cells and tumors has been studied for decades (1). Infiltration of natural killer cells in gastric or colorectal carcinoma or lymphocytes in melanoma, colorectal, or ovarian carcinoma has been associated with a favorable prognosis (2–5). In contrast, tumors that contain infiltrates of other immune cell types, such as macrophages in breast carcinoma or mast cells in non-small cell lung carcinoma (NSCLC) and melanoma are associated with a more unfavorable clinical prognosis (6–9). Finally, immune cells can also release inflammatory mediators that have antiangiogenic or antimetastatic effects (10, 11).

Recently, studies have focused more on three components of the host immune response (innate, adaptive, and suppression) and their role in tumorgenesis. Specifically in colorectal cancer, the presence of cells involved with the T-cell-mediated adaptive immunity component of the host immune response was a better predictor of survival than the current staging method (12). We hypothesized that coordinate expression of the genes associated with different host immune responses (“gene signatures”) may hold prognostic implications across cancers from different primary sites. To test this, we obtained expression data from several large sets of tumors annotated for clinical outcome, performed hierarchical clustering of each set of tumors based upon the expression of the previously identified immune-related genes, and assessed the prognostic significance of distinct groups of samples defined by genes involved in specific immune processes. Overall, consistent associations were identified between the different elements of the host immune system and prognosis, although the specific associations were more related to the type of cancer.

MATERIALS AND METHODS

Cross-platform gene mapping

Analyzing the prognostic value of genes associated with the immune signature required defining common genes present on multiple distinct microarray platforms. The previously identified probes of the 18 genes associated with immune host response to colorectal cancer (12), including T_{H1} adaptive immunity (NM_004131, NM_006433, NM_000619, NM_002198, NM_000734, NM_001768, NM_013351), inflammation (NM_000963,

NM_001712, NM_002423, NM_001168, NM_000584), and immune suppression (NM_000660, NM_003376, NM_025240, NM_003844, NM_000616, NM_000572) were identified on the Affymetrix U133A and U95 platform. Primary tumor gene expression and clinical outcome data for breast [GEO:GSE2034 (13, 14); GEO:GSE3494 (15)], prostate [http://www.broad.mit.edu/cgi-bin/cancer/datasets.cgi; (16)], lymphoma [http://www.broad.mit.edu/cgi-bin/cancer/datasets.cgi; (17)], lung [GEO:GSE3593; (18)], and gliobastoma multiforme [GEO:GES4412; (19)] were obtained from the Gene Expression Omnibus (GEO) Web site (http://www.ncbi.nlm.nih.gov/geo/) or as specified.

Hierarchical clustering

Using the GenePattern software, the array data were log transformed, mean-centered, and then normalized prior to clustering. Hierarchical clustering was performed using complete linkage with a Pearson correlation metric on the preprocess data to organize all of the data elements into a single tree with the highest levels of the tree representing the discovered classes or groupings (20).

Statistical analysis

Kaplan–Meier survival curves and the log-rank *p* values were used to assess the outcome differences between clusters of samples as defined by the expression of immune-related expression using Prism4 statistical software (GraphPad Inc., San Diego, CA, USA).

RESULTS

Development of immune signature

Previous work had identified 18 genes associated with specific components of the host immune response to prognostic outcomes in colorectal cancers (12). We used gene expression profiling to determine if these same genes hold prognostic import across multiple classes of solid tumors. Specifically, after mapping the 18 genes to probes on two commonly used microarray platforms (Affymetrix U95Av2 and U133A), expression of these genes were used to organize large collections of breast (14), prostate (16), lung (18), lymphoma (17), and gliobastoma multiforme tumors (19). Similar to the previous work establishing an association between a gene signature for metastasis and prognosis (20), hierarchical clustering was performed to organize both tumor samples and genes; the highest levels of the tree representing the discovered classes and the genes with increased expression defining the clusters were used to implicate specific immune processes.

