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Association of changes in the gene expression profile of blood cells with the local tumor inflammatory response in a murine tumor model

Yoshio Sakai [a,b], Isamu Tatsumi [c], Mami Higashimoto [c], Akihiro Seki [c], Alessandro Nasti [c], Keiko Yoshida [c], Kazunori Kawaguchi [c], Takashi Wada [b], Masao Honda [c], Takuya Komura [c], Shuichi Kaneko [a,c,*]

[a] Department of Gastroenterology, Kanazawa University, Japan
[b] Department of Laboratory Medicine, Kanazawa University, Japan
[c] Disease Control and Homeostasis, Kanazawa University, Japan

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ABSTRACT

Cancer tissue is frequently associated with the host inflammatory response, which involves blood cells. Using DNA microarrays, we examined the gene expression profiles of blood and tumors in a murine subcutaneous hepatocellular carcinoma model, in which tumors develop during the initial 10 days and then diminish and disappear by day 25 after implantation. Immunohistochemical and gene expression analysis indicated that tumor tissues were associated with an active immune response, particularly the CD4+ T cell-mediated immune response, on day 10. The genes commonly up-regulated in blood and the fraction enriched with tumor-associated inflammatory cells on day 10 also suggested the involvement of CD4+ T cells. Unsupervised hierarchical clustering analysis of gene expression of peripheral blood cells on days 0, 10, 15, 20, and 25 indicated two major clusters: the tumor-existence cluster on days 10, 15, and 20, and the tumor-free cluster on days 0 and 25. Additionally, sub-clusters were detected on each day. These results suggest that the gene expression profile of whole blood cells is affected by the local tumor condition, and is associated with the local host immune response. Its analysis will facilitate exploration of the underlying important features of the host immune response to tumors.

1. Introduction

Cancer is one of the most serious diseases if early diagnosis fails or in cases of recurrence after radical treatment [1]. Therefore, elucidating the detailed biological features of cancer is important for development of new useful diagnostic and therapeutic methods to improve the prognosis of cancer patients [2]. Immunity is an important physiological homeostasis system that protects the host from various diseases. Cancer is frequently associated with the immune reaction of the host [3], although cancer originates from self tissues. Peripheral blood contains a substantial number of immune-mediating cells that can respond to affected tissues or pathogens; therefore, they are a crucial population that reacts to diseases, including cancer, to protect the host.

Whereas the host responds to lesions in the body, the blood reveals consequent characteristic manifestations—*i.e.*, neutrophilia for bacterial infection [4] or leukopenia for viral infection [5]. In the host with cancer, circulating blood contains characteristic immune-mediating cells—*e.g.*, cytotoxic T cells [6,7] and regulatory T cells [8,9]—due to the immune response to the disease. Assessment of these immune-mediating cells in blood is useful for evaluations of prognosis and therapeutic effect because cytotoxic T cells play a role in cancer eradication, whereas regulatory T cells inhibit the host anti-cancer immune response. Therefore, assessment of blood cells is a useful approach to evaluating host immune status in various diseases, including cancer [10,11].

Whole-genome expression analysis using DNA microarray technology allows examination of various biological processes [12–14]. We have reported previously that peripheral blood exhibited a characteristic gene expression profile in cancer [15,16]. Although these studies suggest the usefulness of peripheral blood gene-expression analysis for exploration of the pathophysiological features/conditions of cancer, how the altered gene expression profile of blood cells contributes to understanding of the condition of individuals with cancer remains unknown.

We have established subcutaneous hepatocellular carcinoma (HCC) murine models that show the unique course of cancer: transient development followed by diminishing. This course was mediated by the immune response, particularly CD4+ T cells. Altered gene expression in whole blood cells as well as local

Abbreviation: HCC, hepatocellular carcinoma.

* Corresponding author. Address: Department of Gastroenterology, Kanazawa University, 13-1 Takara-machi, Kanazawa, Ishikawa 920-8641, Japan. Fax: +81 76 234 4250.
E-mail address: skaneko@m-kanazawa.jp (S. Kaneko).

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tumor-associated inflammatory cells implied CD4+ T cell-involvement. Furthermore, the gene expression profiles of blood differed depending on the condition of cancer tissues, suggesting that blood-cell gene expression is associated with tumor condition and that its analysis is useful for investigation of the biological features of the host immune response to cancer.

2. Materials and methods

2.1. Cell culture

Hepa1-6 (ATCC, Manassas, VA), an established murine HCC cell line, was maintained in Dulbecco's modified Eagle's medium (Life Technologies, Carlsbad, CA) supplemented with 10% heat-inactivated fetal bovine serum (Life Technologies).

2.2. Subcutaneous HCC murine model

C57Bl/6 female mice (8 weeks old; Charles River Laboratories, Yokohama, Japan) or BALB/c athymic female mice (8 weeks old) were injected subcutaneously with 1×10^7 Hepa1-6 cells. Tumor size was monitored and the volume was calculated using the following formula:

$$\text{Tumor volume}(\text{mm}^3) = (\text{the shortest diameter})^2 \times (\text{the longest diameter})/2$$

Animal experiments were approved by the institutional review board.

