Application Note

SOMAmer® Reagents for Oncology Biomarker Analysis by Fluorescent Cell Microscopy

Introduction

SOMAmer reagents (Slow Off-rate Modified Aptamers) are DNA-based high affinity (average KD < 1 nM) protein binding reagents with proprietary chemical modifications that provide hydrophobic characteristics not present in natural DNA. These modifications enhance protein binding through direct hydrophobic contacts with the target protein, resulting in increased binding affinities and slower complex dissociation rates compared to unmodified aptamers.

Due to their high specificity and strong affinity for protein targets, SOMAmer reagents can be used in many life science research applications that traditionally rely on antibodies. Since SOMAmer reagents are chemically-synthesized, modified DNA, product consistency and stability are superior to antibodies. Antibodies are produced in fundamentally variable biological systems and may suffer from stability issues inherent to protein-based reagents.

Fluorescent cell microscopy is used as a tool to determine protein expression level and subcellular location. Target proteins are traditionally detected either by target-specific primary antibodies conjugated to a fluorescent reporter molecule or with a primary antibody and fluorescent secondary antibody combination. We have used SOMAmer reagents with a 5' biotin functional group in conjunction with fluorescently-labeled streptavidin as our labeling strategy. This technical note describes the use of SOMAmer reagents for observing, by fluorescent cell microscopy, the cell surface expression of two receptor tyrosine kinases implicated in cancer biology: Epidermal growth factor receptor (EGFR or ERBB1) and Receptor tyrosine kinase erbB2 (ERBB2).

Materials and Methods

Cell culture

Cell lines SKBR3, MDA-MB-231, and MDA-MB-468 were obtained from the American Type Culture Collection (ATCC). All cell lines were cultured in DMEM supplemented with 10% Fetal Bovine Serum and 10mM L-Glutamine in clear 96 well tissue culture plates.

SOMAmer Reagents

Biotinylated SOMAmer Reagents against ERBB2 (SomaLogic, Inc., product #910-00029), ERBB1 (SomaLogic, Inc., product #910-00037), and Coagulation Factor VII (SomaLogic, Inc., product #910-00116) were diluted to 1 µM in 5 mM HEPES, 1 nM EDTA, pH 8 and heated to 95° C for 3 minutes, then slowly cooled in a 25 °C water bath for 20 minutes prior to dilution in cell staining buffer.

Polyanionic Competitor

Inclusion of a competitor during or after cell labeling can be valuable in distinguishing between specific and non-specific binding in all SOMAmer reagent applications. While many applications do not involve high levels of proteins with high random DNA affinity, they often include very high concentrations of proteins with modest affinity (e.g., some highly abundant plasma proteins that can bind DNA). Addition of a competitor can help reduce artifacts that may arise from random DNA binding layered on specific SOMAmer reagent binding. SomaLogic's Polyanionic Competitor [Product Number 910-00001] or dextran sulfate at concentrations ranging from 0.1 µM to 10 mM should be evaluated for use in specific applications (see SomaLogic Application Note on Polyanionic Competition). In these experiments, 10 µM SomaLogic Polyanionic Competitor and 2 mM Dextran Sulfate were used during incubation of cells with SOMAmer reagents.

Cell labeling

Media was aspirated from plates and cells were briefly rinsed with PBS before fixation with 4% formaldehyde for 15 minutes at 25° C. Fixative was removed and cells were washed twice with PBS for 5 minutes at 25° C. SOMAmer reagents were diluted to 100 nM in cell labeling buffer (PBS with 5 mM MgCl2, 1% BSA, 10 µM SomaLogic Polyanionic Competitor (SomaLogic, Inc., product #910-00001), and 2 mM Dextran Sulfate). Cells were incubated in the above SOMAmer labeling solution for 30 minutes at 25° C. Cell labeling solution was removed and cells were washed 3 times with PBS with 5 mM MgCl2 for 10 minutes at 25° C. Streptavidin-phycoerythrin conjugate (Moss, Inc., Product# SAPE-001) was added to cells at 0.5 ug/mL in PBS with 5 mM MgCl2 and incubated for 30 minutes at 25° C. Cell labeling solution was removed and cells were washed 3 times with PBS with 5 mM MgCl2 for 10 minutes at 25° C. Cell nuclei were stained with DAPI at 300 nM in PBS with 5 mM MgCl2. Cells were washed with two times with PBS with 5 mM MgCl2 for 5 minutes at 25° C. Labeled cells were stored in PBS at 4° C until imaging.

Cell Imaging

Cells in PBS with 5 mM MgCl2 were imaged on an EVOS FI digital inverted fluorescence microscope (Advanced Microscopy Group, Thermo Fisher). Epifluorescence images were captured in the RFP and DAPI channels

Results

The ERBB1 and ERBB2 SOMAmer reagents efficiently and specifically labeled cell lines that expressed each SOMAmer reagent's intended target protein. None of the three cell lines studied (SKBR3, MDA-MB-468, or MDA-MB-231) were labeled by the negative control SOMAmer reagent which targets the secreted protein Coagulation Factor VII (Figure 1). The ERBB1-specific SOMAmer reagent only labeled the MDA-MB-468 cell line which is known to express high levels of ERBB1 and low levels of ERBB21. Labeling by the ERBB2-specific SOMAmer reagent was restricted to the well-characterized ERBB2 overexpressing cell line SKBR3 which expresses low level of ERBB12. None of the SOMAmer reagents that were tested generated any significant fluorescent labeling of the ERBB1 and ERBB2 low-expressing cell line MDA-MB-2313. We conclude that these reagents are highly specific and effective binding reagents for the detection of ERBB1 and ERBB2 in a fluorescent cell microscopy application.

