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ScienceDirect
Cancer Letters 265 (2008) 98–106
CANCER Letters
www.elsevier.com/locate/canlet

Phenotypic protein profiling of different B cell sub-populations using antibody CD-microarrays

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Received 8 November 2007; received in revised form 28 January 2008; accepted 3 February 2008

Abstract

Antibody microarrays enable extensive protein expression profiling, and provide a valuable complement to DNA microarray-based gene expression profiling. In this study, we used DotScan™ antibody microarrays that contain antibodies against 82 different cell surface antigens, to determine phenotypic protein expression profiles for human B cell sub-populations. We then demonstrated that the B cell protein profile can be used to delineate the relationship between normal B cells and malignant counterparts. Principle component analysis showed that the lymphomas did not cluster with the normal memory B cells or germinal centre B cells, but they did cluster with germinal centre founder cells and naïve B cells.

Keywords: CD antigens; Antibody microarrays; DNA microarrays; B cell sub-populations; Lymphoma

1. Introduction

The integration of transcriptomics and proteomics is a key issue for the emerging field of systems biology. Transcriptomics has for the last 5–10 years been dominated by DNA microarray-based approaches, and today the entire human transcriptome can be analyzed on a single array. Transcriptomics has also proven useful for delineating the molecular mechanisms behind different diseases [1], development of new diagnostic approaches (www.roche-diagnostics.com) and identification of novel biomarkers [2]. However, mRNA analysis needs to be complemented by analysis of the proteome, as many gene products are post-translationally modified and regulated. Proteome analysis has traditionally been based on a sample separation technique, e.g. 2D gels or liquid chromatography, combined with mass-spectrometry [3]. Alternative approaches, such as antibody microarrays are rapidly emerging, providing valuable tools for multiplex detection of proteins [4–8].

However, antibody microarrays available today can only target a fraction of the proteome. For the analysis of leukocyte sub-populations and haematopoietic malignancies, the human cluster of differentiation (CD) antigens comprise a defined and

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doi:10.1016/j.canlet.2008.02.006

highly relevant part of the proteome [9]. The CD antigens are a well-characterised set of molecules united by their common expression on the surface of leukocytes and their essential roles in the immune system. The DotScan antibody microarray technique, developed by Belov et al. [10–12], is based on the capture of live cells on the microarray slide, in contrast to conventional antibody microarrays, which detect soluble antigens. DotScan microarrays contain antibodies against 82 different cell surface antigens (mainly CD-markers), and recently, these arrays have been used to analyze a large set of acute leukemias and B-lymphoproliferative disorders, demonstrating that expression profiles alone were sufficient to correctly classify these leukemias [13].

B cells are a sub-population of leukocytes that play a key role in the humoral immune system and their primary function is to produce antibodies against pathogens. On their developmental path to antibody-producing plasma and memory B cells, they pass through several different stages of differentiation. The phenotypic delineation of these different B cell sub-populations was outlined for the human lineage by Liu et al. [14] and recently revised by Högerkorp and Borrebaeck [15]. An example of this differentiation is when naïve B cells (IgD^+, $CD23^-$) encounter an antigen in conjunction with the appropriate T cell stimulation, they become activated and differentiate into germinal centre (GC) founder B cells (IgD^+, $CD38^+$). A few GC founder B cells will then expand clonally and form a GC, containing GC B cells (IgD^-, $CD38^+$) that will give rise to memory cells (IgD^-, $CD38^-$) and affinity matured antibody-producing plasma cells (IgD^-, $CD38^{++}$).

The different stages of B cell development involve clonal expansion, somatic hypermutation, and class switch recombination, events that make the B cell vulnerable to malignant transformation. Since several features of a non-malignant B cell are retained in the transformed malignant B cell counterpart [16], it is believed that B cell leukemias and lymphomas may resemble the B cell developmental stage from which they originated [17]. For this reason, it is important to delineate common features between malignant and non-malignant B cells to distinguish clinical markers for more accurate diagnosis and therapeutic strategies. In this study, we have used DotScan™ microarrays to immunophenotype several different mature B cell sub-populations and lymphoma samples. The resulting CD expression profiles distinguished the normal B cell sub-populations and were subsequently used for analysis of the lymphoma samples. In addition, we have compared the CD antigen levels with transcriptional data obtained from high-density oligonucleotide arrays.

2. Materials and methods

2.1. B cell sub-populations

Human tonsils were obtained from 6 paediatric patients (aged between 2 and 16 years) undergoing routine tonsillectomy at Lund University Hospital (Lund, Sweden). Briefly, tonsils were minced and T cells were removed by rosetting with neuraminidase-treated sheep red blood cells. Mononuclear, T-cell-depleted, cells were isolated by density centrifugation, using Ficoll-Isopaque (Amersham Pharmacia Biotech AB, Uppsala, Sweden). The interphase fraction, containing predominantly mononuclear B cells, was washed in PBS containing FBS (10%). The total T cell depleted fraction, approximately $1–1.5 \times 10^9$ cells, was labeled with fluorescent antibodies in a stepwise process: (1) anti-IgM (BD Biosciences, Franklin Lakes, NJ), (2) goat anti-mouse-PE-Texas Red (Caltag, Carlsbad, CA), (3) anti-CD27-FITC (BD Biosciences), anti-IgD-PE (Dako, Glostrup Denmark), anti-CD38-PECy7 (BD Biosciences), anti-CD19-APCCy7 (BD Biosciences), anti-CD3-PB (BD Biosciences) and anti-CD14-PB (BD Biosciences). Cell sorting was performed on a FACS Aria (BD Biosciences). The different B cell populations were isolated as naïve ($CD19^+$, $CD3/14^-$, IgD^+, IgM^+, $CD38^-$, $CD27^-$), GC founders ($CD19^+$, $CD3/14^-$, IgD^+, IgM^+, $CD38^+$, $CD27^{+/-}$), GC B cells ($CD19^+$, $CD3/14^-$, IgD^-, IgM^-, $CD38^+$, $CD27^{+/-}$), Plasma cells ($CD19^+$, $CD3/14^-$, IgD^-, IgM^-, $CD38^{++}$, $CD27^+$) and Memory cells ($CD19^+$, $CD3/14^-$, IgD^-, IgM^-, $CD38^-$, $CD27^+$). Purity was confirmed by reanalysis and was typically >97%. Each population was sorted in three independent experiments, where the starting material for each round of selection was tonsillar lymphocytes pooled from two donors.

2.2. Isolation of lymphoma cells

Tumor samples were collected as diagnostic material from patients at Uppsala University Hospital. Seven lymphomas were analyzed, four Diffuse Large B Cell Lymphoma with non-GC phenotype [18] (DLBCL non-GC), one Chronic Lymphocytic Leukemia (CLL) and two Follicular Lymphomas (FL). The tumor samples were kept frozen as single-cell suspensions in media supplemented with 10% DMSO. After diagnosis, the cells were thawed at 37 °C and suspended in growth medium (1% L-glutamine/10% FCS/1% non-essential aminoacids/RPMI 1640). Cells were stained with anti-CD19-PB, anti-CD3-FITC (BD Biosciences) anti-immunoglobulin lambda light chain-PE (Dako) and anti-immunoglobulin kappa light chain-APC (BD Bioscience). The cells were sorted as $CD19^+$, $CD3^-$, $lambda^+$ or $kappa^+$ (or $lambda^-$ and $kappa^-$). An analytic