Prognostic implication of the immune signature in adenocarcinoma

Localized adenocarcinomas including those of the prostate, breast, and lung consistently organized in clusters with prognostic significance. Similar to previously published colorectal data, genes associated with T_{H1}-mediated adaptive immunity genes were consistently associated with improved disease-free survival.

When the immune signature was applied to a set of prostate cancer patients, increased expression of both the T_{H1}-mediated adaptive immunity genes and the inflammation genes (Cluster 4) conferred improved prognosis when compared to those without the T_{H1}-

mediated adaptive immunity genes (clusters 1 and 2; $p = .03$, hazard ratio [HR] = 0.36) or inflammation genes ($p = .03$, HR = 0.36; Figure 1).

Similarly in breast cancer specimens, the genes implicating a host T_{H1} response (Cluster 2) were associated with improved prognosis in younger women (<45 years of age; $p = 0.04$, HR = 0.42; Figure 2). This association is also present in two independent data sets of breast cancer specimens (Figures 3 and 4). Interestingly, when breast cancer specimens were organized together without accounting for age (Figure 5) or for tumors from patients older than 65 years (Figure 6), no statistically significant clusters were identified. Finally, when a set of NSCLC samples consisting of only adenocarcinomas were clustered, expression of the T_{H1}-mediated adaptive immunity genes, immune suppression genes, or inflammation genes did not appear to confer improved prognosis (Figure 7).

Prognostic implication of immune signature in non-adenocarcinoma

Two examples of non-adenocarcinoma cancers were also organized based upon the immune-related genes, including collections of lymphoma and glioblastoma multiforme samples. In both sets, T_{H1}-mediated adaptive immunity was associated with poorer prognosis and inflammation genes were associated with better prognosis. Specifically, in patients with lymphoma, decreased expression of T_{H1}-mediated adaptive immunity genes and increased expression of inflammation and immune suppression genes (clusters 1 and 2) had better prognosis than those expressing the T_{H1}-mediated adaptive immunity genes (cluster 3; $p = .01$, HR = 0.43; Figure 8). Similarly, in glioblastoma multiforme, the expression of inflammation genes (cluster 3) conferred improved prognosis than those expressing immune suppression genes and T_{H1} adaptive immunity genes (cluster 2; $p = .05$, HR = 0.62; Figure 9).

DISCUSSION

The role of the host immune response in cancer progression remains controversial. Both animal and clinical studies have shown that the immune system can either promote or suppress tumor growth, and recent studies have shown that the tumor microenvironment involves interactions between the tumor cells, supportive stroma, tumor-associated vasculature, and the immune system (21). Furthermore, many cancer patients will develop both an innate and an adaptive immune response during the course of their disease, and the importance of identifying which component of the host immune response is contributing to the tumorigenesis becomes critical for developing new therapeutic targets. Genomic approaches provide an opportunity to study the immune component of the tumor microenvironment and offer the possibility of identifying cytokines and signaling molecules that are important for limiting protumorigenic responses and enhancing antitumor immune responses.

In general, a T_{H1} signature suggestive of an interferongamma-producing adaptive immune response is generally thought to be predictive of a productive antitumor response. Indeed, recent studies in colorectal cancer have supported this hypothesis, showing that tumors with high immune cell densities (CD3, CD8, etc.) had a lower risk of recurrence than those without such a signature (12). Tumors from patients without recurrence had higher immune

cell densities (CD3, CD8, granzyme B [GZMB], and CD45RO) within each tumor region than did those from patients whose tumors had recurred; the nonrecurrent colorectal cancers also had a dominant cluster of comodulated genes for T_{H1} adaptive immunity (genes encoding T-box transcription factor 21, interferon regulatory factor 1, IFN-μ, CD3, CD8, granulysin, and GZMB). However, other studies have clearly shown the existence of a regulatory T-cell population capable of suppressing antitumor immunity by down-modulating an adaptive immune response through the secretion of interleukin-10 (IL-10) and TGF-B. Thus, in the presence of significant numbers of T_{reg}, an adaptive T_{H1} response might not be associated with improved prognosis. Studies have shown that adaptive immunity is modulated by the presence of regulatory T cells (T_{regS}) that potentially suppress the action of T_{H1} cells by releasing IL-10 and TGF-β. Thus, in the presence of significant numbers of regulatory T cells, an adaptive T-cell response characterized by a T_{H1} signature might not prove beneficial, as expected.

