2012 2nd International Conference on Environment Science and Biotechnology
IPCBEE vol.48 (2012) © (2012) IACSIT Press, Singapore
DOI: 10.7763/IPCBEE. 2012. V48. 28

Detection of Abnormal Gene in Leukemia by Magnetic Nanoparticles

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Abstract. A novel technique for abnormal gene detection was developed by using combination of magnetic nanoparticles (MNPs) with reverse transcriptase-polymerase chain reaction (RT-PCR) and enzyme substrate system. In this study, the *BCR/ABL* gene targeting presented in chronic myeloid leukemia (CML) was a model for fusion gene detection. MNPs were used as the solid phase for molecular binding of *BCR* forward primer via carbodiimide method whereas *ABL* reverse primer was bound to biotin. After finished RT-PCR, external magnetic field was applied to separate the PCR products bound MNP and, finally, the optical detection can occur after subsequently adding of streptavidin horseradish peroxidase (HRP), peroxidase substrate and hydrogen peroxide to the product. Our result showed high sensitivity to detect the abnormal *BCR/ABL* gene with detection limit of lower than 1 pg of RNA in the sample, and high specificity to K562, *BCR/ABL* positive cell line.

Keywords: *BCR/ABL* gene, magnetic nanoparticle (MNPs), chronic myeloid leukemia (CML)

1. Introduction

Magnetic nanoparticles (MNPs) have been used in combination with ligand and molecular probes for efficient diagnosis in many diseases such as infection or cancer [1-2]. Chronic myelogenous leukemia (CML) is one of the major causes of cancer-related death occurred by chromosomal translocation (t(9,22)) or *BCR/ABL* fusion gene which is normally used as a model for abnormal gene detection [3]. Although CML patients can be diagnosed by examination of complete blood count but *BCR/ABL* gene is needed to identify for diagnostic confirmation. Therefore, the *BCR/ABL* gene is become more important for disease monitoring after treatment by quantitative-PCR (qPCR) which requires the experienced staffs and costly [4-5].

Herein, we have developed a new technique by using MNPs in combination with conventional PCR and enzyme substrate system for detection of abnormal gene level of CML [6]. The technique was practical and provided high sensitivity and specificity.

2. Materials and Methods

2.1. Samples Collection

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K562 (human chronic myelogenous leukemia cell line) and L929 (fibroblast cell line) were cultured with RPMI-1640 and supplemented with 10% heat-inactivated fetal calf serum (FCS), were used as positive and negative controls. Bone marrow sample from leukemia patients, two CML and two acute myeloid leukemia (AML), were collected from Ramathibodi Hospital (Bangkok, Thailand). White blood cell (WBC) was separated by centrifugation technique [7].

2.2. Immobilization of Primers on MNPs

MNPs with carboxyl surface functional groups were used in the experiment. After washing twice with 25 mM 2-(N-morpholino)ethanesulfonic acid (MES) buffer (pH 6.0), these carboxylated MNPs were immobilized with NH2-C6-modified BCR forward primer (Bio Basic Canada Inc., Canada) by using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) as a coupling agent [6]. The immobilized MNPs were then resuspended in Tris-EDTA buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0) before storage at 4°C.

2.3. Amplification Steps

RNA of cell lines and WBC were extracted by phenol-chroloform method and cDNA was synthesized by reverse transcriptase. The RNA concentration measured by NanoDrop (Thermo Scientific, USA) was varied by 10X serial dilution from K562 cells. The PCR (DNA Engine Peltier Thermal Cycler, Bio-Rad, USA) was performed by using BCR forward primer bound MNPs and biotinylated ABL reverse primer (5' Biotin–ABL reverse primer) (Bio Basic Canada Inc., Canada) [6] with taq DNA polymerase (RBC, Taiwan). The temperature for denaturation, annealing and elongation was at 95ºC, 63ºC and 72ºC, respectively.

2.4. Detection steps

PCR products were separated by magnetic field, and then incubated with HRP labeled streptavidin. Addition of 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) and hydrogen peroxide was then carried out to generate colored oxidized product. The optical density (OD) of the product was measured at 405 nm by using spectrophotometer (DTX800, Beckman Coulter).

