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Biochemical and Biophysical Research Communications 331 (2005) 194–202
BBRC
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Expression profiles of mouse dendritic cell sarcoma are similar to those of hematopoietic stem cells or progenitors by clustering and principal component analyses

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Received 15 March 2005
Available online 30 March 2005

Abstract

We isolated and screened two tumor cell clones DD1 and DG6 with different capacity of metastasis from the same parent cell line, a mouse dendritic cell (DC) sarcoma, using limited dilution method. The genome-wide expressions of DD1 and DG6 cells were detected by Affymetrix's MOE-430A microarray. The expression profiles related with mouse DC development were downloaded from GEO at NCBI and ArrayExpress at EBI database. In order to compare the expression of DC sarcoma and DC developmental arrays which was performed by MG-U74av2, we had screened the best matched probesets between MOE-430A and MG-U74av2 according to the probe identities from Affymetrix technical annotation. After the normalization of 11 housekeeping genes across the 34 arrays (2 DC sarcoma and 32 DC developmental arrays), all these expression profiles were analyzed by the methods of hierarchical clustering, principal component analysis, nearest-neighborhood, and self-organizing maps. The results indicate that expression profiles of DC sarcoma are closer to those of the DC progenitors and hematopoietic stem cells from bone marrow compared with the sorted DCs from spleen. The results support the hypothesis that cancers (tumors or sarcomas) arise from stem cells. It is suggested that the DC sarcomas are more similar to the DC progenitors and hematopoietic stem cells than the relative mature DCs in gene expressions on the large-scale.

Keywords: Mouse dendritic cell sarcoma; Gene expression profiles; Hematopoietic stem cells/progenitors; Clustering and principal component analyses

We succeeded in isolating and identifying the highly and poorly metastatic subclones (named DD1 and DG6) from mouse dendritic cell (DC) sarcoma cell line. Both the DD1 and DG6 cells had the capability of undergoing tumorigenesis, however they showed different metastatic capacity to lung [1,2]. The two cells were very similar in the gene expression profiles which were detected by MOE-430A of Affymetrix microarrays. We tried to determine the expression profiles of DC sarcoma closer to which stages in the DC developments. Meanwhile, the compared results also are of benefit to identify DC sarcoma that we isolated to see whether it is similar to the known DCs in gene expression profiles.

Dendritic cells (DCs) that are highly specialized in antigen presentation play a pivotal role in immune response. Generally, the DCs are regarded as being derived from hematopoietic stem cells (HSCs) from bone marrow, via the stages of DC progenitors, DC precursors, and become

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0006-291X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2005.03.131

the terminal differentiated cells both in lymphoid and nonlymphoid tissues [3–5]. Some expression profiles that associate with DC development can be downloaded from public biological databases which have collected hundreds of microarray data for online browsing, query, and retrieval, such as Gene Expression Omnibus (GEO) at NCBI [6] ArrayExpress at EBI [7].

Here, the downloaded expression profiles of DC developmental samples have all used Affymetrix platform mouse U74Av2. This type of array is composed of 16–20 probe pairs (perfect match and mismatch) that each interrogates a different part of the sequence of a gene, making up what is known as a probeset. Some recent GeneChips of Affymetrix, such as mouse 430 and human U133 arrays, have used as few as 11 probes in a probeset [8]. The intensity values from each of the probes in a probeset are combined together to get an expression measure. Although most of the microarray databases do not provide the original values of each of probes, the global scaling of each array including DC sarcoma and developmental cells is applied to Avg Difference intensity values [9], which is the default setting of Affymetrix GeneChips. It is an advantage for comparing the expression results of the same or similar arrays that were used by Affymetrix platform.

In order to compare the expression profiles of DC sarcoma with those of DC developmental cells, two steps should be processed to the experimental (DC sarcomas) and downloaded expression data (DC developments): one is to establish the matched probesets between mouse U74Av2 and 430A which have 16 and 11 probesets, respectively; the other is data normalization across all

Table 1
The mouse DC sarcoma and developmental cells for expression profiling analyses