Here, we find a consistent association between the same genes previously associated with the T_{H1} immune response in colorectal cancer and good prognosis in prostate cancer and breast cancer in women more than 45 years old, but not with lung cancer (Table 1). However, unlike colon cancer, where only the T_{H1} adaptive immunity genes hold prognostic significance, in prostate cancer it was a combination of the T_{H1} adaptive immunity and inflammation genes that conferred an improved prognosis, suggesting that adaptive immunity alone is not sufficient to maintain tumor control. The specific association of T_{H1} response and prognosis in young women is interesting, as with age, host immunity declines, and this also may reflect a difference in the underlying biology of tumors in the different subsets of patients as seen previously in breast cancer (22).

We also investigated the prognostic significance of these immune signatures in lymphoma as well as in glioblastoma multiforme as examples of localized non-adenocarcinomas (Table 1). Interestingly, genes associated with inflammation were better correlated with prognosis in this group, while T_{H1}-mediated adaptive immunity genes were in fact linked to a worse outcome. These two tumors have unique local tumor microenvironments that may explain this observation, as lymphomas are in direct contact with several types involved in both adaptive immunity and inflammation, while brain tumors may be partially immunoprivileged due to the blood–brain environment. Furthermore, macrophages in the vicinity of tumor cells may have a dual role depending on the type of signals they receive and the cytokines they produce (23). This dynamic interaction emphasizes not only the importance of the presence of immune cells but also the physiological state of the tumor (24). Cancer cells also develop immunoevasive strategies to resist the action of the immune system, and tumor cells can evade the host immune system by masking their identity, repressing tumor antigens, and avoiding apoptosis by inducing immunocyte apoptosis or neutralizing intracellular cytokines.

The shared association between different types of localized adenocarcinoma and non-adenocarcinoma between immune and inflammatory signatures and prognosis supports a compelling role for the immune system in the determination of disease outcome. The strength of our observations is the consistency with which the coordinated expression of genes implicating specific immune activities are associated with prognosis; the novelty and

interest of this work lie in the clear difference between adenocarcinoma and non-adenocarcinoma with respect to how each immune component is associated with good or bad patient outcome. Importantly, by demonstrating consistent associations between the host immune system and tumor behavior, our study further underscores the significant potential for immunotherapy. Specifically in patients with localized disease at risk for recurrence, modulating the host response toward T_{H1} may be an effective adjunctive strategy for adenocarcinoma if the individual's tumor lacks evidence for adoptive immunity. However, alternative approaches are required for patients with lymphoma or glioblastoma. As many alternative methods of immunotherapy are under investigation in phase II and phase III trials, it will be important to determine the predictive value of these immune response signatures and the interaction between existing immune responses to tumors and the clinical benefit of immunotherapy.

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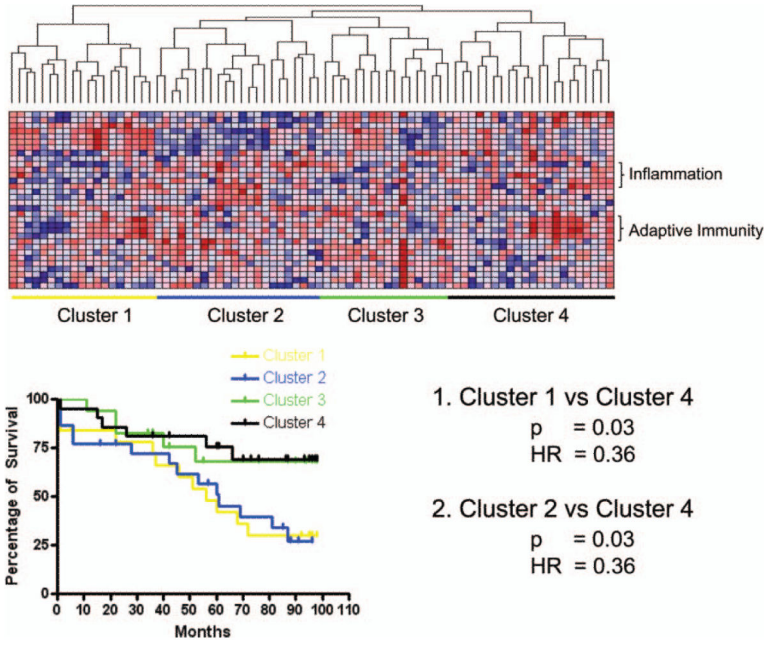

