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Gene Expression Profiles of Cutaneous B Cell Lymphoma

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We studied gene expression profiles of 17 cutaneous B cell lymphomas that were collected with 4–6 mm skin punch biopsies. We also included tissue from two cases of mycosis fungoides, three normal skin biopsies, and three tonsils to create a framework for further interpretation. A hierarchical cluster algorithm was applied for data analysis. Our results indicate that small amounts of skin tissue can be used successfully to perform microarray analysis and result in distinct gene expression patterns. Duplicate specimens clustered together demonstrating a reproducible technique. Within the cutaneous B cell lymphoma specimens two specific B cell differentiation stage signatures of germinal center B cells and plasma cells could be identified. Primary cutaneous follicular and primary cutaneous diffuse large B cell lymphomas had a germinal center B cell signature, whereas a subset of marginal zone lymphomas demonstrated a plasma cell signature. Primary and secondary follicular B cell lymphoma of the skin were closely related, despite previously reported genetic and phenotypic differences. In contrast primary and secondary cutaneous diffuse large B cell lymphoma were less related to each other. This pilot study allows a first glance into the complex and unique microenvironment of B cell lymphomas of the skin and provides a basis for future studies, which may lead to the identification of potential histologic and prognostic markers as well as therapeutic targets.* ***Key words: cutaneous B cell lymphoma/diffuse large B cell lymphoma/follicular lymphoma/marginal zone lymphoma/microarray. J Invest Dermatol 120:865–870, 2003***

Cutaneous B cell lymphomas comprise a heterogeneous spectrum of disease (Burg *et al*, 1985). Several studies have shown that primary cutaneous lymphomas behave differently from nodal-based lymphomas that involve the skin secondarily (Willemze *et al*, 1997; Willemze, 2000). Although the two entities share similar histologic and immunophenotypic features, as a rule primary cutaneous B cell lymphomas carries a much better prognosis than its nodal counterpart and therefore usually requires less aggressive therapy (Santucci *et al*, 1991).

At present at least two classification schemes are in use. One system was devised by the European Organization for Research and Treatment of Cancer (EORTC) and pertains only to primary lymphomas of the skin, whereas the system by the World Health Organization (WHO) was derived from the Revised European-American Classification of Lymphoid Neoplasms for hematologic malignancies and covers nodal as well as extranodal lymphomas (Willemze *et al*, 1997; Harris *et al*, 2000). Both classification systems take into account a constellation of morphologic, immunologic, genetic, and clinical criteria.

The EORTC recognizes three prognostic categories of primary cutaneous lymphomas. In this classification most of primary cutaneous B cell lymphomas represent follicle center cell lymphomas (FCCL) or marginal zone lymphomas (MZL) and are characterized by an indolent clinical behavior. Primary cutaneous FCCL in the EORTC classification include follicular lymphomas (FL) as well as diffuse cutaneous B cell lymphomas of small cell, mixed, or large cell type. The EORTC excludes only diffuse large cell lymphomas occurring on the leg from this category. This is in contrast to the WHO classification that separates follicular center cell derived B cell lymphomas into follicular and diffuse growth patterns and, furthermore, subclassifies the FL according to the predominant cell type into grades 1–3. The rationale to combine FL and diffuse large B cell lymphomas as one entity by the EORTC was largely based on the observation of an overall indolent clinical course of these lymphomas, rather than on the architectural growth pattern, as applied by WHO. Primary cutaneous diffuse large B cell lymphoma of the leg is a rare lymphoma that may have a more aggressive clinical behavior and therefore was classified separately as intermediate cutaneous B cell lymphomas in the EORTC system (Willemze *et al*, 1997; Grange *et al*, 2001).

The different subcategories of lymphoid malignancies are thought to develop and transform from particular stages of lymphocyte differentiation and retain many characteristics of their normal counterparts (Harris *et al*, 2001). Lymphomas that correspond to immature and proliferating stages of differentiation are likely to be aggressive, whereas those that correspond to naive or mature effector stages are more likely to be indolent. From genotypic and immunophenotypic data, it has been proposed that the neoplastic cells in primary cutaneous FCCL originate from the lymphocytes of the germinal center and the neoplastic cells in primary cutaneous MZL correspond to the normal B cells of the marginal zones in the spleen in their

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Abbreviations: FL, follicular lymphoma; FCCL, follicle center cell lymphoma; MZL, marginal zone lymphoma; MF, mycosis fungoides.