Cell groups	Names	Sources	Descriptions
Dendritic cell sarcoma	DD1	Peking Union	Subclone, highly metastatic to lung
	DG6	Medical College	Subclone, poorly metastatic to lung
		Yuqin Liu et al.	
Dendritic cells from spleen	GSM4697	National Institute of	Sorted CD11c high, $CD4^+$ population (exp 1)
	GSM4707	Allergy and	Sorted CD11c high, $CD4^+$ population (exp 2)
	GSM4708	Infectious	Sorted CD11c high, $CD8^+$ population (exp 1)
	GSM4709	Diseases (NIAID),	Sorted CD11c high, $CD8^+$ population (exp 2)
	GSM4710	Edwards et al. [14]	Sorted CD11c high, $CD8^-$, $CD4^-$ population (exp 1)
	GSM4711		Sorted CD11c high, $CD8^-$, $CD4^-$ population (exp 2)
Dendritic cells from spleen cultivated for 2 h	GSM4757		Sorted CD11c high, $CD4^+$ population, cultivated for 2 h (exp 1)
	GSM4758		Sorted CD11c high, $CD4^+$ population, cultivated for 2 h (exp 2)
	GSM4772		Sorted CD11c high, $CD8^+$ population, cultivated for 2 h (exp 1)
	GSM4773		Sorted CD11c high, $CD8^+$ population, cultivated for 2 h (exp 2)
	GSM4774		Sorted CD11c high $CD8^-$, $CD4^-$ population, cultivated for 2 h (exp 1)
	GSM4775		Sorted CD11c high $CD8^-$, $CD4^-$, cultivated for 2 h (exp 2)
Dendritic cell progenitors from bone marrow	GSM10877	Max Delbrueck	$FLT3^+/CD11b^+$ dendritic cell progenitor (sample 1)
	GSM10878	Center (MDC) for	$FLT3^+/CD11b^+$ dendritic cell progenitor (sample 2)
	GSM10879	Molecular Medicine,	$FLT3^+/CD11b^+$ dendritic cell progenitor (sample 3)
	GSM10880	Hacker et al. [4]	$FLT3^+/CD11b^+$ dendritic cell progenitor (sample 4)
	GSM10881		$FLT3^+/CD11b^+$ dendritic cell progenitor (sample 5)
Dendritic cell precursors from bone marrow	GSM8635	Lineberger Cancer	Wild-type B6, day 10 dendritic cells (expt 2)
	GSM8636	Center, Wong	CIITA −/−, day 10 dendritic cells (expt 2)
	GSM8637	et al. [15]	IAB −/−, day 10 dendritic cells (expt 2)
	GSM8638		Wild-type B6, day 10 dendritic cells (expt 3)
	GSM8639		CIITA −/−, day 10 dendritic cells (expt 3)
	GSM8640		IAB −/−, day 10 dendritic cells (expt 3)
Hematopoietic progenitor cell line	MHH0083	GBF, Department	0 h control (two samples for an array)
	MHH0084	of Pediatric	6 h control (two samples for an array)
	MHH0085	Hematology/	6 h GM-CSF (5 ng/ml) (two samples for an array)
	MHH0086	Oncology, Rathinam	24 h control (two samples for an array)
	MHH0087	et al. [16]	24 h GM-CSF (5 ng/ml) (two samples for an array)
	MHH0088		48 h control (two samples for an array)
	MHH0089		48 h GM-CSF (5 ng/ml) (two samples for an array)
Hematopoietic stem cells		MDC for Molecular	Sorted hematopoietic stem cells from bone marrow
	GSM10882	Medicine [4]	Lin-c-kit+Sca1+ hematopoietic stem cells (sample 1)
	GSM10883		Lin-c-kit+Sca1+ hematopoietic stem cells (sample 2)

(CIITA−/−) deficient of the major histocompatibility complex (MHC) class II transactivator.
(IAB−/−) deficient of the major histocompatibility complex (MHC).
(GM-CSF) granulocyte–macrophage colony-stimulating factor.

the samples (arrays) [10]. We screened the best matched probesets between the MOE-430A and MG-U74av2 arrays from Affymetrix's annotation. The identity percent of the two target sequences was over 96%. Meanwhile, as many as 11 housekeeping genes were used to normalize each array because of their stable and high expressions across these arrays [11]. After selecting the best matched probesets between two types of arrays and housekeeping gene normalization, mouse DC sarcoma can be compared with the DC developmental cells downloaded from GEO and ArrayExpress databases, and can be further processed by clustering and principal component analyses.

Materials and methods

The isolation and screening of highly and poorly metastatic DCs. We cultured DC sarcoma cells (collection of tumor cell lines from Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences [1,2]) in RPMI-1640 medium with 10% fetal bovine serum and 2 mM penicillin–streptomycin. These cells were incubated in mid-log phase. The collection of these cells was stained with trypan blue, and the survival rate of live cells was more than 95%. These cells were diluted to the concentration of 10 cells/ml by series limited dilution. The cell suspension was subsequently added to the 96-well plates (0.1 ml for each well). After 2 h of cell adhesions, the wells with single cell were marked under invert microscope. These wells were marked repeatedly for five consecutive days to make sure that single cell clones were selected. The cell clones with obviously different growth rates were chosen and transferred to the 6-well plates for cell amplification. Screened by the tests of cell migration and proteolysis in vitro, we established two subclones, the typical highly metastatic DD1 and poorly metastatic DG6.