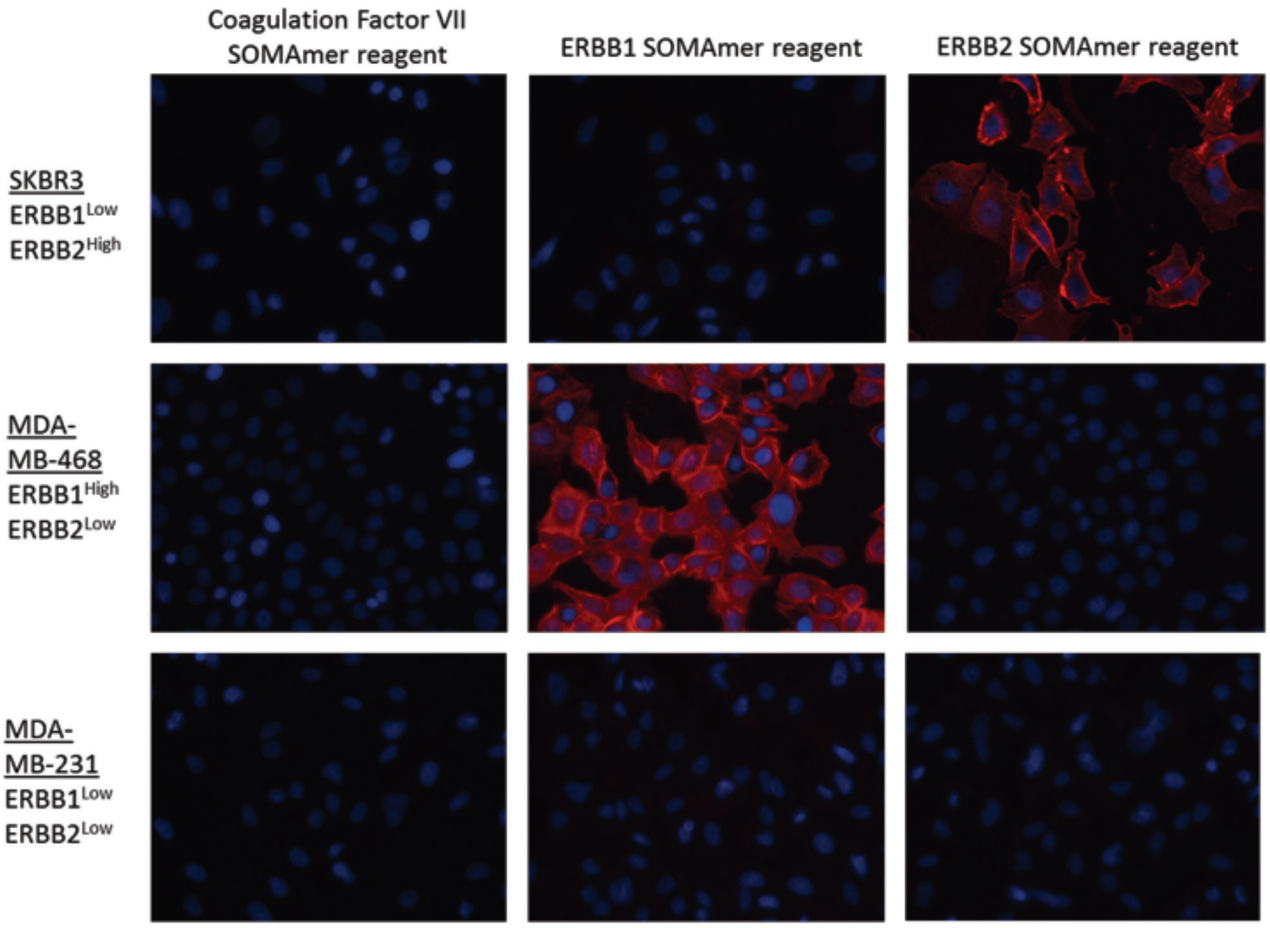

Figure 1: *Fluorescent cell microscopy using SOMAmer reagents as primary detection reagents. ERBB1-specific SOMAmer reagent labeling is limited to the ERBB1High cell line MDA-MB-468. ERBB2-specific SOMAmer reagent labeling is limited to the ERBB2High cell line SKBR3. The ERBB1Low, ERBB2Low cell line MDA-MB-231 is not labeled by either SOMAmer reagent. The control SOMAmer reagent against Coagulation Factor VII does not label SKBR3, MDA-MB-468, or MDA- MB-231 cell lines.*

References

1. Goldenberg, A. et al. Imaging of human tumor xenografts with an indium-111-labeled anti-epidermal growth factor receptor monoclonal antibody. J. Natl. Cancer Inst. 81, 1616–25 (1989).
2. Van de Vijver, M. J., Mooi, W. J., Wisman, P., Peterse, J. L. & Nusse, R.Immunohistochemical detection of the neu protein in tissue sections of human breasttumors with amplified neu DNA. Oncogene 2, 175–8 (1988).
3. Lee, C. M. & Tannock, I. F. The distribution of the therapeutic monoclonal antibodies cetuximab and trastuzumab within solid tumors. BMC Cancer 10, 255 (2010).

DCN 16-064 Rev. 1
AN-15-010 Effective: 3/18/2016

2945 Wilderness Place
Boulder, CO 80301
U.S. Technical Support: 800-324-0783
Main Number: 303-625-9000