Figure 1.

Hierarchical clustering of prostate adenocarcinoma samples based on immune genes revealed four distinct clusters. Cluster 4 with increased expression of adaptive immunity and inflammation genes was identified to have improved prognosis (p = .03). Red represents high expression, and blue represents low expression.

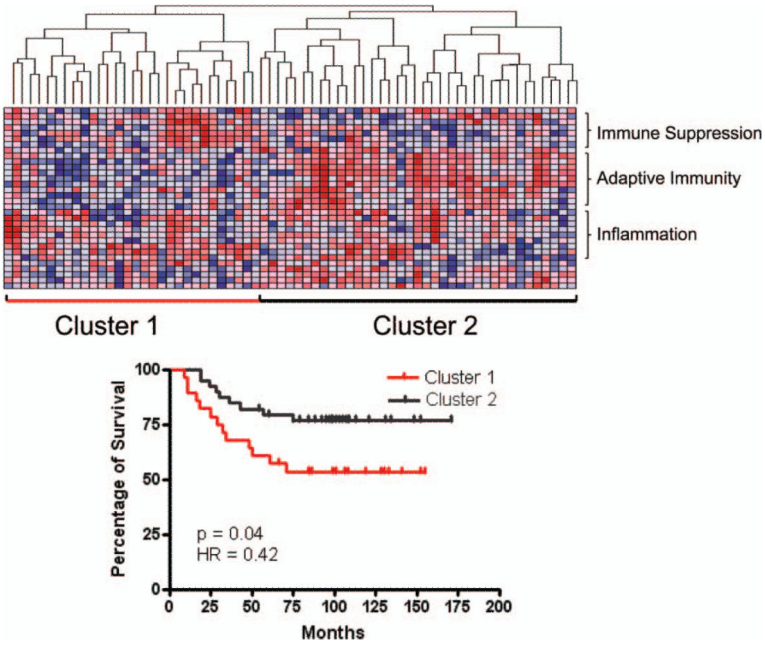

Figure 2.

Hierarchical clustering of breast adenocarcinoma samples (age <45 years) based on immune genes revealed two distinct clusters. Cluster 2 with increased expression of adaptive immunity genes was identified to have improved prognosis (p = .04). Red represents high expression, and blue represents low expression.