Genome-wide expression profiles of DD1 and DG6. DD1 (highly metastatic) and DG6 (poorly metastatic) were cultured and high quality RNAs were extracted with RNeasy Mini Kit (Qiagen, Valencia, CA) from DD1 and DG6 cells in flasks. Then we processed and hybridized the total RNA to the MOE-430A oligonucleotide microarray (Affymetrix, Santa Clara, CA), which contained 22,690 gene probeset (see manufacturer's manual for detailed protocol). The expression profiles of DD1 and DG6 were generated from the scanning signals by Affymetrix GeneChip Operating Software version 1.0 (GCOS 1.0) with default processing.

Microarray data download and preprocessing. Altogether 32 expression profiles of mouse dendritic cells, DC precursors, progenitor cells, and hematopoietic stem cells were downloaded from Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo/) and ArrayExpress database (http://www.ebi.ac.uk/arrayexpress/). These data included dendritic cells which were sorted by CD11c (high +), CD4(+/−) or CD8(+/−) population from spleen (GSM4697–GSM4711) and the same sorted cells were cultivated for

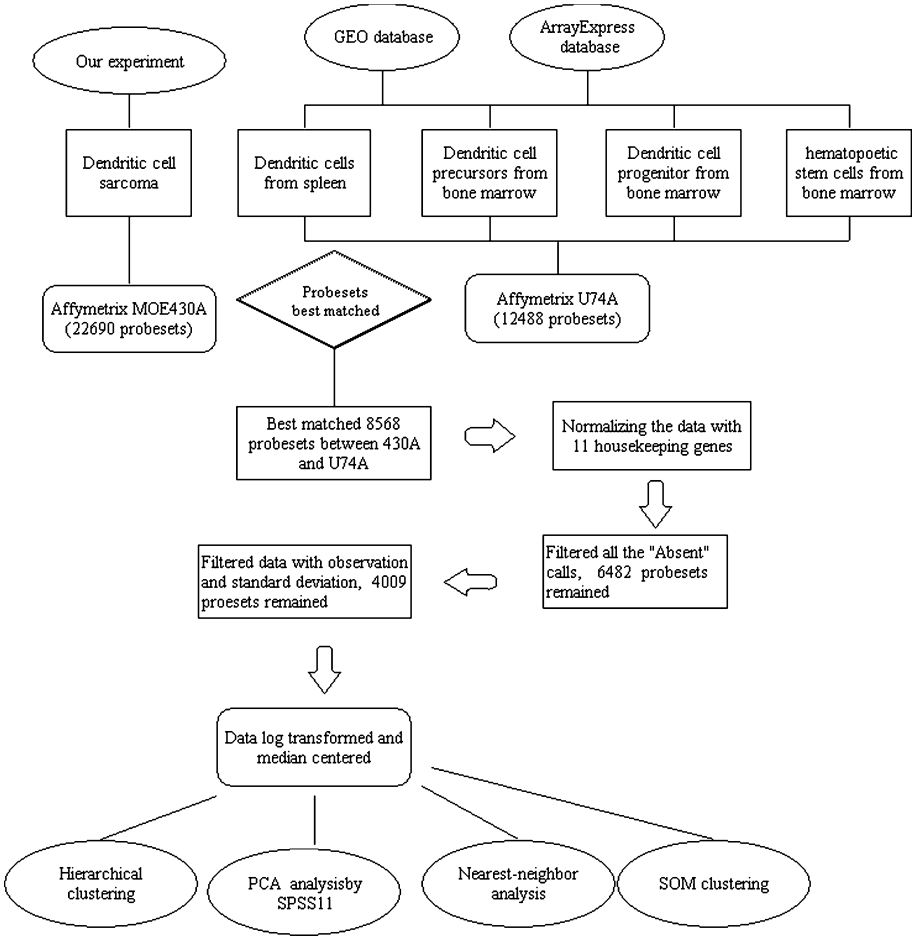

Fig. 1. Flowchart of data preprocessing and analyses on the expression of DC sarcoma and DC developmental cells.