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differentiation (Pandolfino *et al*, 2000). For primary cutaneous diffuse large B cell lymphomas the normal counterpart in B cell lymphocyte ontogeny is postulated to be a germinal center B cell or a cell at a later stage of differentiation (Pandolfino *et al*, 2000).

As a step toward a more complete molecular characterization of cutaneous B cell lymphomas we used cDNA microarrays with over 40,000 genes. By analyzing large-scale gene expression profiles of cutaneous B cell lymphomas we address if genomic profiling can distinguish between the different subtypes of cutaneous B cell lymphomas and whether distinct gene expression profiles correlate with clinical parameters, outcome data, or current classification systems. The 17 cutaneous B cell lymphoma samples used in this study were classified according to the WHO classification.

MATERIALS AND METHODS

Patients' material All frozen tumor tissue was collected in the Department of Dermatology, Division of Medical Oncology, Stanford University Medical Center (Stanford, CA, U.S.A.) and archived in the lymphoma tissue bank in the Department of Pathology, Stanford University. **Table I** summarizes the characteristics of patients investigated. A total of 25 tissue specimens were used in this study, including 17 B cell lymphoma specimens. Twelve of the 17 cutaneous B cell lymphoma cases represented primary cutaneous B cell lymphomas with five FL (including two tumor biopsies of the same patient), two diffuse large B cell lymphomas, and five MZL. The remaining five cases showed cutaneous and extracutaneous involvement at presentation and were diagnosed as three diffuse large B cell lymphomas and two FL. Whereas two of these five cases clearly represented secondary skin involvement by a lymph node based lymphoma (FL 98-023b, diffuse large B cell lymphoma 98-007), the remaining three cases (97-022b, 97-022D, 98-020, 98-010b) may represent an extranodal lymphoma involving the skin secondarily or a primary cutaneous lymphoma that had disseminated to extranodal sites prior to diagnosis. We refer to these five cases as secondary cutaneous lymphomas, as all of them had extracutaneous involvement at presentation.

Additionally, we included two cases of mycosis fungoides (MF), three normal skin biopsies, and three tonsillectomy specimens. A frozen section was cut from each specimen prior to RNA isolation to confirm that the archived material was representative of the case. The lymphoma samples in this study are classified according to the WHO classification.

RNA isolation and amplification Frozen tissue was homogenized in TRIzol reagent and total RNA was isolated according to the manufacturer's instructions (Invitrogen, Life Technologies, Carlsbad, CA). For most biopsies the total RNA yield was insufficient to meet the required input of 30–50 μg of total RNA for direct labeling cDNA microarrays and thus all samples were amplified. The yield of total RNA from the lymphoma samples ranged from 20 to 200 μg, and normal skin yielded 10–20 μg of total RNA. Total RNA samples were cleaned using the QIAamp RNA Mini Protocol (Qiagen, Valencia, CA) to create a high-quality template for subsequent RNA amplification. For comparative hybridization we used a universal Human Reference RNA (Stratagene, La Jolla, CA). Two micrograms of total tumor RNA from each sample and of reference RNA were subjected to one round of RNA Amplification (RiboAmp Kit, Arcturus, Mountain View, CA). The amplification process yielded 100–300 x of amplified anti-sense RNA (aRNA). For hybridization 3 μg of tumor and reference aRNA was used.

aRNA labeling and hybridization to spotted cDNA microarrays DNA microarray experiments were done essentially as described previously (Eisen and Brown, 1999). The reverse transcribed reaction was primed using 9 μg of $pd(N)_6$ random hexamer primer (Amersham Biosciences, Piscataway, NJ). cDNA probes were labeled using the Cy3 dye (green fluorescent) for the reference aRNA and the Cy5 dye (red fluorescent) (both Amersham Biosciences) for each tumor specimen. Each Cy5-labeled experimental cDNA probe was combined with the Cy3-labeled reference probe and the mixture was hybridized to spotted cDNA microarrays containing 42,000 spots. cDNA microarrays were obtained from the core microarray facility at Stanford. All