2.3. Immunohistochemistry

Hepa1-6 subcutaneous tumor tissues were removed and embedded in optimal cutting temperature compound (Sakura Finetek, Torrance, CA), snap-frozen in liquid nitrogen, and cryostat-sectioned. Tissues were also fixed with neutral-buffered formalin, embedded in paraffin, cut into 4-μm sections, mounted on microscope slides, and stained with hematoxylin and eosin. Cryostat sections of frozen tissues were fixed in cold acetone, and endogenous biotin/avidin in the tissue specimens were blocked using a blocking kit (Vector Laboratories, Inc., Burlingame, CA). Slides were incubated with the appropriately diluted primary antibodies, anti-mouse CD4, CD8, CD11b (BD Pharmingen, Sparks, MD), and Gr-1 (eBioscience, San Diego, CA), respectively. The reactions were visualized using EnVision kits (DAKO, Glostrup, Denmark), followed by counterstaining with hematoxylin.

2.4. RNA isolation

Blood was obtained by retro-orbital venipuncture from mice and immediately stored as RNAlater aliquots (Ambion, Austin, TX). Total RNA of the obtained whole blood was extracted using the Mouse RiboPure™-Blood RNA Isolation Kit (Ambion), according to the manufacturer's protocol. A portion of Hepa1-6 tumor tissues was obtained and stored in RNAlater, followed by RNA isolation using the RNeasy Mini Kit (QIAGEN, Tokyo, Japan). The tumor fraction enriched with tumor-associated inflammatory cells was obtained using Histopaque®. Briefly, tumor tissues were minced and overlaid on the Histopaque® aliquot. After centrifugation, the visible separated monolayer was collected. RNA was isolated from the collected fraction using the microRNA Isolation Kit (Stratagene, La Jolla, CA), according to the manufacturer's protocol. The quality of purified RNA was analyzed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA).

2.5. Gene expression analysis by DNA microarray and data analysis

Isolated RNA was amplified and labeled with Cy5 using the Quick Amp Labeling Kit (Agilent Technologies) according to the manufacturer's protocol. For reference, RNA isolated from the blood of normal C57Bl/6 or BALB/c athymic female mice was amplified and labeled with Cy3. The labeled objective and reference cRNA was mixed and hybridized using the Whole Mouse Genome 4×44K Array kit (Agilent Technologies). The slide was scanned using a DNA Microarray Scanner (Model G2505B; Agilent Technologies). Microarray data were deposited in NBCI Gene Expression Omnibus (GSE ID: GSE 39075).

Gene expression analysis was performed using the GeneSpring analysis software (Agilent Technologies). Each measurement was divided by the 75th percentile of all measurements in that sample at per chip normalization. Hierarchical clustering and principal component analysis of gene expression were performed. Welch's *t*-test with Benjamini and Hochberg's false discovery rate were used to identify genes that were differentially expressed in the groups of interest. Analysis of biological processes and networks was performed using the MetaCore software suite (GeneGo, Carlsbad, CA).

3. Results

3.1. Subcutaneous Hepa1-6 HCC cells in C57Bl/6 mice grew transiently and subsequently diminished due to the T-cell immune response

Hepa1-6 murine HCC cells were subcutaneously injected into C57Bl/6 female mice and tumor size was monitored. Until day 10, implanted Hepa1-6 cells proliferated continuously, forming a consistent tumor. From days 10 to 15, tumor size started to decrease, and the established tumors were completely eradicated by day 25 (Fig. 1A). In contrast, when Hepa1-6 cells were subcutaneously injected into BALB/c athymic mice, which were deleted of most, if not all, T cells due to the lack of a thymus, tumor tissues were established and continued to grow during the 37-day observation period without stabilization or diminishment (Fig. 1B). Histologically, tumors in C57Bl/6 mice obtained on days 10 and 15 were filled with viable Hepa1-6 cells (Fig. 1C). Tumor tissues obtained on day 20 showed fibrous tissues with few viable Hepa1-6 cells (Fig. 1C). Immunohistochemical analysis of immune-mediating cells in tumors showed that a substantial number of CD4+ T cells were found on day 10, the frequency of which was reduced after day 15 (Fig. 1D). In contrast, fewer CD8+ T cells were detected during any phase (Fig. 1D). CD11b+ and Gr-1+ cells were observed during all tumor phases (Fig. 1D). We also examined infiltrating inflammatory cells in the Hepa1-6 tumors after 10 days' growth in athymic mice. In contrast to the intensively infiltrating CD4+ T cells in Hepa1-6 tumors in C57Bl/6 mice on day 10 (Fig. 1D), few CD4+ T cells and no CD8+ T cells were observed in Hepa1-6 tumors from athymic mice (Supplementary Fig. S1). CD11b+ cells were diffusely present and a substantial number of infiltrating Gr-1+ cells were found (Supplementary Fig. S1). These results suggest that the characteristic feature of the immune response in subcutaneous Hepa1-6 tumors of C57Bl/6 mice on day 10 was CD4+ T cell-involvement.

