Supporting Information

Detection and Quantification of the Bcr/Abl Chimeric Protein on Biochips using LDI-TOF MS

Seol-Hye Hong, Jae Il Kim, Hyunook Kang, Soojin Yoon, Dong-Eun Kim, Woong Jung, and Woon-Seok Yeo*

1. Materials and Methods

Materials. AuNPs (40 nm in diameter) were prepared by using the previously explicated method.[1] Gold chips were prepared by vacuum deposition of titanium (10 Å) followed by gold (50 Å) onto glass slides. Sodium borohydride, sodium citrate, cobalt chloride, ammonium acetate, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), dimethyl sulfoxide (DMSO), and all chemical reagents for the synthesis were purchased from Sigma-Aldrich (St. Louis, MO, USA), unless otherwise specified. Ethylene glycol-D4 (isotope labeling percentage 98%) was purchased from Cambridge Isotope Laboratories, Inc. (Fr. Andover, MA, USA). Hydrogen tetrachloroaurate(III) hydrate ($HAuCl_4$) was purchased from Kojima chemicals Co., Ltd. (Sayama-shi, Japan). Ethanol was purchased from Merck (Darmstadt, Germany). Anti-Abl, Anti-Bcr, and anti-β-actin were purchased from Santa Cruz Biotechnology, Inc. (CA, USA). Polyvinylidene difluoride membrane was purchased from Millipore (Billerica, MA, USA). Phosphate buffered saline (PBS), RPMI-1640 medium, bovine calf serum, Genecticin (G418), and antibiotics (100 U/mL penicillin G and 100 mg/mL streptomycin) were purchased from WelGene biopharmaceuticals (Daegu, Korea).

Preparation of anti-Abl presenting biochips. Gold chips were cleaned in piranha solution (sulfuric acid : hydrogen peroxide = 7:3. Caution! Extremely hot and corrosive) before use. The gold chips were incubated in a mixed solution of tri(ethylene glycol)-terminated alkanethiol (1mM in ethanol) and carboxylic penta(ethylene glycol)-terminated alkanethiol (1mM in ethanol) in a ratio of 95 : 5 for 12 h. After incubation, the gold chips were washed with ethanol, and dried under a stream of nitrogen. 7 μL NHS (10 mg/mL in PBS) and 3 μL EDC (20 mg/mL in PBS) were applied to carboxylic acid-presenting gold chips for 2 h and then the chips were washed with PBS. The resulting NHS-activated ester-presenting chips were incubated with 5 μL anti-Abl (0.87 μM in phosphate buffer (PB)) for 1h. The resulting antibody-immobilized gold chips were washed with PB and stored at 4 °C until use.

Preparation of anti-Bcr presenting gold nanoparticles (AuNPs). As-made AuNPs (1 mL of 3.3 nM) were washed with tri-distilled water by centrifugation three times and incubated with a mixed solution of tri(ethylene glycol)-terminated alkanethiol (100 μM in ethanol) and carboxylic acid penta(ethylene glycol)-terminated alkanethiol (100 μM in ethanol) in a ratio of 95 : 5 for 12 h. The resulting AuNPs were washed with absolute ethanol by using centrifugation at 14,000 rpm for 3 min three times. Acid-presenting AuNPs (3.3 nM in ethanol) were treated with 350 μL NHS (10 mg/mL in DMSO) and 150 μL EDC (20 mg/mL in DMSO) for 2 h, and washed with DMSO and phosphate buffer (PB) by centrifugation at 14,000 rpm for 3 min. 200 μL anti-Bcr (1.03 μM in PB) was incubated with the above NHS-activated AuNPs for 1 h at 4 °C. The resulting antibody immobilized AuNPs were then washed with distilled water by centrifugation at 7,000 rpm for 3 min three times and stored at 4 °C until use.

Preparation of internal standard-coated AuNPs. As-made AuNPs (1 mL of 1.1 nM) were washed with tri-distilled water by centrifugation three times and incubated with a solution of deuterated tri(ethylene glycol)-terminated alkanethiol (100 μM in ethanol) for 12 h. The

resulting AuNPs were washed with absolute ethanol using centrifugation at 14,000 rpm for 3 min three times and stored at 4 °C.

Cell culture and lysis. Murine pro-B lymphoid cell line Ba/F3 cells that express Bcr/Abl chimeric proteins were cultured in RPMI1640 medium, supplemented with heat inactivated 10% bovine calf serum and 1% antibiotics (100 U/mL penicillin G and 100 mg/mL streptomycin) at 37 °C in humidified 5% CO_2 atmosphere. For the sub-culture, 10% G418 (50 mg/mL) was added to media. The cells were collected from the petri dishes, then vortexed in PB (10 mM, pH 8), and treated with protease inhibitor cocktail (1×). The mixture was subjected to sonication for 2 min at 4 °C. The lysed cells were then centrifuged at 14,000 rpm for 5 min, and the supernatant was placed in a fresh tube.