Table I. Characteristics of patients investigated

CBCL (WHO)	Sample	Sex	Age	Site of biopsy	Extent of skin involvement	Extracutaneous involvement	EORTC classification
FL[b]	94-001	F	86	Scalp	Regional	No	PCFCCL
FL[b]	96-002	F	88	Scalp	Regional	No	PCFCCL
FL	99-003	M	38	Scalp	Regional	No	PCFCCL
FL	97-009	M	67	Scalp	Regional	No	PCFCCL
FL[a]	97-022b/D	M	61	Cheek, face	Regional	Lymph node	Not primary cutaneous lymphoma at presentation
FL	98-023b	M	34	Forehead, face	Generalized	Lymph node bone marrow	Not primary cutaneous lymphoma (secondary cutaneous involvement by nodal based lymphoma)
FL	95-025b	M	58	Face	Generalized	No	PCFCCL
DLBCL[a]	89-004b/D	M	43	Scalp	Regional	No	PCFCCL
DLBCL	01-012b	F	79	Neck	Generalized	No	PCFCCL
DLBCL	98-010b	M	50	Arm	Generalized	Testis	Not primary cutaneous lymphoma at presentation
DLBCL	98-007	M	64	Arm	Regional	LN	Not primary cutaneous lymphoma (secondary cutaneous involvement by nodal based lymphoma)
MZL/DLBCL	98-020	M	73	Flank	Generalized	Bone marrow	Not primary cutaneous lymphoma at presentation
MZL	92-013b	M	53	Shoulder	Regional	No	PCIC/PCMZL
MZL	91-015	F	63	Arm	Generalized	No	PCIC/PCMZL
MZL	97-016	F	54	Arm	Regional	No	PCIC/PCMZL
MZL	01-018	M	72	Lateral thigh	Generalized	No	PCIC/PCMZL
MZL	96-005	M	57	Unspecified	Regional	No	PCIC/PCMZL
MF	01-002b	M	75	Forearm	Generalized	No	MF
MF	01-003b	M	67	Left flank	Generalized	No	MF

[a]Done in duplicates.
[b]Sequential biopsies of the same patient.
CBCL, cutaneous B cell lymphoma; PCFCCL, primary cutaneous follicle center cell lymphoma; DLBCL, diffuse large B cell lymphoma; PCIC, primary cutaneous immunocytoma; PCMZL According to the WHO classification for lymphoid tumors, FL is a neoplasm of follicle center B cells/cleaved follicle center cells (FCC) and centroblast/noncleaved FCC, which has at least a partially follicular pattern. Diffuse large B cell lymphoma is a diffuse proliferation of large neoplastic B lymphoid cells with nuclear size equal to or exceeding normal macrophage nuclei or more than twice the size of a normal lymphocyte. Extranodal marginal zone B cell lymphoma of mucosa associated lymphoid tissue (MALT lymphoma, MZL) MALT lymphoma is an extranodal lymphoma compromising morphologically heterogeneous small B cells including marginal zone cells (centrocyte-like) cells, cells resembling monocytoid cells, small lymphocytes, and scattered immunoblast and centroblast-like cells. There is a plasma cell differentiation in a proportion of the cases. The infiltrate is in the marginal zone of reactive B cell follicles and extends into the interfollicular region. Additionally, cutaneous B cell lymphomas cases were also diagnosed according to the EORTC classification for primary cutaneous lymphomas, PCFCCL, primary cutaneous immunocytoma/MZL (PCIC/primary cutaneous MZL) according to the described standard criteria (Willemze *et al*, 1997).

our image files are stored in the Stanford Microarray Database and are accessible at http://genome-www.stanford.edu/micorarray.