3.2. Gene expression profiles of Hepa1-6 tumors on days 10 and 15

Tumors established in C57Bl/6 on days 10 and 15 revealed a similar appearance microscopically (Fig. 1C), whereas more CD4+ T cells infiltrated into tumors on day 10 than on day 15. To compare the molecular biological features of tumors on days 10 ($n = 3$) and 15 ($n = 3$), we assessed gene expression profiles using

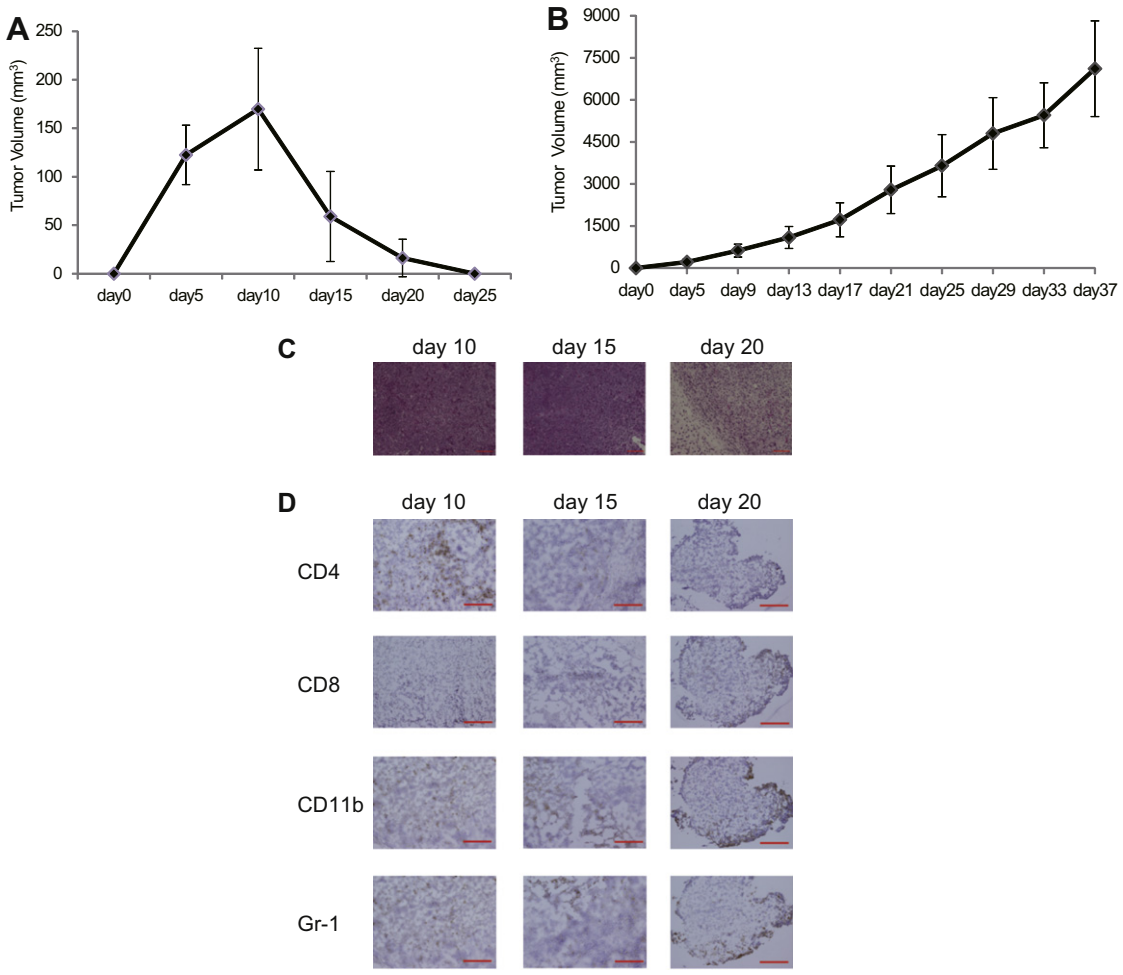

Fig. 1. The subcutaneous Hepa1-6 tumor model. Ten million Hepa1-6 HCC cells were subcutaneously inoculated into C57Bl/6 mice or Balb/c athymic mice. The size of tumors was monitored and tumor tissues were examined histologically as well as immunohistochemically. (A) Tumor growth in C57Bl/6 mice. (B) Tumor growth in Balb/c athymic mice. Bars, standard errors. (C) Histological features of inoculated Hepa1-6 subcutaneous tumors in C57Bl/6 mice on days 10, 15, and 25. Hematoxylin and eosin staining. Bars, 100 μm. (D) Immunohistochemical analysis of subcutaneous Hepa1-6 tumors in C57BL/6 mice on days 10, 15 and 25. Bars, 200 μm.

DNA microarrays. We identified 118 gene probes twofold differentially expressed between days 10 and 15 with $p < 0.05$ (Supplementary Table S1). Unsupervised hierarchical clustering and principal component analysis showed formation of two complete clusters on days 10 and 15 (Fig. 2). Network analysis of these 118 genes probes showed that regulation of T-cell proliferation was associated with the Stat5b, Stat5a, Jak2, Cyclin D1, and androgen receptor genes, whereas muscle filament sliding was associated with the Myh4, Myosin-IIA, Prelid2, Acta1, and Actc genes (Table 1). Thus, the T-cell immune responses to tumors on days 10 and 15 were markedly different.