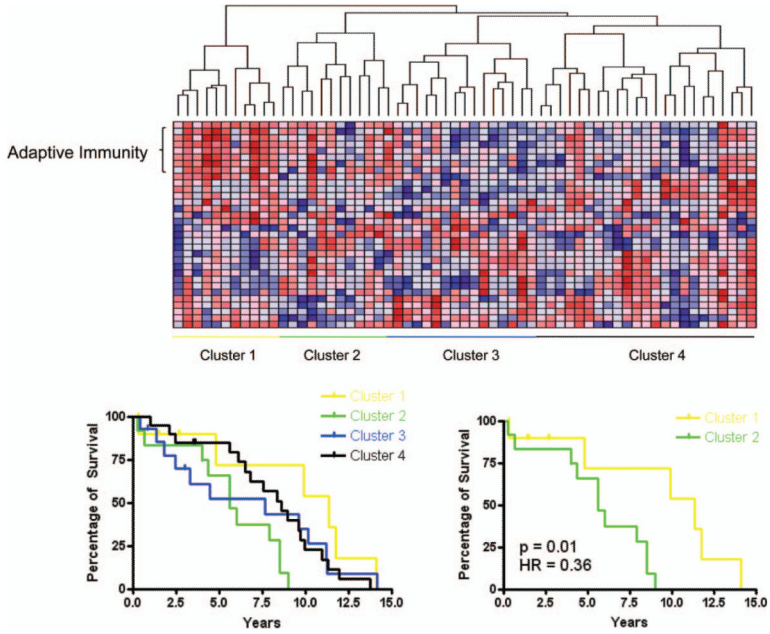

Figure 3.

Hierarchical clustering of breast adenocarcinoma samples (age <45 yaers) based on immune genes revealed four distinct clusters. Cluster 1 with increased expression of adaptive immunity genes was identified to have improved prognosis (p = .01).

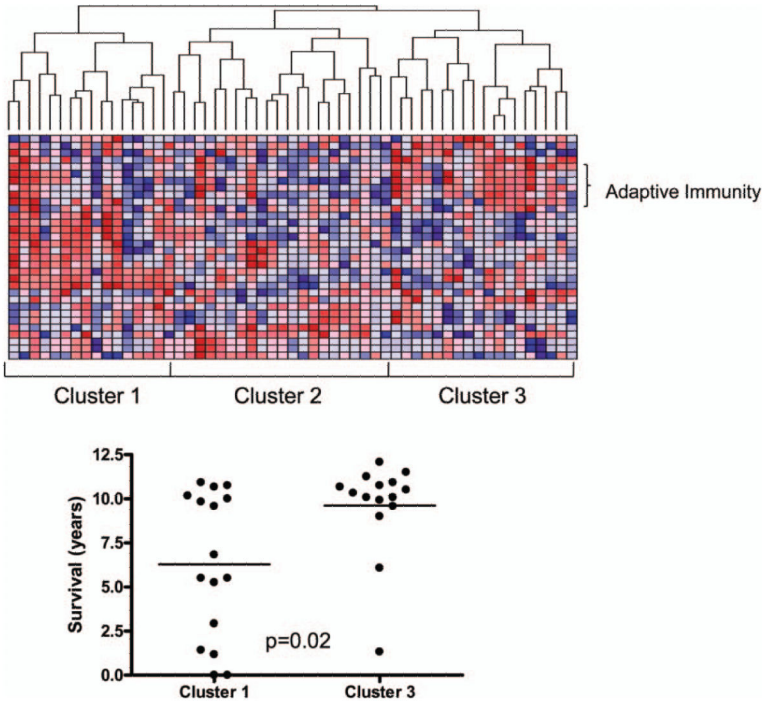

Figure 4.

Hierarchical clustering of breast adenocarcinoma samples (age <45 years) based on immune genes revealed three distinct clusters. Cluster 3 with increased expression of adaptive immunity genes was identified to have improved prognosis (p = .02).

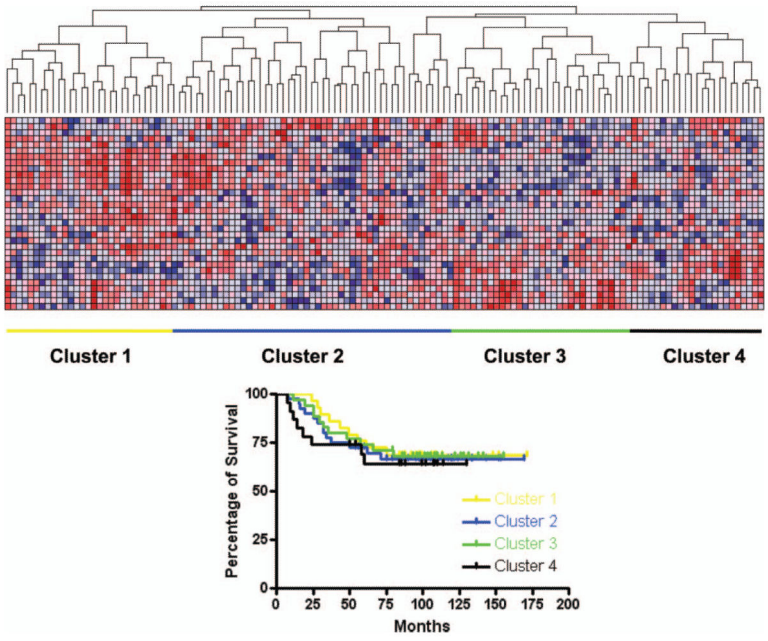

Figure 5.