2 h (GSM4757–GSM4775), dendritic cell precursors from bone marrow (GSM8435–GSM8440), dendritic cell progenitors from bone marrow (GSM10877–GSM10881), hematopoietic stem cells from bone marrow (GSM10882–GSM10883), and hematopoietic progenitor cell line (MHH0083–MHH0089) (Table 1). All the downloaded data were performed with the platform of mouse Affymetrix's MG-U74av2 microarray which had 12,488 probesets and each probeset had 16 probe pairs. By removing the not properly matched probesets between mouse MOE-430A and MG-U74av2 microarrays, the identity percent of the two matched probesets was very high (over 96%). Furthermore, we eliminated the probesets (genes) with all "Absent Call" across the arrays and truncated each negative value to 0.01.

Normalization and data filtering. The matched expression values of two dendritic cell sarcoma and 32 downloaded arrays that were associated with DC developments were normalized by the average values of 11 housekeeping genes across the arrays [9]. These housekeeping genes included: mouse β-actin (probeset ID: AFFX-b-Actin-Mur/M12481_M_at, M12481_5_at, M12481_3_at), GAPDH (AFFX-GapdhMur/M32599_M_at, M32599_5_at, M32599_3_at), β glucuronidase (97538_at), β-2 microglobulin (93088_at), hypoxanthine guanine phosphoribosyl transferase 1 (Hprt1, 160107_at), phosphoglycerate kinase 1 (93346_at), ribosomal protein L14 (99653_at), transferrin receptor (AFFX-TransRecMur/X57349_3_at), adenosine

Table 2
The extracted principal components and cumulative percent of variances

Principal components	Initial eigenvalues		
	Total values	Percent of variance (%)	Cumulative percent (%)
1	9.515	27.986	27.986
2	5.505	16.191	44.176
3	4.078	11.995	56.172
4	2.625	7.721	63.893
5	2.017	5.932	69.825
6	1.746	5.137	74.962
7	1.306	3.841	78.803

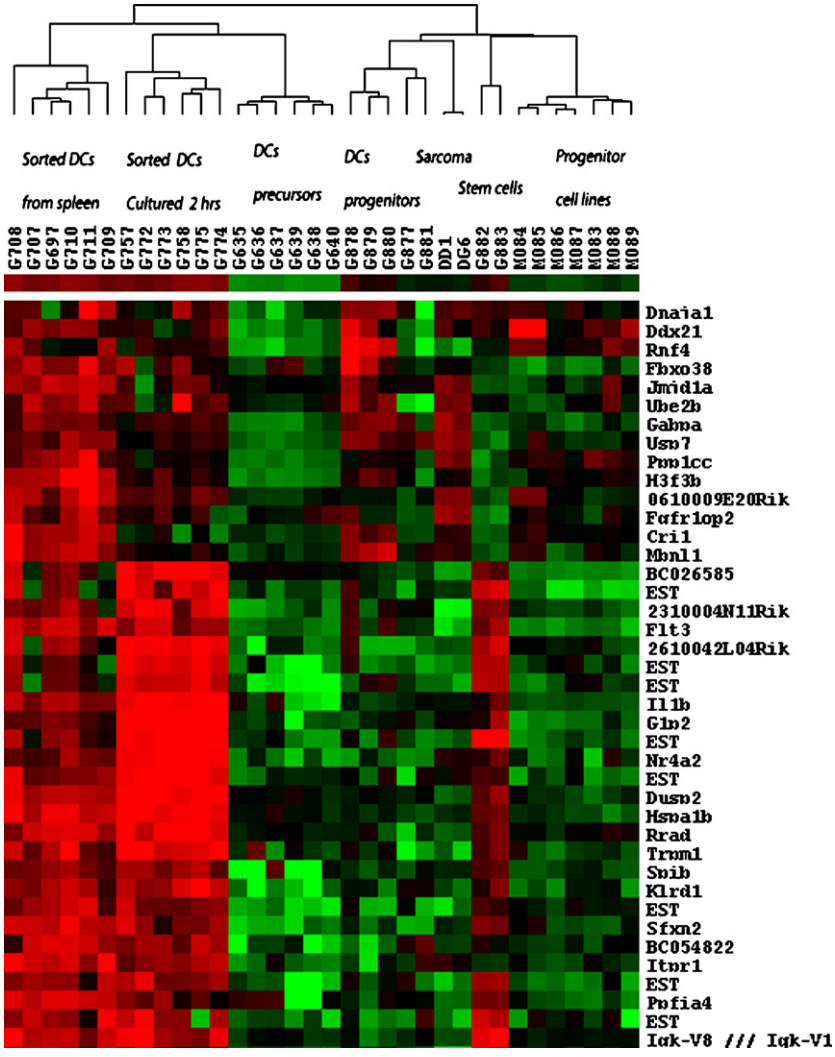

Fig. 2. Unsupervised hierarchical clustering analysis of DC sarcoma and DC developmental cells (partial graph). The gene-expression values are represented by using a red-green color scheme, with the red corresponding to higher than median expression values, the black corresponding to equal to median, and the green corresponding to lower than median expression values. The array names in the top of the figure are combined with the first letter and the last three numbers. The gene symbol names are listed in the right of the figure. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this paper.)

deaminase (98632_at), ATP synthase 5c1(92800_i_at), and 18S ribosomal RNA (AFFX-18SRNAMur/X00686_3_at) genes.