Detection of the Bcr/Abl chimeric protein in cell lysates. Lysed cell supernatant (100 μL) was incubated with 50 μL of 100 pM anti-Bcr-presenting AuNPs for 30 min and Bcr/Abl-bound AuNPs were separated by centrifugation at 7,000 rpm for 3 min. The AuNPs were dispersed with 15 μL PB (10 nM, pH 8) and were treated on the anti-Abl presenting biochips for 30 min. The chips were rinsed with distilled water to remove unbound AuNPs and dried under a stream of nitrogen, then analyzed by LDI-TOF MS without an organic matrix.

Quantification of Bcr/Abl chimeric proteins. Lysed cell supernatants (100 μL) from various numbers of cells were incubated with 50 μL of 100 pM anti-Bcr presenting AuNPs for 30 min and Bcr/Abl-bound AuNPs were separated by centrifugation at 7000 rpm for 3 min. The AuNPs were dispersed with 15 μL PB (10 nM, pH 8), and were treated on the anti-Abl-presenting biochip for 30 min. The chip was rinsed with distilled water to remove unbound AuNPs and dried under a stream of nitrogen. Next, the biochip was treated with 5 μL internal standard (IS; 200 pM in ethanol) and dried at ambient conditions. The chip was then transferred to a PCR tube containing 200 μL ethanol and subjected to sonication for 30 s to separate AuNPs and ISs from the chip. The chip was removed and AuNPs and ISs were

separated by centrifugation at 14,000 rpm for 5 min. Finally, AuNPs and ISs were dispersed with 15 μL ethanol and 1 μL of the dispersion was analyzed by LDI-TOF MS without an organic matrix. The standard deviations were obtained from three independent experiments.

Immunoblot analysis. The cells were centrifuged at 13,000 rpm for 10 min and the pellet was lysed in RIPA buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.1% SDS, 0.5% deoxycholate, 1.0% NP40, and 1 mM phenylmethanesulfonylfluoride) for 1 h on ice. The cell suspension was disrupted by sonication and pelleted at 13,000 rpm for 20 min at 4 °C. Total protein was separated by gradient SDS-PAGE (4–12% polyacrylamide). Proteins were transferred to a polyvinylidene difluoride membrane at 80 mA for 90 min. The membrane was blocked in 5 % skimmed milk for 1 h and incubated at 37 °C, and the membrane was immunoblotted with anti-c-Abl monoclonal antibody (1:500) and anti-β-actin antibody (1:1500). The membrane was incubated with horseradish peroxidase-conjugated anti-goat immunoglobulin, and signals were detected with an enhanced chemiluminescence system (Millipore).

LDI-TOF MS analysis. Mass analysis was performed using an Autoflex III MALDI-TOF mass spectrometer (Bruker Daltonics) equipped with a smartbeam laser as an ionization source. The spectra were acquired with a 19 kV accelerating voltage, a 100 Hz repetition rate, and a positive mode with an average of ~700 shots.

2. Additional data

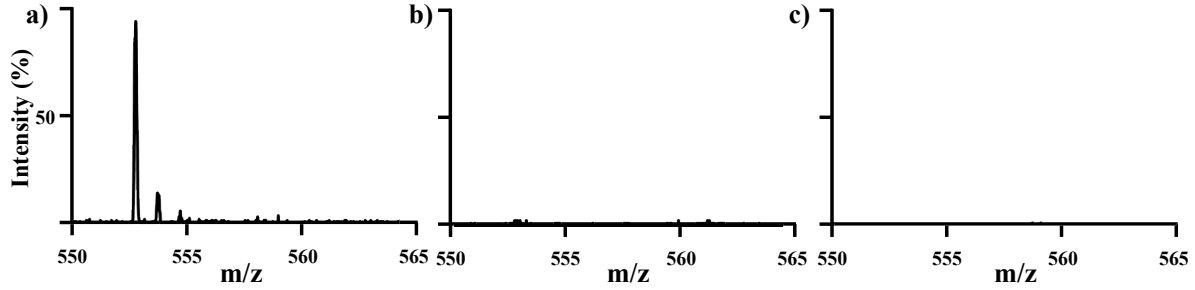

Figure S1. Detection of the Bcr/Abl chimeric protein in cell lysates using LDI-TOF MS. a) Am-tag signal at m/z 553 was clearly observed using anti-Bcr-coated AuNPs and anti-Abl-coated biochip. Control experiments using the anti-PSA instead of anti-Bcr on AuNPs (b) and instead of anti-Abl on a biochip (c) did not afford the peak at m/z 553.

Reference

1. Schwartzberg A M, Olson T Y, Talley C E and Zhang J Z *J. Phys. Chem. B.* **2006**, *11 0*, 19935.