Data analysis The fluorescence ratio for each gene spot on the hybridized arrays was obtained with a Genepix 4000 scanner (Axon Instruments, Foster City, CA), and analyzed with Genepix 3.0 software (Axon Instruments). Uninterpretable spots were manually flagged and excluded. Hierarchical clustering was performed to analyze the gene expression data as described previously (Eisen *et al*, 1998). Genes chosen for cluster analysis were selected using the following criteria. All nonflagged spots with a fluorescent intensity greater than 1.5 × the local background of the red or green channel were included. Fluorescence ratios were centered for each gene by subtracting (in log space) the median ratio observed across all samples. We only included genes that were at least 3-fold upregulated or downregulated relative to the median in two arrays and that passed these filter criteria in 80% of the hybridized arrays.

RESULTS

Overview of the dendrogram We analyzed 25 specimens (19 lymphomas, three normal skin, and three normal tonsil specimens) using a hierarchical cluster algorithm. This method arranges the genes and tissue samples according to their degree of similarity in the pattern of gene expression. The relationships among the tissue samples are shown here in the form of a dendrogram where branch lengths reflect the degree of similarity between the samples. Very similar tumors are connected by short branches and longer branches display diminishing similarity of the tumors. Based on the described filtering criteria in *Materials and Methods* 1440 unique cDNA elements were selected that we used for hierarchical clustering. **Figure 1** shows the dendrogram that depicts the degree of similarity in gene expression among the 25 investigated tissues. The duplicate experiments (89-004D, 89-004b, 97-022D, 97-022b) clustered in immediately adjacent columns. The sequential tumor biopsies of one patient (FL94-001, 96-002) were taken 2 y apart and did not cluster next to each other but remained within the same branch. The two MF samples (01-002b, 01-003b) clustered side by side on the right main branch. Primary and secondary cutaneous FL were captured on the left main branch. In contrast primary and secondary cutaneous diffuse large B cell lymphomas did not cluster together. Whereas the primary diffuse large B cell lymphomas were found adjacent to primary and secondary FL, all three secondary diffuse large B cell lymphomas clustered on the right main branch. Primary cutaneous MZL figured on both main branches. MZL comprise a heterogeneous mixture of small lymphocytes, monocytoid B cells, and plasmacytoid and plasma cells, and usually contain reactive germinal centers (Cerroni *et al*, 1997), which may explain their wide distribution in the cluster. Two of the MZL showed prominent plasmacytoid differentiation and clustered closely (91-015, 01-018). Normal skin and tonsil showed a distinct gene expression signature. Tonsil was found adjacent to the primary and secondary cutaneous FL and the primary cutaneous diffuse large B cell lymphomas. Normal skin clustered on the right branch together with the MF samples and secondary cutaneous diffuse large B cell lymphomas.

Gene expression patterns in cutaneous B cell lymphomas **Figure 2(*A*,*B*)** give an overview of gene expression profiles after hierarchical clustering using 1440 genes. Most prominent gene clusters seen in **Fig 2(*A*)** are genes that are either upregulated or downregulated in normal skin and normal tonsil. As this study aims at gaining a first look at the molecular level of cutaneous B cell lymphomas, we will focus on the more subtle gene expression clusters that are associated with distinct lymphocyte lineage and developmental stages [T cell indicated in orange (2), GC B cell indicated in blue (1, 3–7, 10), plasma cell indicated in green (8–9)] and are shown in detail in **Fig 2(*B*)**.

The T cell signature was highly expressed in the MF specimens and variably expressed in the FL and diffuse large B cell