Hierarchical clustering of breast adenocarcinoma samples (all ages) based on immune genes revealed four distinct clusters. No cluster was identified to have improved prognosis.

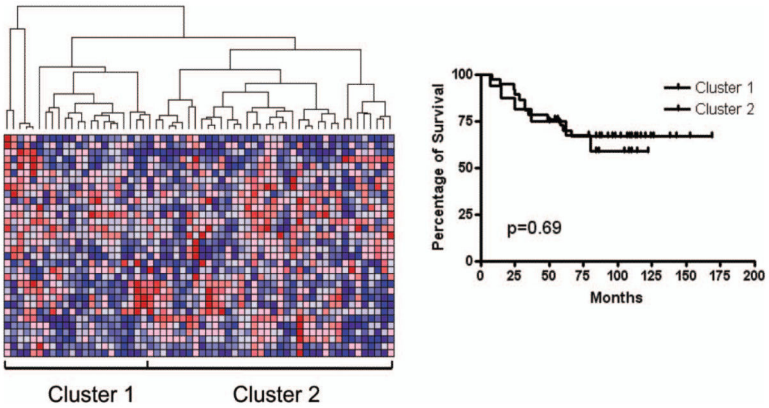

Figure 6.

Hierarchical clustering of breast adenocarcinoma samples (age >65 years) based on immune genes revealed two distinct clusters. No cluster was identified to have improved prognosis.

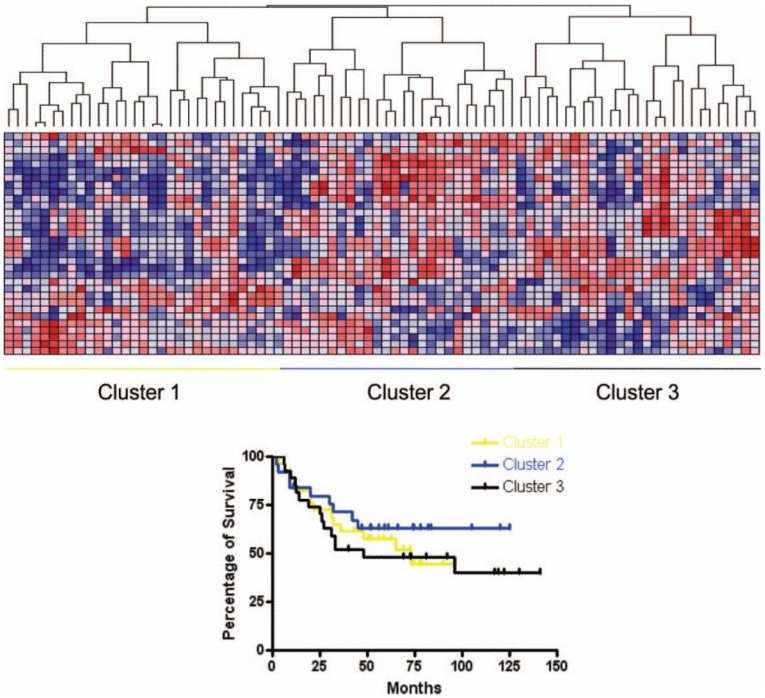

Figure 7.

Hierarchical clustering of lung adenocarcinoma samples based on immune genes revealed three distinct clusters. No cluster was identified to have improved prognosis.