We filtered the probesets whose normalized value SDs (standard deviations) as well as maximum minus minimum values were less than 2.0. We also removed the probesets without any value that were normalized by housekeeping genes greater than 2.0 in these arrays. All these values were transformed to logarithm (base 2).

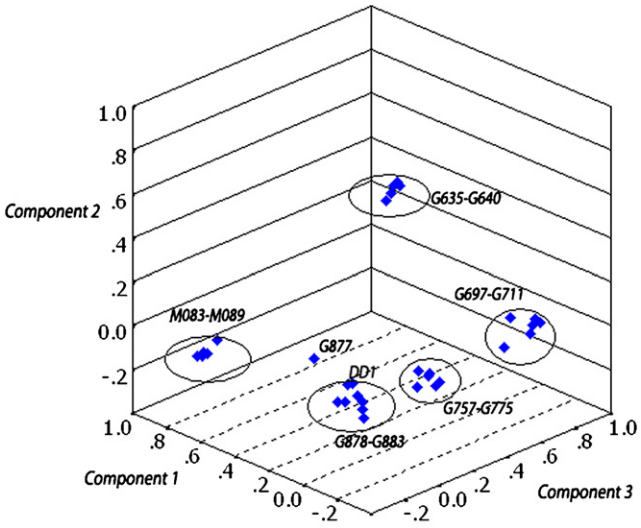

Fig. 3. The principal component analysis on DC sarcoma and DC developmental arrays. From the 3D graph, these DC-associated cells can be divided into five groups obviously. The arrays of DC sarcoma are clustered to those of DC progenitors and HSCs. The names of array in the figure are abbreviated with the first letter and the last three numbers.

Clustering, principal component, and nearest-neighborhood analyses. Unsupervised hierarchical clustering analysis was carried out by Cluster3.0 [10, updated by Michiel de Hoon] and Treeview software [12] using median-centered Pearson correlation and complete linkage. The principal component analysis (PCA) was performed by SPSS11 program "factor analysis" module. Clustering of self-organizing maps (SOM) was performed by GeneCluster2.0 package and 12 class (4×3) SOMs were constructed. The nearest-neighborhood analysis was also performed by GeneCluster2.0 package [13] to obtain the nearest-neighboring scores (nodes). For the data preprocessing and analyses on the expression of DC sarcoma and DC developmental cells please see the flowchart (Fig. 1).

Results and discussion

Unsupervised hierarchical clustering on DC sarcoma and DC developmental cells

From Fig. 2, it can be found that two arrays of DC sarcoma and 32 arrays associated with DC developments are classified into seven classes, which are the sorted DCs from spleen, DCs cultivated for 2 h from spleen, DC precursors from bone marrow, DC progenitors from bone marrow, DC sarcoma, HSCs, and hematopoietic progenitor cell line. The former three classes and the latter four classes formed two main groups. The arrays of DC sarcoma are clustered to those of DC progenitors and HSCs. The array names are abbreviated by the first letter and last three figures. For an example, G708 and M089 stand for GSM4708 and MHH0089 arrays, respectively.

