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Research paper

Element-tagged immunoassay with ICP-MS detection: Evaluation and comparison to conventional immunoassays

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ABSTRACT

We have investigated the possibility of using element-tagged antibodies for protein detection and quantification in microplate format using Inductively Coupled Plasma Mass Spectrometry (ICP-MS), and compared the results to conventional immunoassays, such as Enzyme-Linked Immunosorbent Assay (ELISA) and Western blotting. The technique was further employed to detect low levels and measure DNA-binding activity of transcription factor p53 in leukemia cell lysates through its interaction with immobilized oligonucleotides and recognition by element-tagged antibodies. The advantages of ICP-MS detection for routine performance of immunoassays include increased sensitivity, wide dynamic range, minimal interference from complex matrices, and high throughput. Our approach advances the ICP-MS technology and demonstrates its applicability to proteomic studies through the use of antibodies directly labeled with polymer tags bearing multiple atoms of lanthanides. Development of this novel methodology will enable fast and quantitative identification of multiple analytes in a single well.

1. Introduction

There is growing interest in measuring low levels of biological molecules in a complex matrix using affinity products, such as antibodies. A sensitive and dynamic method to quantify antigens is through inductively coupled plasma mass spectrometry (ICP-MS) using element-tagged antibodies. ICP-MS is extensively used as an analytical chemistry tool as well as to study natural and induced metal incorporation into bacteria (Li et al., 2005; Zhang et al., 2003a,b; Binet et al., 2003), plants (Sharma et al., 2005) and in metalloproteomics research (Prange and Profrock, 2005; Szpunar, 2005). In all of these cases, the bio-molecules being analyzed are already associated with a metal component. In contrast, the ICP-MS-linked immunoassays provide a means to determine the concentrations of proteins that do not necessarily contain a metal. To achieve this, ICP-MS immunoassays rely on the use of affinity products that include a metal containing tag (Careri et al., 2007; Zhang et al., 2001, 2002). The most straightforward approach utilizes antibodies that have been raised specifically against the antigens (e.g. proteins) of interest and tagged with metal.

In this investigation, we used antibodies conjugated to a polymer tag containing multiple metal chelates loaded with lanthanide ions. Interchain disulfide bonds in the antibody were partially reduced, and the polymer was attached through its terminal maleimide group to sulfhydryl residues on the Fc portion of the antibody (Lou et al., 2007). Thus, the antigen recognition sites retained their specificity, while inclusion of multiple lanthanide atoms on the polymer increased instrument detection sensitivity. Multiplex analysis for a variety of surface biomarkers has been performed on cell samples using commercial and in-house prepared element-tagged antibodies (Ornatsky et al., 2006; Tanner et al., 2007; Lou et al., 2007). The present investigation focused on the evaluation and application of ICP-MS techniques utilizing element-tagged antibodies for the

Abbreviations: ICP-MS, Inductively Coupled Plasma Mass Spectrometry; ELISA, Enzyme-Linked Immunosorbent Assay; Tb, Terbium; Tm, Thulium.

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quantification of growth and transcription factors in cell lysates. The method is compared to the Enzyme-Linked Immunosorbent Assay (ELISA) and Western blotting. ELISA is considered the gold standard for measuring levels of a single analyte in a sample (Crowther, 2001). We modified both direct and sandwich immunoassays to include lanthanide labeled antibodies rather than enzyme antibody conjugates.

The present study evaluated several model systems for the ICP-MS immunoassays: purified growth factor assay, p53 protein level analysis, and measurement of transcription factor p53 DNA-binding activity in human leukemia cell lysates. The first is the human platelet-derived growth factor (hPDGF), a major mitogenic factor found in serum with a role in connective tissue homeostasis (Claesson-Welsh and Heldin, 1989; Soma et al., 1992). hPDGF exists as a dimer, and has three distinct isoforms: hPDGF-AA, AB or BB. We used the human recombinant PDGF-AA isoform as the target growth factor in our assays. *p53* was chosen primarily due to its presence in many cell lines, availability of well characterized antibodies that recognize wild-type and mutant protein, and reliable short hairpin RNA (shRNA) for human *p53* silencing (Brummelkamp et al., 2002). p53 protein is a transcription factor involved in the regulation of cell growth, DNA repair, and apoptosis in response to environmental stress. Activating signals, such as ionizing radiation, cause the p53 protein to undergo a number of posttranslational modifications (e.g. phosphorylation on key Ser and Thr residues) which correlate with p53 protein accumulation, translocation into the nucleus and increased association with the consensus sequences of p53 responsive genes (Vogelstein et al., 2000).