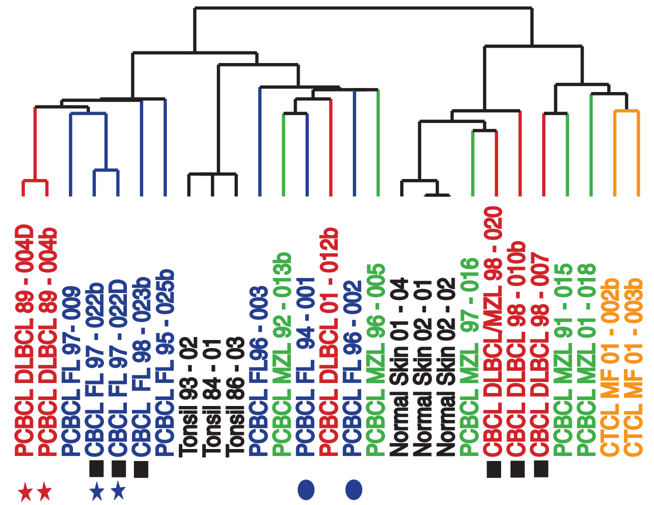


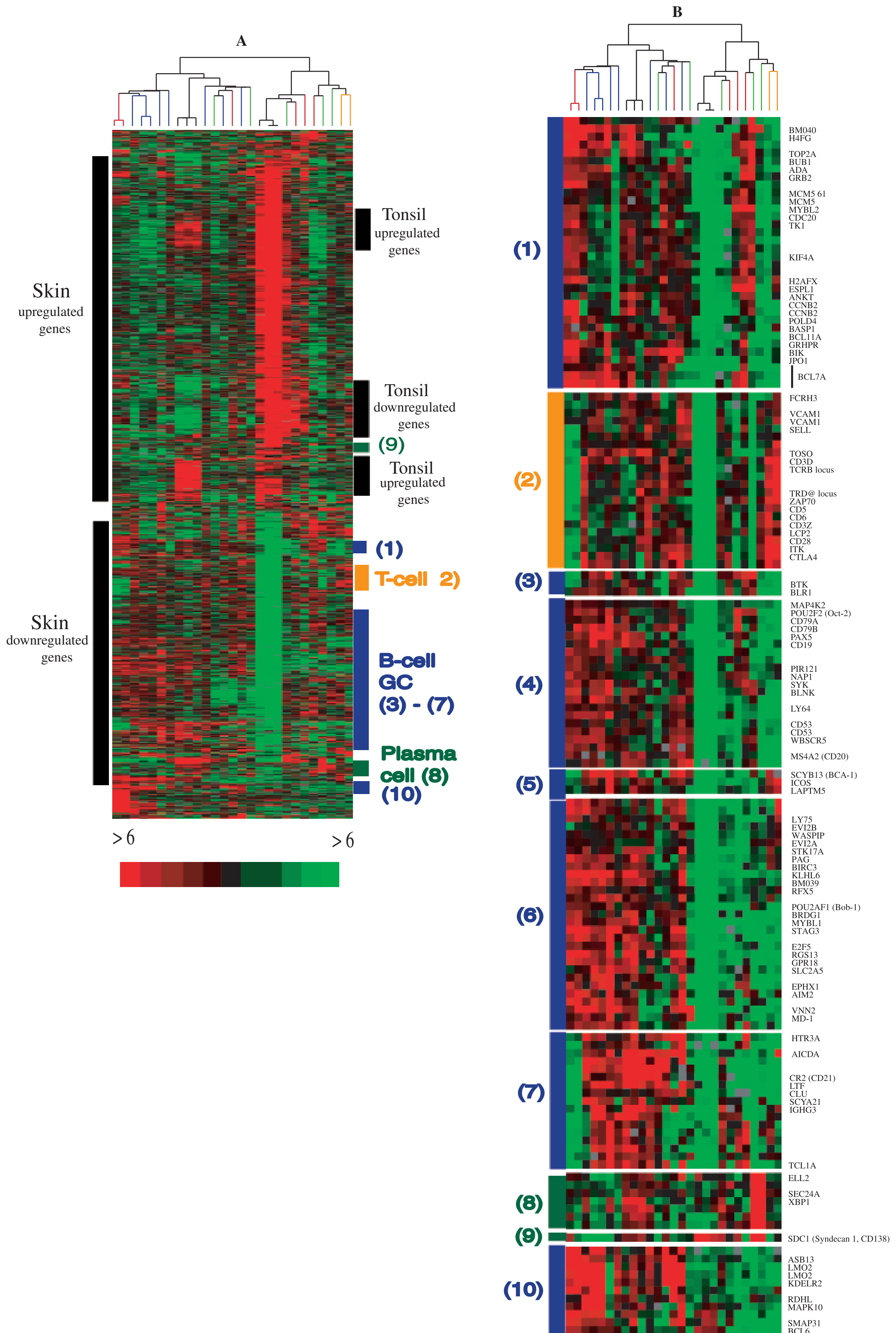
Figure 1. Overview of dendrogram. A total of 25 lymphoma samples including normal skin and tonsil were sorted by hierarchical clustering based on the similarity of gene expression. Patient identification number, the year of biopsy and the WHO classification (blue = follicular lymphoma (FL), red = diffuse large B-cell lymphoma (DLBCL), green = marginal zone lymphoma (MZL), orange = mycosis fungoides (MF), black = nor-normal skin, tonsil) are shown directly below the corresponding branch of the dendrogram. Secondary cutaneous B-cell lymphoma are marked by ■, tumor duplicates by ★ and the tumor pair by ●.