3.3. Genes up-regulated in both blood and tumor-associated inflammatory cells were associated with local tumor conditions

The results shown above suggested that T cells, particularly CD4+ T cells, played an important role in the immune reaction to Hepa1-6 tumors in C57Bl/6 mice on day 10. To evaluate the relevance of local tumor inflammation and blood cells, we determined the genes whose expression was altered in the blood and tumor fraction enriched with tumor-associated inflammatory cells of C57Bl/6 mice ($n = 8$) or Balb/c athymic mice ($n = 3$) with Hepa1-6 tumors on day 10. We identified 127 genes whose expression was commonly twofold up-regulated ($p < 0.05$) between the tumor fraction enriched with tumor-associated inflammatory cells and whole blood cells in C57Bl/6 mice (Supplementary Table S2). The characteristic features involved a network that involved the C3g, IL-2r alpha chain, Shh, CD4, and Tgf-alpha genes (Table 2 and Fig. 3). Other networks were also observed to involve CD4: the network that involved Mkl2 (Mrtf-b), CD4, Dysstrophin, Pthr1, and Col1a2 and that involving CD4, Cdk1 (p34), iNOS, Tmem107, Dlx4 (Bp1). Sixty-five genes whose expression was commonly twofold up-regulated ($p < 0.05$) between the tumor fraction enriched with tumor-associated inflammatory cells and whole blood cells of athymic mice ($n = 3$) on day 10 were identified (Supplementary Table S3). Gene ontology processes for these genes included transcription, macromolecule metabolic process, regulation of multicellular organismal process, and development-related process, suggesting a role for local tumor-associated inflammatory cells in tumor development (Supplementary Table S4).

Thus, the biological features of genes up-regulated in both local tumor-associated inflammatory cells and blood cells suggested an underlying host CD4+ T cell-mediated immune response to tumors in C57Bl/6 mice, suggesting that the altered gene expression profile in systemically circulating blood cells reflected the local tumor conditions.

3.4. Whole peripheral-blood gene expression profiles were associated with tumor condition

Genes whose expression was altered in the blood of mice with Hepa1-6 tumors implied inflammation local to the tumor. We next

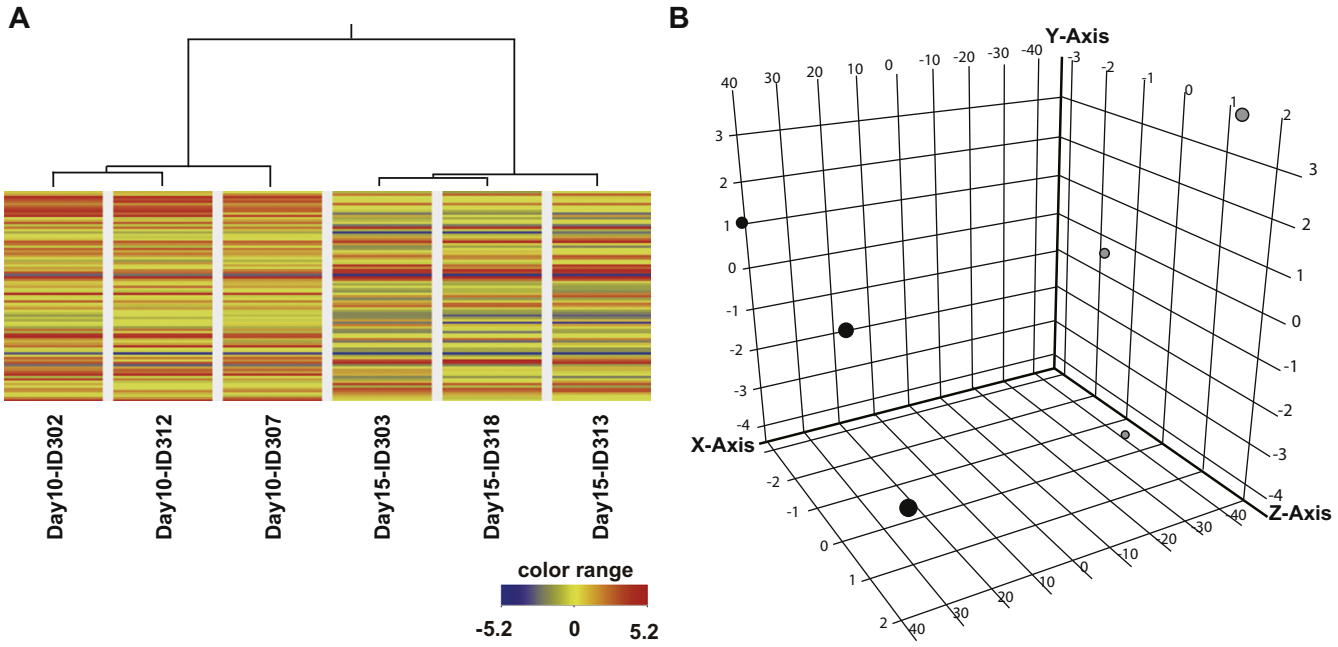

Fig. 2. Hierarchical clustering and principal component analysis of gene expression in subcutaneous Hepa1-6 tumors. Tumor tissues were obtained 10 and 15 days after subcutaneous inoculation of Hepa1-6 cells in C57Bl/6 mice and gene expression levels were evaluated by microarray. Unsupervised clustering (A) and principal component analysis (B) were performed using 118 probes specific for genes whose expression was significantly different at twofold. Filled circles, day 10. Gray circles, day 15.