3. Results

3.1. Specificity of Detection

Determination of specificity of the technique was performed by using K562 and L929 cells as positive and negative control. The OD of enzyme substrate system is shown in Fig 1. Only K562, BCR/ABL positive cell line, showed the increasing of OD while L929, BCR/ABL negative cell line, exhibited the OD closed to blank (DEPC-treated water).

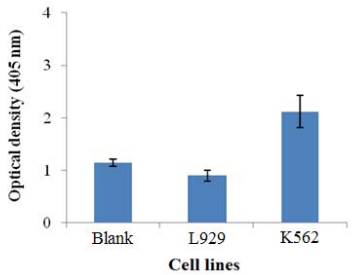

Fig. 1: OD of K562 and L929 cells compared with blank (DEPC-treated water).

Leukemic samples were analyzed with enzyme substrate system and the result was shown in Fig 2. The samples were presented with AML1-2, CML1-2 and blank, respectively. It was clearly seen that the OD of CML 1-2 (OD of CML1= 2.43, CML2=1.86) was significantly higher than that of AML 1-2 (OD of AML1=1.04, AML2=1.08) which exhibited the OD nearby the blank.

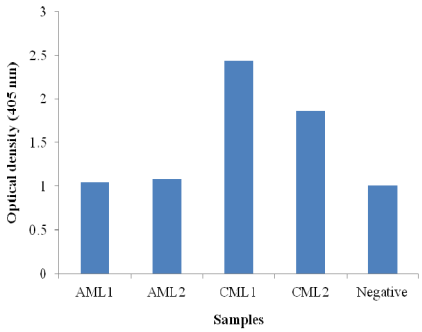

Fig. 2: OD of leukemic patients, AML and CML compared to negative control.

3.2. Limit of Detection

RNA concentrations of K562 cells were measured before reverse transcription and varied from 1 µg to 1 pg for determination of the limit of detection in this technique. The OD of the oxidized colored product at various RNA concentrations was displayed in Fig. 3. It was observed that the OD of the sample ranged from µg to pg was higher than that of the negative control.

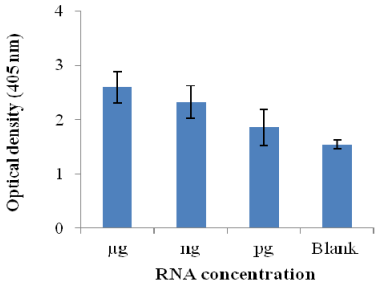

Fig. 3: OD of the oxidized colored product at various RNA concentrations (blank: DEPC-treated water).

4. Discussion

For specificity, the OD was increased only in K562, the *BCR/ABL* gene presented. While L929 cell, the *BCR/ABL* negative cell was not. CML1 and CML2 which presented with *BCR/ABL* gene was increased the OD in similar to what we found in K562 cell line whereas AML was not. We suggested that this technique was highly specific to sample presented the *BCR/ABL* gene.

Interestingly, our technique showed the lowest amount of total RNA was pg level which was in the same scale as that of the real-time PCR, the standard method for gene measurement.

5. Conclusion

In this work, the MNPs in combination with PCR and enzyme-substrate technique were successfully used to detect the *BCR/ABL* abnormal gene of CML patient. The technique was very sensitive (pg of RNA template) and specific to CML-positive cell line and also in leukemic patients. It has a potential to be an alternative method for early diagnosis and disease follow-up in CML patients.

6. Acknowledgements

Research grants from the office of Higher Education Commission and Mahidol University under The National Universities Initiative to KJ, The Thailand Research Fund (TRF)/ the Commission on Higher Education (CHE) (RTA5480007) to PT, and the scholarships from National Science and Technology Development Agency (NSTDA) under Thailand Graduate Institute of Science and Technology (TGIST) (TGIST 01-54-035) to YM and from Faculty of Graduate Studies (academic year 2010), Mahidol University under The 60th Year Supreme Region of His Majesty King Bhumibol Adulyadej Scholarship, were gratefully acknowledged. Rerkarmnuaychoke B, Dittharot Y, Peng-On J and staffs at Ramathibodi Hospital, Mahidol University, are also acknowledged for patient samples and technical support.

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