lymphoma. This signature contained components of the T cell receptor (TCR-α, TCR-β, TCR-γ, TCR-δ, CD3), T cell surface markers (CD5, CD6), including downstream signaling proteins (LAT, ZAP70) (all shown in **Fig 2*B***, T cell 2).

The B cell signature was expressed by the primary and secondary cutaneous FL and the primary cutaneous diffuse large B cell lymphomas and comprised antigens associated with the B cell receptor (CD79a, CD79b, syk, BLNK) (**Fig 2*B***, B cell 4), B cell specific cell surface markers (CD19, CD20) (**Fig 2*B***, B cell 4), and transcription factors necessary for certain stages in B cell development (PAX5, TCL1, Oct-2) (**Fig 2*B***, B cell 4, 7) (Said *et al*, 2001; Shaffer *et al*, 2001). Furthermore, these tumors, including the tonsil samples, expressed genes that are known to be expressed in germinal center B cells [Oct-2, Bob-1, CD21, BCL-6, ICOS, BLR1 (CXCR5), BCA-1 (CXCL13), RGS13, BCL7A, BCL11A, LMO2] (**Fig 2*B***, B cell 3–7, 10) (Forster *et al*, 1996; Kim *et al*, 1996; Schubart *et al*, 1996; Fukuda *et al*, 1997; Gunn *et al*, 1998; Alizadeh *et al*, 2000; Ansel *et al*, 2000; Shaffer *et al*, 2001; Tafuri *et al*, 2001; Reif *et al*, 2002). BCL-6, as a characteristic marker of germinal centers, was also upregulated in normal skin, where it is known to be expressed in keratinocytes (Yoshida *et al*, 1996).

Most notably the three secondary diffuse large B cell lymphoma clustering on the right side of the dendrogram lacked the above-mentioned germinal center B cell signature and only shared some genes associated with cell proliferation (CDC20, thymidine kinase1, cyclin B2, BUB1) (**Fig 2*B***, B cell 1).

The plasma cell signature could be identified in two primary cutaneous MZL with prominent plasmacytoid differentiation (91–015, 01–018) and in three normal tonsil samples, known to contain plasma cells. All expressed the recently described transcription factor XBP-1 (**Fig 2*B***, plasma cell 8), which is essential for terminal differentiation into a plasma cell (Reimold *et al*, 2001). Furthermore, we found that in these two primary cutaneous MZL with plasmacytic differentiation CD19, CD20, BCL6, and PAX5 were downregulated. This finding has been described previously (Calame, 2001) and reflects the essential transcriptional events that take place in the transformation and


A
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H2AFX
ESPL1
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BCL11A
GRHPR
BIK
JPO1
BCL7A
FCRH3
VCAM1
VCAM1
SELL
TOSO
CD3D
TCRB locus
TRD@ locus
ZAP70
CD5
CD6
CD3Z
LCP2
CD28
ITK
CTLA4
BTK
BLR1
MAP4K2
POU2F2 (Oct-2)
CD79A
CD79B
PAX5
CD19
PIR121
NAP1
SYK
BLNK
LY64
CD53
CD53
WBSCR5
MS4A2 (CD20)
SCYB13 (BCA-1)
ICOS
LAPTM5
LY75
EVI2B
WASPIP
EVI2A
STK17A
PAG
BIRC3
KLHL6
BM039
RFX5
POU2AF1 (Bob-1)
BRDG1
MYBL1
STAG3
E2F5
RGS13
GPR18
SLC2A5
EPHX1
AIM2
VNN2
MD-1
HTR3A
AICDA
CR2 (CD21)
LTF
CLU
SCYA21
IGHG3
TCL1A
ELL2
SEC24A
XBP1
SDC1 (Syndecan 1, CD138)
ASB13
LMO2
LMO2
KDELR2
RDHL
MAPK10
SMAP31
BCL6