Table 1
Networks for genes whose expression in tumors of C57Bl/6 mice was differetially expressed between day 10 and day 15.

Network	*p*-Value	GO processes
Stat5b, Androgen receptor, Stat5a, Jak2, Cyclin D1	0.0672	Positive regulation of activated T cell proliferation, cellular response to chemical stimulus, positive regulation of cell proliferation, regulation of activated T cell proliferation, response to hormone stimulus
Myh4, Myosin-IIA, Prelid2, Acta1, Actc	2.29×10^{-19}	Muscle filament sliding, actin-myosin filament sliding, actin-mediated cell contraction, actin filament-based movement, muscle system process
Rev-Eerb-b, Comp, Substance P receptor, Kcrm, Lingo1	7.02×10^{-15}	Regulation of multicellular organismal process, response to external stimulus, intracellular signal transduction, transmembrane receptor protein tyrosine kinase signaling pathway, multicellular organismal process
Bmal1, Stat5b, Pdgf-r-alpha, CD20, Dbp	7.26×10^{-13}	Positive regulation of transcription from RNA polymerase II promoter, regulation of transcription from RNA polymerase II promoter, positive regulation of gene expression, positive regulation of transcription, DNA-dependent, regulation of cellular biosynthetic process
Krt25, Keratin, type I cytoskeletal 25	0.00145	Intermediate filament organization, hair follicle morphogenesis, intermediate filament cytoskeleton organization, intermediate filament-based process, epidermis morphogenesis

explored how the gene expression profiles of whole blood cells changed with altering tumor conditions in mice with Hepa1-6 tumors. Blood was collected from C57Bl/6 mice before and 10, 15, 20, and 25 days after Hepa1-6 subcutaneous implantation, and then total RNA was isolated for gene expression analysis. The number of gene probes twofold up-regulated in the blood of mice with tumors compared with that of mice on day 0 was 968 on day 10 (Supplementary Table S5), 945 on day 15 (Supplementary Table S6), 53 on day 20 (Supplementary Table S7), and 14 on day 25 (Supplementary Table S8), and 418, 338, 61, and 134 gene probes, respectively, were down-regulated (Supplementary Tables S9, S10, S11 and S12). Unsupervised analysis of gene expression using 23,326 DNA microarray gene of that passed quality checking showed two major clusters, one associated with tumor presence on days 10, 15, and 20 (the tumor existence cluster), and the other to being tumor-free on days 0 and 25 (the tumor-free cluster), with minor discernible clusters depending on each day (Fig. 4A). Principal component analysis also revealed that the gene expression profile of each mouse differed depending on the day (Fig. 4B). We identified that 1525 genes probes was the union of 968 and 945 up-regulated genes probes on day 10 and day 15, indicating that 388 genes probes were commonly up-regulated. Despite considerable number of commonly up-regulated genes probes, clustering analysis using 1525 genes probes for gene expression profiles in blood on day 10 and day 15 formed two complete clusters discerning each day, suggesting that gene expression profile of blood was changing during the initiating phases of tumor diminishment. A pathway map analysis was performed on 968 and 945 genes whose expression was up-regulated on days 10 and 15, respectively. Development-related and cytoskeleton-remodeling pathways were the characteristic features of both groups (Supplementary Tables S13 and S14). Pathway maps for cell adhesion—integrin-mediated cell adhesion and migration, CCR4-induced leukocyte adhesion, plasmin signaling, and endothelial cell contacts by non-junctional mechanisms—were the prominent features of up-regulated genes on day 15 (Supplementary Table S14), suggesting enhanced involvement of tissue remodeling. We also analyzed the biological process features of genes differentially expressed between the tumor existence and tumor-free clusters. The number of gene probes whose expression was twofold up-regulated in the tumor existence cluster ($p < 0.05$) was 673, whereas 17 gene probes were down-regulated. The characteristic biological process networks indicated by these 673 gene probes were cytoskeleton, development, cell cycle, apoptosis, and cell adhesion (Supplemen-

Table 2
Networks for genes whose expression was commonly up-regulated in the tumor fraction enriched with tumor-associated inflammatory cells and blood of C57Bl/6 mice on day 10.

