Performance of scintillation proximity assay (SPA) to measure the level of VEGFR 1 protein

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1. Introduction

In medicine, the concentration of a specific protein can be use a biomarker which indicates the risk or progression of a disease, or susceptibility of the disease to a given treatment.

Scintillation proximity assay is a one of radioimmunoassay and can be assayed without the washing or filtration procedures normally used to separate bound from free fractions [1, 2]. Due to its simplicity and high-throughput protocol, it is broadly applicable to immunology, receptor binding, monitoring receptor-ligand interactions and enzyme reactions. Briefly, an antibody or receptor is coated on SPA beads. When a radiolabeled antigen or ligand binds to the beads, the SPA beads stimulate to emit short range electrons. The ^{3}H and ^{125}I are commonly used for radiolabeling and the produced photons are detectable with a liquid scintillation counter (LSC) [1, 2]. A binding affinity of unlabeled ligands can be determined by competitive reaction of the radiolabeled ligands.

Bevacizumab is a recombinant humanized monoclonal antibody, and can stop or delay growth of tumors by inhibiting vascular endothelial growth factor (VEGF) [3]. Bevacizumab was approved by FDA for metastatic cancer such as colorectal cancers, ovarian cancers, breast cancers and glioblastoma multiform of the brain. Recently, Dan G. duda et al. was reported that the concentration of vascular endothelial growth factor receptor-1 (VEGFR-1) in plasma may potentially be used as a negative selection biomarker [4].

In this study, we describe a method using scintillation proximity assay to detect the amounts of VEGFR-1 protein. This method is successfully used to measure the concentration of VEGFR-1 protein in human cell extracts.

2. Methods and Results

2.1 Preparation of cell lysate and determination of VEGFR 1 using western blot

HT-29 human colon cancer cells were obtained from the Korea Cell line Bank and cultured in RPMI1640 (LONZA), and supplemented with 10% fetal bovine serum, 300mg/L L-glutamine, 25mM HEPES, 25mM $NaHCO_3$, 100 units/ml penicillin, and 100 g/ml streptomycin (Sigma Aldrich) in an atmosphere of 5% CO_2 in air at 37℃. Cell lysate was obtained with RIPA lysis buffer (Thermo scientific), centrifuged and quantified. Protein was quantitated using the Bradford method (Bio-Rad Laboratories).

The detection of the VEGFR 1 in HT-29 cell extract was tested using western blot.

30μg of protein was separated by SDS-PAGE, and transferred to nitrocellulose membrane (Millipore). It incubated with the anti-VEGFR 1antibody (Sigma Aldrich) and detected via reaction with HRP-conjugated anti-mouse secondary antibody (Santa Cruz) and ECL chemiluminescence detection system (GE Healthcare).

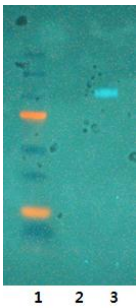

Fig. 1) Analysis of VEGFR 1 expression in HT-29 colon cancer cell by western blot
1; stained size marker (250, 150, 100, 75, 50, 37, 25kDa), 2; cell lysate of HT-29 colon cancer cell, 3; 1 μg of VEGFR 1 protein (standard)

The concentration of the VEGFR-1 protein from the HT-29 cell extract couldn't be determined by comparing the intensity of the standard VEGFR-1 protein detected using western blots.

2.2 Performance of scintillation proximity assay (SPA)

Anti-mouse Yttrium silicate (YSi) SPA beads were purchased from PerkinElmer. SPA beads were suspended in PBS buffer and used directly.

Radiolabeled VEGFR 1 protein as standard was obtained using iodogen. 30μg of VEGFR 1 protein was added in 0.3mCi I-125, and mix by gently. The mixture was reacted for 30 min, and the reaction mixture was transferred to a fresh test tube. The radiochemical purity was determination by instant thin-layer chromatography (TLC). Silica gel (SG) plate and saline as solvent were used.

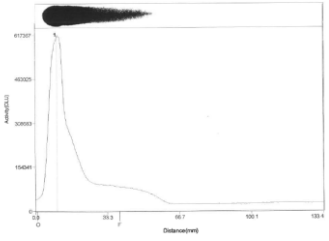

Fig. 2) Determination of I-125 labeled VEGFR 1 protein by iTLC

The I-125 labeled VEGFR 1 was diluted into PBS buffer containing 0.2% Bovine serum albumin (BSA).

For optimization of protein/beads ratio, the amount of SPA beads was varied at fixed concentrations of protein and radioligand.

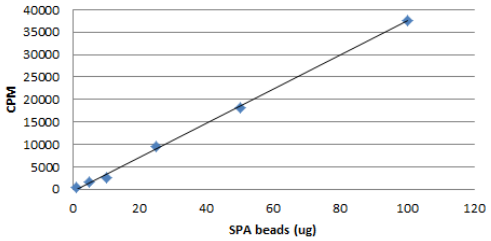

Fig. 3) Binding of fixed amount of I-125 labeled VEGFR 1 to anti-VEGFR 1 at various concentrations of SPA beads

In another experiment, the amount of I-125 labeled VEGFR 1 or anti-VEGFR 1 coupled to SPA bead was varied at fixed concentrations of SPA beads.

The protein/beads ratio was optimized to achieve the maximal signal to background ratio. Based on the specific binding, 5 μg of SPA bead is the minimum required to capture 1 μg of anti-VEGFR 1 and 1 μg of I-125 labeled VEGFR 1, and chosen for the rest of experiments.

The amount of VEGFR 1 in HT-29 colon cancer was measured in the presence of 5 μg of SPA beads, 1 μg of anti-VEGFR 1, 1 μg of I-125 labeled VEGFR 1 and 10- or 100-fold dilution of cell lysate in PBS buffer containing 0.2% BSA.

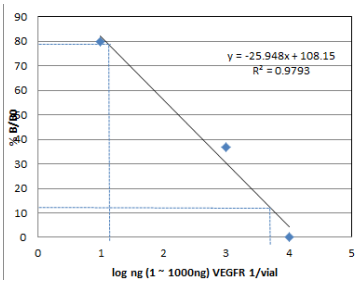

Fig. 4) Standard curve of VEGFR 1 protein

The concentrations of VEGFR 1 in 10- and 100-fold dilution of cell lysate are determined to be 5.6 and 4.9ng, respectively.

3. Conclusions

In summary, a simple and sensitive assay is developed for measuring the amount of VEGFR 1 protein in cancer cell lysate using SPA beads. The antibody coating on the beads and antigen binding are achieved in one mixing step. The beads are mixed with anti-VEGFR 1 antibody, I-125 labeled VEGFR 1 protein, and cell lysate simultaneously in a glass bottle. This simplified assay is readily adaptable for other diagnosis systems.

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