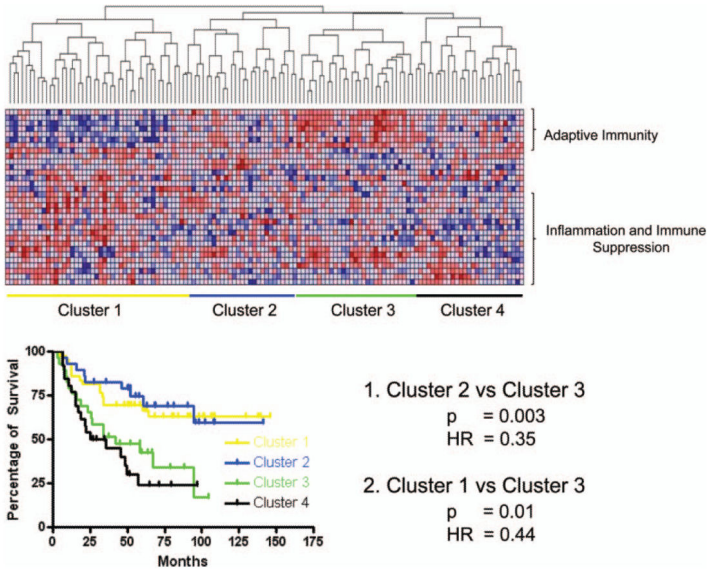

Figure 8.

Hierarchical clustering of lymphoma samples based on immune genes revealed four distinct clusters. Cluster 3 with increased expression of adaptive immunity genes was found to have poorer prognosis (p = .01).

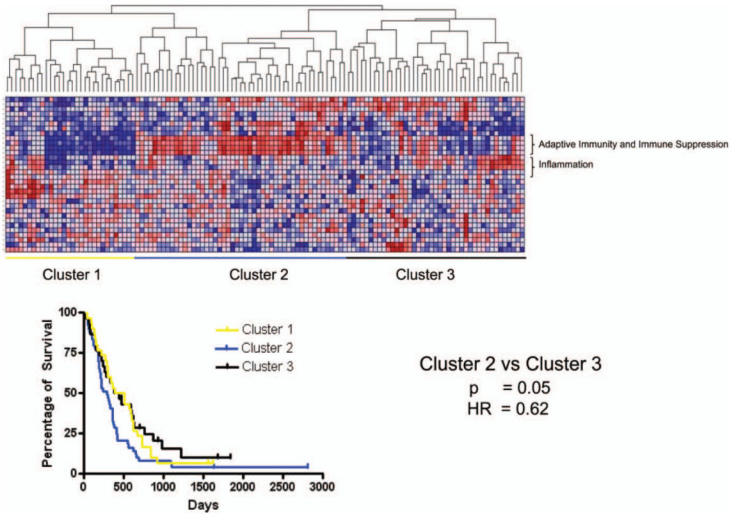

Figure 9.

Hierarchical clustering of glioblastoma multiforme samples based on immune genes revealed three distinct clusters. Cluster 3 with increased inflammation gene was identified to have improved prognosis (p = .05).

Table 1

Presence of Adaptive, Immunity, Inflammation and Immune Suppression in Solid Tumors

	Good Prognosis Cluster			Poor Prognosis Cluster				
Cancer	Adaptive Immunity	Inflammation	Immune Suppression	Adaptive Immunity	Inflammation	Immune Suppression	*p* Value	Hazard Ratio
Breast								
GSE2034	Increased				Increased	Increased	$p = .04$	HR = 0.42
Chin *et al.*	Increased						$p = .01$	HR = 0.36
GSE3494	Increased				Increased	Increased	$p = .02$	
Prostate								
Glinsky *et al.*	Increased	Increased				Increased	$p = .03$	HR = 0.36
Lung								
GSE3593		Increased		Increased			$p = .45$	HR = 1.37
Lymphoma								
Savage *et al.*		Increased	Increased	Increased			$p = .01$	HR = 0.43
Glioblastoma multiforme								
Horvath *et al.*		Increased		Increased		Increased	$p = .05$	HR = 0.65