Network	*p*-Value	Gene ontology processes
C3g, Il-2r alpha chain, Shh, CD4, Tgf-alpha	9.26×10^{-22}	Positive regulation of cell proliferation, regulation of macromolecule metabolic process, cell proliferation, positive regulation of cellular process, regulation of cell proliferation
Cdk1 (p34), Aggrecan, Sox9, PNPase(old-35), Nyd-sp26	3.85×10^{-17}	Regulation of cell proliferation, generation of neurons, cellular developmental process, anatomical structure morphogenesis, neurogenesis
Nanos2, Nanos homolog 2	0.00143	Germ-line stem cell maintenance, negative regulation of meiosis, regulation of meiosis, regulation of meiotic cell cycle, stem cell maintenance
Wdr59, WD repeat-containing protein 59,	0.00143	Biological_process
Cdk1 (p34), Alk, Racgap1, Fibrinogen beta, Mmd	9.51×10^{-11}	Blood coagulation, coagulation, hemostasis, cytoskeleton organization, wound healing
Mkl2(Mrtf-b), CD4, Dystrophin, Pthr1, Col1a2	9.51×10^{-11}	Positive regulation of protein metabolic process, positive regulation of cellular metabolic process, positive regulation of metabolic process, regulation of cell proliferation, positive regulation of cellular process
Dnah12, Dynein, axonemal, heavy chains	0.00286	Microtubule-based movement, ATP catabolic process, ATP metabolic process, microtubule-based process, purine ribonucleoside triphosphate catabolic process
CD4, Cdk1 (p34), iNOS, Tmem107, Dlx4 (Bp1)	9.63×10^{-9}	Positive regulation of cellular metabolic process, interspecies interaction between organisms, positive regulation of metabolic process, positive regulation of macromolecule metabolic process, positive regulation of developmental process
Cdk1 (p34), Pthr1, Centg2, Bex2, Bex1	1.07×10^{-8}	G-protein signaling, coupled to cAMP nucleotide second messenger, cAMP-mediated signaling, G-protein signaling, coupled to cyclic nucleotide second messenger, cyclic-nucleotide-mediated signaling, regulation of catalytic activity
Fam33a, Mkl2(Mrtf-b), Cdk1 (p34), Tank, Pitx2	1.07×10^{-8}	Positive regulation of macromolecule metabolic process, positive regulation of cellular metabolic process, regulation of cellular metabolic process, cell cycle, positive regulation of metabolic process

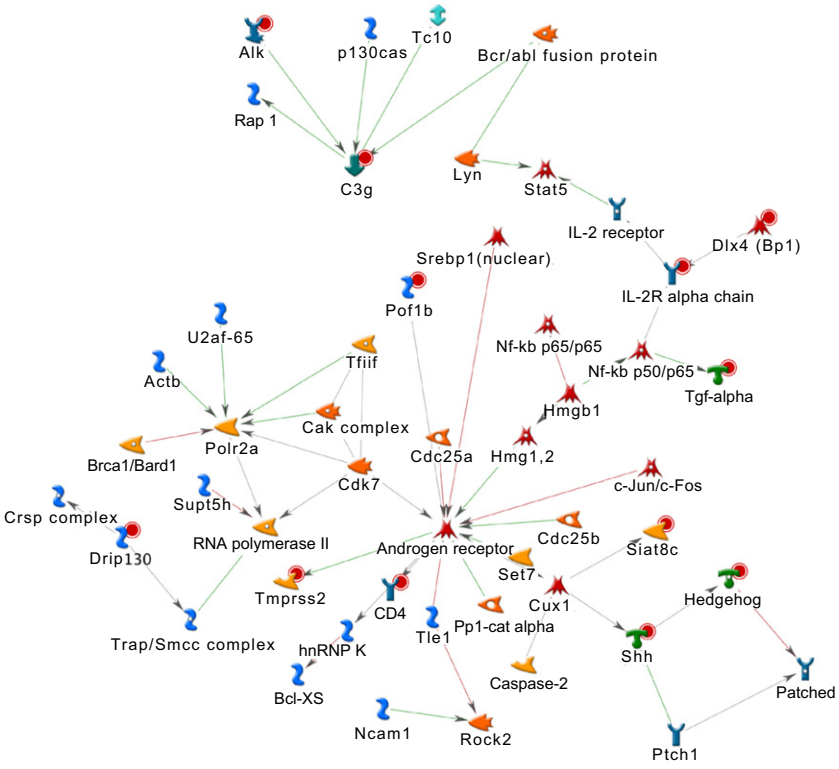

Fig. 3. Network depicting the involvement of C3G, IL-2R alpha chain, SHH, CD4, and TGF-alpha genes. Ten million Hepa1-6 cells were subcutaneously inoculated in C57Bl/6 mice. Blood and tumor tissues were obtained on day 10. Gene expression levels in blood and the fraction enriched with tumor-associated inflammatory cells were assessed by DNA microarray. One-hundred and twenty-seven genes were commonly up-regulated at twofold in blood and the tumor fraction enriched with tumor-associated inflammatory cells, and were related to the gene network that consisted of C3G, IL-2R alpha chain, SHH, CD4, and TGF-alpha plus related genes.

tary Table S15), implying the dynamic role of blood cells in immobilization and cellular kinesis, indicative of tissue remodeling, of tumors associated with the active immune response of the C57Bl/6 mouse.