ICP-MS offers the potential for multivariate biological analysis based on metal-tagged affinity assays. Significant advantages of the approach include universality of tagging antibodies; a wide variety of elemental tags from which to choose (up to 100 stable metal isotopes), and lack of sensitivity to light which allows for laboratory labelling under convenient ambient conditions. These antibody labeling features, combined with the analytical characteristics of ICP-MS instruments, including analyte quantification with high precision; low detection limits; large dynamic range, both for each antigen and between antigens; low matrix effects from other components of the biological sample (i.e. contaminating proteins in the sample have no effect on elemental analysis); low background from plastic plates (i.e., plastic containers do not cause interference on elemental detection as it can with fluorescence), and superior spectral resolution (abundance sensitivity) call for further development and application of this novel methodology in immunological studies.

Here we demonstrated the use of element-tagged ICP-MS immunoassays for protein quantification in microplate format and its equivalence to conventional immunoassays, such as ELISA and Western blotting. The technique is further employed to determine levels of activated transcription factor p53 through the measurement of its binding to p53 consensus oligonucleotides and recognition of the complex by element-tagged antibodies in nuclear lysates of leukemia cell.

2. Materials and methods

2.1. Antibodies and antibody labeling

Monoclonal antibodies against human p53 (DO-1 and Pab1801; Santa Cruz Biotechnology Inc., Santa Cruz, CA), anti-human PDGF-AA, anti-GAPDH antibodies (GeneTex Inc., San Antonio, TX)) and mouse IgG_1 (R&D Systems Inc., Minneapolis, MN) were obtained from commercial sources. Lanthanide tagging was performed according to published protocol (Lou et al., 2007). In brief, antibodies were partially reduced using TCEP, tris(2-carboxyethyl)phosphine (Pierce Biotechnology, Inc., Rockford IL) in 30K Nanosep centrifugal filters (Pall Canada Limited, Mississauga, ON), and incubated for 1 h at 37 °C with a 30-fold molar excess of ligand-bearing polymer, known as MAXPAR™ (DVS Sciences Inc., Richmond Hill, ON). Excess polymer was washed off in the same filters and a 100-fold molar excess of a lanthanide chloride solution was added. After 45 min incubation at 37 °C, excess metal was washed off and the antibodies were stored in TBS (Tris buffered saline) at a concentration of approximately 0.8 mg/mL and used in assays at dilutions 1/200 and 1/400.

2.2. Cell lines

A431, 293T, KG1a and K562 human cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA) and cultured according to manufacturer's specifications at 37 °C, 5% CO_2 and 100% humidity. 293T cells are adenovirus-transformed human kidney cells that express the SV40 large T antigen. The Tex cell line is a human immortalized myeloid cell line derived from cord blood cells transduced with TLS-ERG oncogene. Tex cells were cultured in IMDM supplemented with 15% FCS, 20 ng/mL SCF and 2 ng/mL IL-3 (Amgen Inc., Thousand Oaks, CA) (Warner et al., 2005). Tex cells were obtained from the laboratory of J.E. Dick (University Health Network, Toronto, Canada). Tex-ShRFP (control shRNA against Red Fluorescent Protein), Tex-Shp53, 293T-ShRFP and 293T-Shp53 cell lines were obtained by transduction of the parental cell lines with lentiviral vectors expressing shRNA targeting RFP or p53.

2.3. hPDGF-AA immunoassays

2.3.1. Standard ELISA

Recombinant human PDGF-AA (R&D Systems Inc., Minneapolis, MN) was reconstituted in 4 mM HCl with 0.1% BSA and diluted with carbonate buffer pH 9.0 to concentrations of 0–5 μg/mL. 96-well ReactiBind maleic anhydride microplates (Pierce Biotechnology, Inc., Rockford, IL) were incubated with 50 μL protein solutions overnight at 4 °C and blocked with 1% BSA/PBS with 20 μg/mL mouse IgG (Biomeda Co., Burlingame, CA). Plates were washed 5 times with 0.1% Tween-20/PBS. Unlabeled mouse anti-human PDGF-AA antibodies (R&D Systems Inc., Minneapolis, MN) (at 1/200 dilution of 0.5 mg/mL stock) were added to plates, and after 90 min of incubation at room temperature plates were washed several times. Horseradish peroxidase-conjugated anti-mouse antibody (Cell Signaling Technology, Inc., Danvers, MA) was added to the plates and incubated for 30 min at 37 °C. Excess antibody was washed away, and the TMB, 3,3′,5,5′-tetramethylbenzidine, (Sigma Chemical Co., St. Louis, MO) substrate with hydrogen peroxide were added to develop color. The reaction was allowed to proceed for 30 min at room temperature, and stopped with sulfuric acid. The optical density was determined in a microplate reader (Molecular Devices Co., Union City, CA) at 450 nm.