development from a GC B cell to a mature plasma cell. Another well known cell surface marker of plasma cells Syndecan-1 (CD138) (**Fig 2*B***, plasma cell 9) (Sanderson *et al*, 1989) was also expressed in these two MZL. With the exception of these two MZL with plasmacytoid differentiation, the remaining MZL showed inconsistent clustering behavior, presumably due to their heterogeneous lymphoid infiltrate. Whereas one case shared the germinal center B cell gene expression pattern (92–013b), the rest of the MZL clustered on the right branch with normal skin and secondary cutaneous diffuse large B cell lymphoma cases.

DISCUSSION

The aim of this study was to contribute to the molecular characterization of cutaneous B cell lymphomas through large-scale gene expression analysis. Currently, it is not possible to discern between primary and secondary cutaneous B cell lymphomas on histopathologic and immunophenotypic examination alone. Subtle genetic and phenotypic differences have been shown especially for primary and secondary cutaneous FL. Whereas FL arising in the lymph node mostly demonstrate the t(14;18) translocation, resulting in an overexpression of the anti-apoptotic protein BCL-2, primary cutaneous FL normally lack both of these hallmarks (Yunis, 1988; Cerroni *et al*, 1994, 2000). This finding provoked several investigators to doubt the existence of a true primary cutaneous FL (Slater, 1997) and suggested a closer relationship to MZL (de Leval *et al*, 2001). Intraclonal diversity of the immunoglobulin genes in primary cutaneous FCCL indicating a germinal center cell origin was demonstrated only recently by using a single cell polymerase chain reaction (Gellrich *et al*, 2001).

On large-scale expression profiling primary and secondary cutaneous FL clustered together in our dataset, showing that these tumors share an overall common gene expression pattern despite the known differences. Notably, BCL-2 was not selected with the criteria used for gene selection. In this study, we chose to use genes that are either 3-fold upregulated or downregulated relative to the median across all samples in order to extract meaningful differentially expressed genes.

Another tumor subtype that clustered with FL consisted of primary cutaneous diffuse large B cell lymphomas involving the head area. Despite the differences in histology with a follicular growth pattern on the one hand and a diffuse pattern on the other, on the transcriptional level FL and primary cutaneous diffuse large B cell lymphomas appear to be very similar. Their common signature is characterized by high expression of genes that define germinal center B cells and other cells residing in this special microenvironment, including follicular dendritic cells. Many of these genes are known to play a crucial part in the formation of the follicular architecture of lymph nodes, Peyer patches, and the spleen, but so far have not been well described in skin lymphomas. The overlapping gene expression profiles of FL and primary cutaneous diffuse large B cell lymphomas may reflect the gene expression program of the specific differentiation stage they correspond to. It is important to note that within these tumor subtypes gene expression was quite heterogeneous and no single gene was pathognomonic for this group. Nevertheless, the close relatedness of primary cutaneous FL and diffuse large B cell lymphomas emerging on the molecular level, provides some support for the EORTC classification that groups FL and diffuse lymphomas in sites other than the leg into one group based on their overall indolent clinical behavior. An additional interesting finding in FL and primary cutaneous diffuse large B cell lymphomas, reconfirming the rather indolent nature of these entities, was the lack of gene expression associated with cell proliferation.

Recent gene expression studies in lymph node based lymphomas found a virtually unchanged gene expression signature of GC B cells in FL and some *de novo* diffuse large B cell lymphomas (Alizadeh *et al*, 2000), which is consistent with our data. Furthermore, these large studies identified at least one new subtype of diffuse large B cell lymphoma, the activated B like diffuse large B cell lymphoma, which lacked the GC B cell signature and was associated with a worse clinical outcome (Alizadeh *et al*, 2000; Rosenwald *et al*, 2002).