We also examined whole blood gene expression profiles of BALB/c athymic mice with Hepa1-6 tumors on day 10 compared with that before inoculation of Hepa1-6 cells. Unsupervised clustering analysis of gene expression using 15583 gene probes that passed quality checking showed two clusters, clearly distinguishing between the tumor-existence and tumor-free conditions (Supplementary Fig. S2A). Principal component analysis showed that the gene expression profiles on days 0 and 10 tended to gather depending on the day (Supplementary Fig. S2B). We found that expression of 962 gene probes was up-regulated on day 10 compared with day 0. Pathway map analysis of the 962 gene probes revealed cell kinetics involving transcription, the cell cycle, and cell

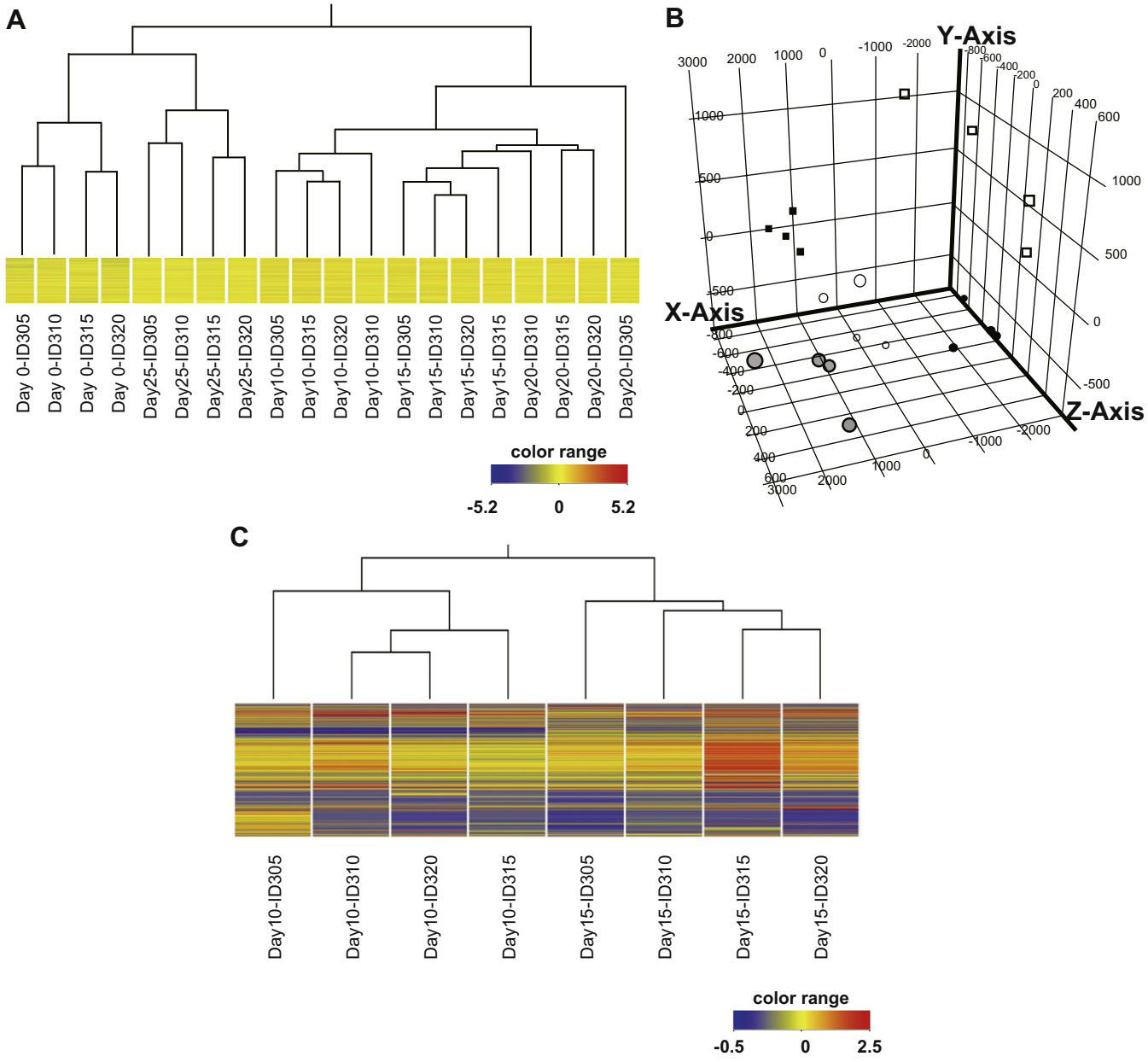

Fig. 4. Hierarchical clustering and principal component analysis of gene expression levels in blood obtained from C57Bl/6 mice inoculated subcutaneously with Hepa1-6 cells. RNA was extracted from the blood of C57Bl/6 mice inoculated with Hepa1-6 cells on days 0, 10, 15, 20, and 25, followed by gene expression analysis by DNA microarray. (A) Unsupervised hierarchical clustering and (B) principal component analysis of gene expression levels on day 0, 10, 15, 20, and 25 using all gene probes that passed a quality check. Open rectangle, day 0; filled rectangle, day 25; filled circle, day 10; gray circle, day 15; open circle, day 20. (C) Unsupervised hierarchical clustering of gene expression levels on day 10 and day 15 using the 1525 union genes probes up-regulated on day 10 and day 15.

adhesion (Supplementary Table S16), suggesting a relationship between blood cells and locally proliferating tumor cells that were inoculated subcutaneously. Thus, the gene expression profiles of whole blood cells in mice with tumors were altered depending on the local tumor conditions.

