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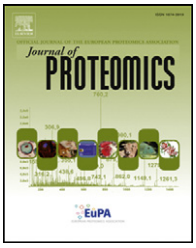

A comprehensive surface proteome analysis of myeloid leukemia cell lines for therapeutic antibody development

Verena Strassberger[a], *Katrin L. Gutbrodt*[a], *Nikolaus Krall*[a], *Christoph Roesli*[a,1], *Hitoshi Takizawa*[c], *Markus G. Manz*[c], *Tim Fugmann*[b,*], *Dario Neri*[a,**]

[a]*ETH Zurich, Department of Chemistry and Applied Biosciences, Wolfgang-Pauli-Strasse 10, 8093 Zurich, Switzerland*
[b]*Philochem AG, Libernstrasse 3, 8112 Otelfingen, Switzerland*
[c]*Division of Hematology, University Hospital Zurich, Raemistrasse 100, 8091 Zurich, Switzerland*

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ABSTRACT

A detailed characterization of the cell surface proteome facilitates the identification of target antigens, which can be used for the development of antibody-based therapeutics for the treatment of hematological malignancies. We have performed cell surface biotinylation of five human myeloid leukemia cell lines and normal human granulocytes, which was used for mass spectrometric analysis and allowed the identification and label-free, relative quantification of 320 membrane proteins. Several proteins exhibited a pronounced difference in expression between leukemia cell lines and granulocytes. We focused our attention on CD166/ALCAM, as this protein was strongly up-regulated on all AML cell lines and AML blasts of some patients. A human monoclonal antibody specific to CD166 (named H8) was generated using phage display technology. H8 specifically recognized AML cells in FACS analysis while demonstrating tumor targeting properties in vivo. After in vitro screening of five potent cytotoxic agents, a duocarmycin derivative was used for the preparation of an antibody–drug conjugate, which was able to kill AML cells in vitro with an IC_{50} of 8 nM. The presented atlas of surface proteins in myeloid leukemia provides an experimental basis for the choice of target antigens, which may be used for the development of anti-AML therapeutic antibodies.

Biological significance
The ability to discriminate between malignant and healthy, essential cells represents an important requirement for the development of armed antibodies for the therapy of hematological malignancies. Our proteomic study is, to our knowledge, the first large scale comparison of the accessible cell surface proteome of leukemia cells and normal blood cells, facilitating the choice of a suitable target for the treatment of acute myeloid leukemia (AML).

* *Corresponding author*. Tel.: +41 43 5448813; fax: +41 43 5448809.
** *Corresponding author*. Tel.: +41 44 6337401; fax: +41 44 6331358.
E-mail addresses: tim.fugmann@philochem.ch (T. Fugmann), neri@pharma.ethz.ch (D. Neri).
[1] Current address: Junior Research Group Biomarker Discovery, Deutsches Krebsforschungszentrum (DKFZ) and Heidelberg Institute for Stem Cell Technology and Experimental Medicine (HI-STEM gGmbH), Im Neuenheimer Feld 280, 69120 Heidelberg, Germany.

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An antibody drug conjugate was generated recognizing the CD166 antigen which was found to be strongly up-regulated in all AML cell lines and AML blasts of some patients. This antibody drug conjugate SIP(H8)-Duo might be further characterized in therapy experiments and might lead to a new targeted treatment option for AML.

1. Introduction

Monoclonal antibodies are gaining importance for the treatment of hematological malignancies. In addition to approved products, such as Rituximab for the treatment of non-Hodgkin lymphoma (NHL) [1] and chronic lymphocytic leukemia (CLL), Ofatumumab for CLL and Alemtuzumab for CLL [2], several products are currently in advanced clinical development (e.g., Daratumumab for the treatment of multiple myeloma [3]) and we anticipate that a number of additional products will become routinely available for clinical use in the next few years.

While traditionally intact antibodies have been used for therapeutic applications, there has been a recent trend towards arming antibodies with suitable payloads, such as drugs [4,5], radionuclides [6] or cytokines [7]. Indeed, Brentuximab vedotin is an antibody drug conjugate recently approved for the treatment of last-line post-transplant Hodgkin lymphoma and systemic anaplastic large cell lymphoma [8], while the radiolabeled anti-CD20 antibodies Ibritumomab tiuxetan and Iodine I 131 tositumomab have received marketing authorization for NHL treatment [8]. Moreover, the use of bispecific antibodies has exhibited promising results in NHL and ALL [9].