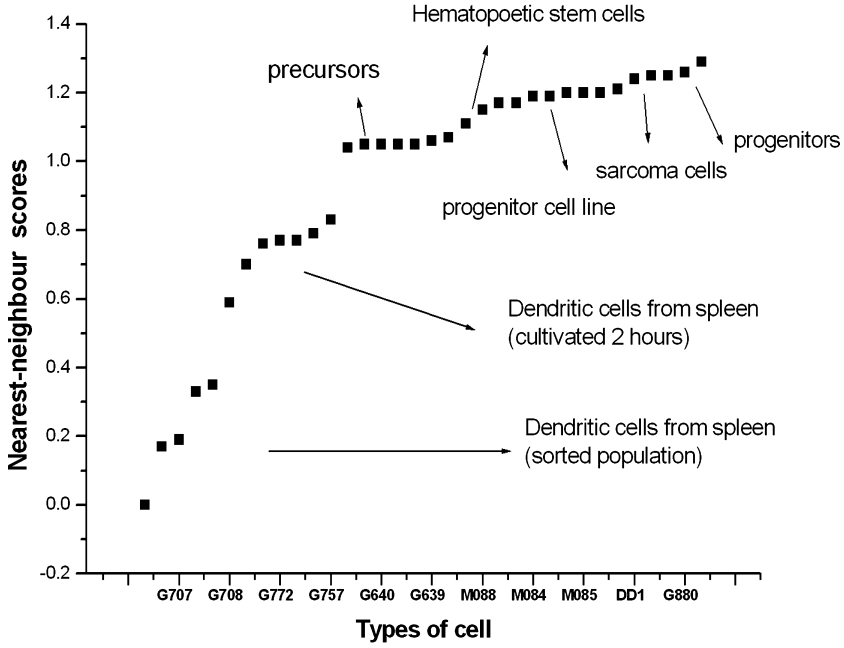

Fig. 4. The scatter plot of nearest-neighborhood analysis on DC sarcoma and DC developmental arrays. These arrays can be separated into three main parts if taking GSM4697 (one experiment of sorted DCs from spleen) as the baseline. The arrays (spots) attribute to the DC populations or HSCs that the arrows indicate.

Principal component analysis on DC sarcoma and DC developmental cells

There are seven extracted principal components whose eigenvalues are over 1.0 from 34 arrays. These components and the cumulative percent of variances are listed in Table 2.

The first three components whose eigenvalues are over 4.0 are used to make scatter plot in three-dimension. The cumulative percent of variances of these components is greater than 56%. From the 3D plot (Fig. 3) of principal component analysis (PCA), five main groups are formed obviously across the 34 arrays except GSM10877 (an array for DC progenitors from bone marrow). The expression profiles of DC sarcoma are closer to those of DC progenitors and HSCs from bone marrow (GSM10878–GSM10883). These eight arrays are centered to one group. The arrays of DC sarcoma do not cluster to the hematopoietic progenitor cell lines (MHH0083–MHH0089). The other four groups are composed of DCs from spleen (GSM4697–GSM4711), DCs cultivated for 2 h from spleen (GSM4757–GSM4775), DC precursors from bone marrow (GSM8635–GSM8640), and hematopoietic progenitor cell line (MHH0083–MHH0089).

PCA is used commonly for reducing the dimensionality of complex data and is useful as a visualization technique [17,18]. The first principal component captures more variation than the second, the second than the third, and so on. The principal component can be

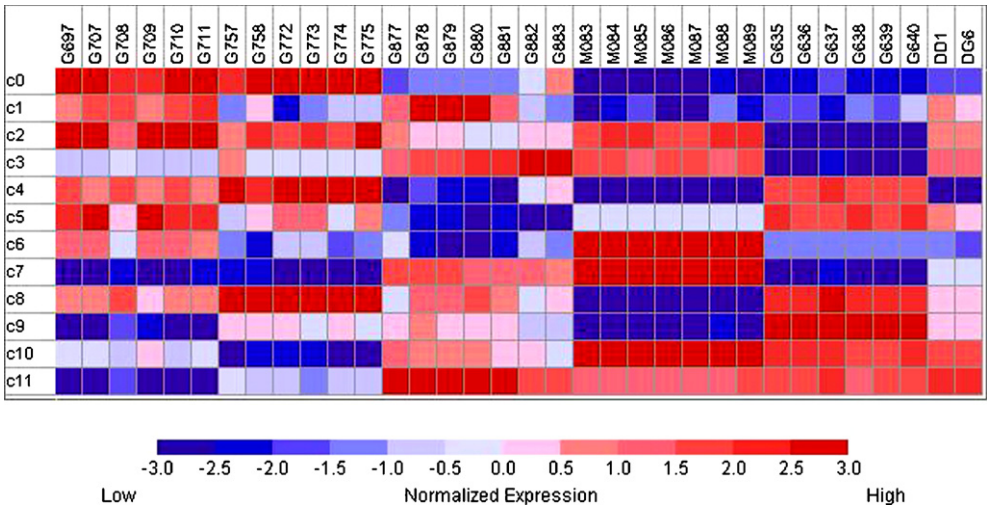

Fig. 5. SOM clustering of (4 × 3) classes on 34 arrays. The red color represents the higher values of gene expression and the blue represents for lower values. The colors at bottom of the figure represent the relative expression levels. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this paper.)