4. Discussion

The subcutaneously inoculated Hepa1-6 cells in C57Bl/6 mice demonstrated the unique course of tumor development/diminishment. Eradication of tumors was mediated by the T-cell immune response, particularly CD4+ T cells. Interestingly, genes up-regulated in both whole blood and the tumor fraction enriched with tumor-associated inflammatory cells on day 10 indicated a biological network involving CD4+ T cells, suggesting that gene expression alteration of whole blood cells was associated in part with the local inflammatory response to tumor tissues. Additionally, the gene expression profiles of whole blood cells in mice with Hepa1-6 tumors were associated with the tumor condition, and their biological features during tumor existence were suggestive of tissue remodeling-related processes.

The genes commonly affected in both blood cells and the tumor fraction enriched with tumor-associated inflammatory cells in C57Bl/6 mice suggested a biological network involving CD4+ T cells. Thus, CD4+ T cells may contribute to the altered blood gene expression profile on day 10. However, the blood gene expression profile of athymic mice with Hepa1-6 tumors was discernible from that of tumor-free mice, suggesting the involvement of other immune-mediating cells in the altered blood gene expression profile.

The gene expression profiles of whole blood cells of C57Bl/6 mice with tumors indicated biological processes related to tissue remodeling, such as development, cell adhesion, and the cytoskeleton. A fraction of circulating blood cells are involved in tissue remodeling/repair, such as monocytes/macrophages [17–19]. Thus, some blood cells likely contributed to these biological features as a consequence of tumor formation. The mechanism(s) underlying the association between blood gene expression profile and tumor condition should be determined.

We reported previously that features of the gene expression profile of peripheral blood mononuclear cells of HCC patients are shared with local inflammatory cells of HCC tissues [16]. In that analysis, the shared biological features were characterized by the local immune responses of the host to tumor tissues, tumor microenvironment—such as antigen presentation, response to hypoxia, and oxidative stress—ubiquitin-proteasomal proteolysis, mRNA processing, and the cell cycle. Peripheral blood mononuclear cells are devoid of cell types such as polymorphonuclear cells; therefore, the collected peripheral blood mononuclear cell populations rather contain inflammatory cells that are the major players in cancer immunity: the antigen-presenting cell population (monocytes/macrophages and dendritic cells), and the lymphocyte population (T and B cells) [20]. In the current study, gene expression of peripheral blood involved all cell populations therein. However, the genes up-regulated in both blood and the tumor fraction enriched with tumor-associated inflammatory cells suggested the importance of local tumor-associated CD4+ T cells, which played an important role in the C57Bl/6 mouse Hepa1-6 tumor model. Blood gene expression profiles of C57Bl/6 and athymic mice with Hepa1-6 tumors also depended on the presence and/or condition of a tumor. However, Gr-1+ cells were the major local inflammatory cells in the Hepa1-6 tumors of athymic mice. Because the network of genes up-regulated in blood and tumor-associated inflammatory cells in the athymic mouse model involved cellular kinetics and development, not directly suggestive of Gr-1+ cells, the role of local Gr-1+ cells in the alteration of blood gene expression profiles is unclear. Further studies should determine how immune-mediating cells in both blood and local tumor-infiltrating inflammatory cell populations alter blood gene expression depending on tumor condition.

The immune reaction of the host to cancer is complex: natural killer cells are well-characterized anti-cancer immune cells [21], and the innate immune system is represented by monocyte/macrophages [22]. In terms of acquired immunity, Th1 cells and cytotoxic T cells are known to be important due to the presence of antigen-presenting dendritic cells [23]. In contrast, the contradictory immune response is present concomitantly; this involves, for instance, regulatory T cells [8,9]. When anti-cancer immune cells predominate, cancer tissues will diminish. Conversely, when immune cells that work as suppressors of anti-cancer immunity are the major effectors, the tumor tissue will continue to grow. Thus, qualitative elucidation of host immune status is extremely important for assessing both prognosis and therapeutic efficacy. Blood gene expression analysis has been investigated extensively and has been shown to be useful in terms of diagnosis, prediction of a therapeutic effect, or prognosis in, for example, renal cell carcinoma [24], breast cancer [25], and digestive disease cancers [26]. Our data suggest that the blood gene expression profile of a murine cancer model was associated with the local tumor condition. The utility of blood cell gene expression profiling for further elucidation of the overall immune condition in terms of the presence of immune-mediating cell types should be investigated.

In conclusion, we observed that gene expression features of peripheral blood cells were altered depending on tumor condition. Additionally, biological features associated with CD4+ T cells, which play a pivotal role in Hepa1-6 C57Bl/6 tumor models, were implicated in the gene expression profiles common to both blood and tumor-infiltrating inflammatory cells. Further studies are needed to understand the systemic effect of cancer on blood gene expression profiles in other cancer-related conditions —*e.g.*, recurrent cancer or vaccination—for development of both novel diagnostic tools and effective treatments.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.10.004.

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