The systemic diffuse large B cell lymphomas secondarily involving the skin that were included in our study did not show a GC B cell signature. According to recent findings (Alizadeh *et al*, 2000; Rosenwald *et al*, 2002), we expected to find the activated B like diffuse large B cell lymphoma expression signature in these three tumors. Surprisingly, this was not the case and we could only identify a few genes associated with cell proliferation (CDC20, thymidine kinase 1, cyclin B2) that were shared by two of these three secondary diffuse large B cell lymphomas (98–010b, 98–007). But for the most part no common gene expression signature was evident in these three tumors. This different finding may be due to the underlying heterogeneity and the small number of investigated cases in this study. The three cases consisted of one immunoblastic lymphoma (98–007), one centroblastic lymphoma (98–010b) and one lymphoma that represented large cell transformation of a prior MZL (98–020) and perhaps, therefore, have failed to show a common expression pattern.

Another interesting finding was the close relatedness of the one immunoblastic variant of diffuse large B cell lymphoma (98–007) with the plasmacytoid MZL. It is known that upon antigen encounter, mature nave B cells appear to move into the T cell zone of lymphoid tissues, where they transform into large B blasts and then proliferate and differentiate into short-lived, IgM-producing plasma cells or into B cells that acquire the capacity to initiate a germinal center reaction (McHeyzer-Williams *et al*, 2001). The overlapping global gene expression profile found in one immunoblastic diffuse large B cell lymphoma and two plasmacytic MZL may reflect the similarities of gene expression repertoires in these entities.

Whereas primary cutaneous MZL with plasmacytic differentiation showed a plasma cell signature and clustered closely, primary cutaneous MZL with no plasmacytic differentiation showed inconsistent clustering behavior, which most likely is due to their heterogeneous composition. The plasma cell fate has been shown to be associated with a loss of molecules (CD19, CD20, BCL-6, Pax5), which are essential for the GC B cell, and a gain of molecules (XBP-1, CD138), which are needed for the plasma cell function (Calame, 2001) and can be observed in our result. MZL contain reactive as well as colonized follicles that may influence their cluster behavior toward germinal center derived lymphomas.

In summary, we have shown that microarray analysis can be performed successfully using a small amount of skin tissue as starting material. We have gained a first insight into the specialized microenvironment of the most common subtypes of cutaneous B cell lymphomas and have set the starting point for a larger and more elaborate study that will also include primary

Figure 2. Cluster Analysis. (A) Overview of the hierarchical clustering (average linkage) of 1440 genes selected from 25 lymphoma samples including normal skin and tonsil. Each row represents a separate cDNA clone on the microarray and each column a separate tissue sample. Red, green and black squares indicate that expression of the gene is greater, less than or equal to the median level of expression acrossall 25 tissues, respectively. Gray represents missing or poor quality data. The scale bare reflects the fold increase (red) or decrease (green) for any given gene relative to median level of expression across all samples. (B) Lineage and stage specific signatures could be identified. The blue bar indicates gene clusters associated with the B-cell receptor, B-cell surface markers, transcription factors and genes associated with germinal centers. The orange bar depicts genes associated with the T-cell receptor, T-cell surface markers and signaling molecules. The green bar shows genes particularly expressed in plasma cells. Numbers 1-10 indicate each specific gene cluster and corresponding position in the cluster overview.

cutaneous diffuse large B cell lymphomas of the leg. The specific gene expression signatures found in FL and primary cutaneous diffuse large B cell lymphomas, not involving the leg, give further support for a GC origin of these entities, which was shown previously (Kerl and Kresbach, 1984; Gellrich *et al*, 2001) and may explain the overall favorable prognosis of these patients. A larger set of tumor samples may help to identify single or multiple genes, which will be of diagnostic, prognostic, and therapeutic value, and will intensify our understanding of the diverse biology of these tumors. Potential candidate genes could furthermore be quantitated using real-time PCR as shown previously (Korz *et al*, 2002).

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