Antibodies recognize surface antigens on target cells. A comprehensive analysis of the surface proteome promises to be useful for the identification and validation of targets, which may be considered for the development of antibody-based therapeutics. Traditionally, the characterization of membrane proteins by conventional methods (such as two-dimensional gel electrophoresis) has been difficult, because these proteins tend to be insoluble in water and are not very abundant. However, the selective chemical modification of accessible proteins on the cell surface (e.g., by reaction of the proteins' primary amino groups [10,11] or of carbohydrate moieties [12] with reactive derivatives of biotin) may facilitate their enrichment and the subsequent mass spectrometry-based proteomic analysis of tryptic peptides.

We have previously studied the surface proteome, either enriching accessible proteins by in vitro biotinylation of cell lines [11] or the in vivo biotinylation of the vasculature in healthy organs and at sites of disease [10]. Biotinylated proteins were purified on streptavidin resin, followed by on-resin digestion, yielding tryptic peptides which could be analyzed by mass spectrometry. While proteomics technologies continue to improve rapidly, the relative quantification of protein-derived peptides remains a challenge [13].

In this article, we present the results of a comparative surface proteome analysis of four acute myeloid leukemia cell lines (AML), one chronic myeloid leukemia (CML) cell line and of granulocytes isolated from normal human peripheral blood. Cell surface biotinylation, followed by capture on streptavidin resin, tryptic digestion and DeepQuanTR-assisted mass spectrometric analysis, allowed the identification and relative quantification of 823 proteins. We studied in more detail CD166 or Activated Leukocyte Cell Adhesion Molecule (CD166/ALCAM), as this protein was found to be strongly up-regulated in all AML cell lines, compared to CML and granulocyte controls. An anti-CD166 human monoclonal antibody (termed H8) was isolated, characterized and coupled to potent cytotoxic agents, in order to selectively kill AML cells. Finally, the expression of CD166 on human peripheral blood and bone marrow cells of AML patients and healthy donors was analyzed.

2. Materials and methods

2.1. Cell culture

NB4 (ACC 207), THP1 (ACC 16), K562 (ACC 10), PLB985 (ACC 139) (all DSMZ) and HL60 (ATCC® CCL240™) cells were maintained in RPMI1640/L-Glutamine (Life Technologies, Inc., Carlsbad, CA), supplemented with 10% FBS (20% for HL60) (Life Technologies) and 1× antibiotic–antimycotic (Life Technologies) at 37 °C and 5% CO_2.

For the localized xenograft models 10^7 NB4 cells were subcutaneously injected into the right shoulder of 7 to 9 weeks old BALB/c nude mice (Charles River Laboratories, Sulzfeld, Germany). All animal experiments were performed on the basis of project license (42/2012) granted by the Veterinaeramt des Kantons Zuerich and approved by all participating institutions.

2.2. Purification of human polymorphonuclear leukocytes (PMN)

From each of four different probands, 40 ml of normal human venous blood was collected with sodium citrate S-Monovette (Sarstedt, Nuembrecht, Germany) and processed and analyzed individually. Informed consent was obtained from each subject before blood collection. PMNs were purified using Polymorphprep™ (Axis-Shield PoC AS, Oslo, Norway) following the manufacturer's guidelines. Erythrocyte contamination was removed with 16.2 ml H_2O for 20 s followed by 5.4 ml 0.6 M KCl and 33.2 ml PBS. At least 99.5% purity was reached as assessed by Wright Giemsa staining.

2.3. Cell surface biotinylation

3.4×10^7 cells were washed twice with ice cold PBS by 4 min centrifugation at 252 ×*g* and biotinylated for 5 min at room temperature on an orbital shaker with 6 ml 411 μM EZ-link sulfo-NHS-LC-biotin (Thermo Fisher Scientific, Inc., Rockford, IL) in PBS followed by addition of 25 μmol Tris–HCl (pH 7.4) and washing once with PBS. Cells were lysed in PBS containing 2% Nonidet P-40, 0.2% SDS, 10 mM EDTA by sonication for 9 s before and after incubation on ice for 30 min with repeated vortexing. The lysate was centrifuged at 16,100 ×*g* for 10 min at 4 °C and protein concentration of