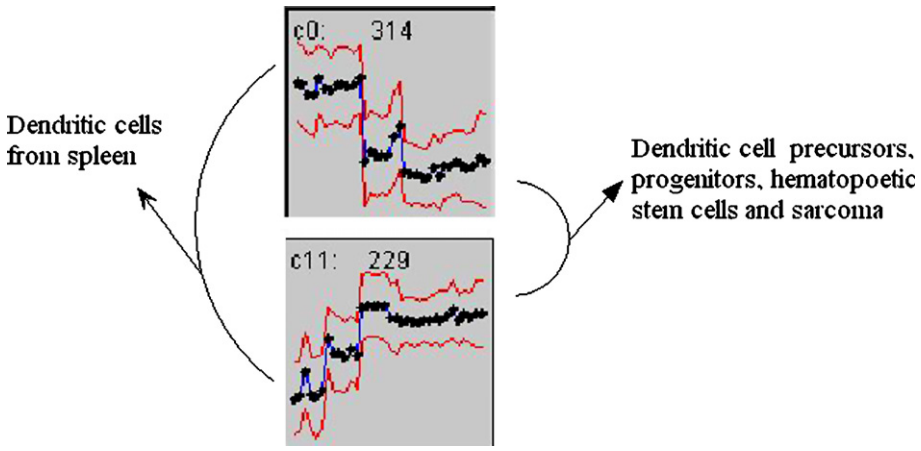

Fig. 6. The expression patterns in C_0 and C_{11} that are generated by SOM clustering. The arrays are in the same order as in Fig. 5. From the left to right, the black points represent sorted DCs from spleen, sorted DCs that cultivated for 2 h from spleen, DC progenitors, HSCs, DC precursors, and DC sarcomas.

considered as a combined biological sample and represents the maximum variation in gene expression [11,15]. We use this method to view the expression profiles of two DC sarcoma arrays closer to which stage in the DC developmental arrays. The results show that there are common characteristic features in expression profiles of DC sarcoma and HSCs/DC progenitors.

Nearest-neighborhood analysis on DC sarcoma and DC developmental cells

The nearest-neighbor method can be used in an unsupervised manner as well as a supervised one [13,17]. This technique is to find individual arrays that are most similar to the hypothesis sample (baseline sample). The other samples (arrays) can be compared to this hypothesis sample and ranked by their similarity in expression profiles.

To take GSM4697 (one experiment of sorted DCs from spleen) as the baseline, the other 33 arrays are compared with this array and the nearest-neighbor scores are calculated with the Pearson distance of neighbor analysis from GeneCluster 2.0 program [11]. From the scatter plot (Fig. 4), the arrays can be separated into three parts. Part 1 includes the sorted DCs from spleen (GSM4697–GSM4711). Part 2 includes the sorted DCs that cultivated for 2 h from spleen (GSM4757–GSM4775). The other arrays formed Part 3 which included the DC precursors, DC progenitors, HSCs, and DC sarcomas. The nearest-neighbor scores of DC sarcoma are closer to those of DC progenitors and HSCs. The results indicate that the expression profiles of DC sarcoma are closer to those of the DC progenitors or HSCs compared with the relative mature sorted DCs.

Clustering of self-organizing maps on DC sarcoma and DC developmental cells

Altogether 4009 probesets with log transformation and centered by medians were further analyzed by clustering of self-organizing maps (SOMs). This process was performed by GeneCluster2.0 package and constructed 12 (4×3) classes (Fig. 5).

SOM is a clustering algorithm where a grid of two-dimensional nodes (clusters) is iteratively adjusted to reflect the global structure in the expression dataset [5]. The DC developmental process in gene expression is largely controlled at the transcriptional level as well as in DC sarcomas. Which process of DC development is more similar to DC sarcoma can indicate the common gene expression between them. The SOM can extract biologically meaningful groups of genes and provide a survey of expression patterns.

From C_0 to C_{11} 12 classes, we find that two classes (C_0 and C_{11}) show the special features across the 34 arrays. The 314 genes in class C_0 are over-expressed in the

Table 3
The top 30 genes in C_0 and C_{11} classes by SOM clustering

Probesets	Distances	Genes or ESTs
C_0		
1421134_at	0.6949	Areg
1460671_at	0.6807	Gpx1
1421529_a_at	0.6321	Txnrd1
1416144_a_at	0.6108	Dhx15
1426721_s_at	0.6087	Tiparp
1451264_at	0.6048	4930488L10Rik
1431359_a_at	0.6014	1110007C09Rik
1422932_a_at	0.5858	Vav1
1448050_s_at	0.5637	Map4k4
1426334_a_at	0.5492	Bcl2l11
1415692_s_at	0.5450	Canx
1417714_x_at	0.5404	Hba-a1
1450295_s_at	0.5402	D7Ertd458e
1416868_at	0.5188	Cdkn2c
1448267_at	0.5140	Stx5a
1451714_a_at	0.5065	Map2k3
1460590_s_at	0.5051	Ywhaq
1449818_at	0.5017	Abcb4
1425462_at	0.4927	Fbxw11
1415675_at	0.4896	EST
1423678_at	0.4815	BC017643
1449119_at	0.4780	Arih2
1425052_at	0.4699	2610034N03Rik
1423721_at	0.4672	Tpm1
1448672_a_at	0.4648	Zfp289
1434133_s_at	0.4601	D1Ucla4
1442745_x_at	0.4546	C79248
1454887_at	0.4521	Pak2
1460394_a_at	0.4405	Inppl1
1435458_at	0.4398	Pim1
C_{11}		
1417607_at	0.5046	Cox6a2
1450062_a_at	0.4992	Maged1
1417771_a_at	0.4986	Psmc6
1426875_s_at	0.4771	Npn3
1426459_s_at	0.4744	AW549877
1418674_at	0.4686	Osmr
1425565_at	0.4679	Rest
1417566_at	0.4671	Abhd5
1428249_at	0.4634	1190004M21Rik
1434392_at	0.4565	Usp34
1426529_a_at	0.4554	Tagln2
1460357_at	0.4537	Ythdf2
1418840_at	0.4531	Pdcd4
1434935_at	0.4477	D6Ertd245e
1448700_at	0.4404	G0s2
1434403_at	0.4383	Spred2
1433618_at	0.4367	C330006A16Rik
1419914_s_at	0.4354	EST
1416156_at	0.4330	EST
1420808_at	0.4320	Ncoa4
1417013_at	0.4316	Hspb8
1435800_a_at	0.4313	Csda
1416740_at	0.4306	Col5a1
1422931_at	0.4223	Fosl2
1418172_at	0.4204	Hebp1
1455090_at	0.4149	EST
1448819_at	0.4085	Eif2s2
1418532_at	0.4076	Fzd2
1454610_at	0.4072	7-Sep
1418666_at	0.4068	Ptx3

sorted DCs from spleen and down-regulated in DC progenitors, HSCs, DC precursors, and DC sarcomas except for GSM10883 (a hematopoietic stem cell). On the contrary, 229 genes in class C_{11} are up-regulated in the latter cells and down-regulated in the former cells (Fig. 6). The results show that expression patterns of the relatively mature DCs (sorted DCs from spleen) are significantly different from their progenitors, HSCs or sarcomas. The top 30 genes that have large distances in two classes are listed in Table 3. These genes may be regarded as the candidate genes that associate with the immature DCs and the DC sarcomas.

DCs are assumed to originate from HSCs or hematopoietic progenitor or precursor cells from bone marrow. There are several DC subsets which have been identified and these subsets are found to differ in phenotype, activation state, and location [19,20]. From the results of hierarchical clustering, PCA, nearest-neighborhood, and SOM analyses, the expressions of DC sarcoma are closer to those of the DC progenitors and HSCs from bone marrow compared with the sorted DCs from spleen. The results support the hypothesis that cancers (tumors or sarcomas) arise from stem cells or cancer is a problem of developmental biology [17]. Stem cells and cancer cells share some characters including self-renewal and proliferation. The similar expressions in DC sarcomas and DC progenitors or HSCs indicate that there are common genes which may co-express in some related biological process. These common genes may be regarded as the candidate genes that associate with the early DC developmental cells as well as sarcoma cells.

Conclusions

By screening the best matched probesets between two types of Affymetrix arrays and normalizing with 11 housekeeping genes, the expression profiles of DC sarcoma can be compared with those of the DC developmental arrays. The results of hierarchical clustering, PCA, nearest-neighborhood, and SOM analyses suggest that the DC sarcomas are more similar to the DC progenitors and hematopoietic stem cells than the relative mature DCs in gene expressions on the whole. The method that we presented here is also useful for picking up the candidate genes co-expressed in DC sarcoma and DC progenitors or hematopoietic stem cells.

Acknowledgments

The authors thank Dr. Yun Bai and Qikuan Hu for their helping in RNA extraction from DC sarcomas and the experiment with Affymetrix microarrays. This work was supported by grants from Chinese National Natural Science Foundation (No. 60121101), National 863 project (No. 2002AA2Z2041), and National 973 project (No. 2002CB513100-2).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2005.03.131.

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