CROSSTALK-ELIMINATED QUANTITATIVE DETERMINATION OF AFLATOXIN B1-INDUCED CANCER STEM CELLS IN HEPATOCELLULAR CANCER

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Abstract - In this work, it was found that hepatocellular cancer stem cell (CSCs) were produced from HepG2 cells by aflatoxin B_1-induced mutation and their amount were quantitatively determined using a crosstalk-eliminated multicolor cellular imaging based on quantum dot (Qdot) nanoprobes and an acousto-optical tunable filter. Hepatocelluar CSCs were acquired from HepG2 cells via magnetic bead-based sorting and observed using concurrent detection of three different markers CD133, CD44 and aldehyde dehydrogenase1 (ALDH1). The DNA mutation of HepG2 cells caused by aflatoxin B_1 was quantitatively observed via absorbance spectra of aflatoxin B_1-8,9-epoxide-DNA adducts formed in HepG2 cells by the treatment of HepG2 cells with aflatoxin B_1. The number percentages of hepatocellular CSCs formed in the entire HepG2 cells were determined to be 9.77±0.65%, 10.9±1.39%, 11.4±1.32%, and 12.8±0.7%, respectively at the control, 5μM, 10μM, and 20μM aflatoxin B_1. The results were well matched with those obtained using flow cytometry used as a general tool for the detection of hepatocellular CSCs. This study demonstrates that accurate quantitative measurement of aflatoxin B_1-induced hepatocellular CSCs can be accomplished using a constructed multicolor cellular imaging system that eliminates the crosstalk among hepatocellular CSC biomarkers.

Index Terms - Aflatoxin B_1, cancer stem cell, quantum dot, multicolor cellular imaging.

I. INTRODUCTION

Early diagnosis of HCC is an essential issue to prolong the lifetime of liver cancer patients. DNA sequence has been used mainly to analyze mutagenic effects of Aflatoxin B_1 on genomic DNAs. The identification of HCC markers with high sensitivity and specificity is another effective way for the diagnosis of HCC. Recently, tumor-initiating cell termed as cancer stem cell (CSC) has received great attention in cancer therapy due to its anticancer drug and radiation resistance. The resistive properties of CSCs have been considered to be the source of postoperative recurrence and metastasis. Functional liver CSCs have been reported to be found in HCC cell lines. CD133 has been used as a CSC surface marker for the isolation and detection of liver CSCs.

The cancer diagnosis using quantum dot (Qdot) nanoprobe-based high-content cellular imaging has various advantages which may be appropriate for clinical application for early cancer diagnosis. Concurrent detection of cancer markers at the single cell level can provide a promising information on identification, pathological condition, and prognosis of cancer. Qdots possess excellent physical properties such as high sensitivity and strong photostability as imaging nanoprobe. In addition, Qdot nanoprobe is much more suitable than conventional dyes for the simultaneous detection of cancer markers because of its narrow wavelength range of emission spectrum and larger absorbance at shorter wavelength in the range of ultraviolet to visible wavelength. These properties enable concurrent monitoring of many Qdots through excitation at a single wavelength and promise more accurate cancer diagnosis. In this work, for the first time, quantitative high-content determination of aflatoxin B_1-caused hepatocellular CSCs is reported. The quantitative determination was executed using a hypermulticolor cellular imaging system based on a acousto-optical tunable filter (AOTF) and Qdot nanoprobes. The AOTF-based hypermulticolor cellular imaging was used very efficiently for the identification of hepatocellular CSCs induced by aflatoxin B_1. This was achieved by spectral overlap-free simultaneous monitoring of CD133, CD44, aldehyde dehydrogenase 1 (ALDH1) markers, which are specific to hepatocellular CSCs.

II. MATERIALS AND METHODS

A. Cell sorting using microbead

HepG2 cells were treated with aflatoxin-B_1 as a function of concentration (5 μM, 10 μM, and 20 μM) for 48 h at 37℃ under 5% CO_2. CD133, CD44 and ALDH1 cells were isolated from aflatoxin-B_1 treated cells. The cells were isolated according to the manufacturer's instructions (microbead kit human;MACS Miltenyi Biotec)

B. Quantum dot-antibody conjugation and imaging cytometry

CD133, CD44 and ALDH1 antibodies were conjugated to quantum-dot (QD) 705, QD525 and QD625 (Quantum Dot Conjugation Kit; Invitrogen, Carlsbad, CA, USA), respectively. QD has a specific

emission wavelength so that the QD-antibody conjugations were detected at their own emission wavelengths. For the simultaneous detection of CD133, CD44 and ALDH1, the AOTF was scanned as a function of wavelength, and the transmitted emissions of biomarkers were detected on the CCD.

C. Flow cytometry

HepG2 cells were treated with aflatoxin-B_1 as a function of concentration (5 μM, 10 μM, and 20 μM) for 48 h at 37℃ under 5% CO_2. The HepG2 cells were treated with the accutase for 20 min at 37℃. The attached cells were treated with 4% formaldehyde and 0.2% saponin under the same conditions used in the imaging cytometry. The cells were then treated with 1:200 diluted CD44 antibody-QD conjugation and CD133 antibody-QD conjugation for 2 h at room temperature. After 2 h, the cells were washed with 1 mL 1xPBS solution and centrifuged at 230 g for 3 min twice. The supernatant was removed, and the pallet was resuspended with 2 ml 1xPBS solution. Then, the resuspended cells were subjected to flow cytometric analysis using a FACS Calibur (FACS Calibur; BD Bioscience).

III. RESULTS AND DISCUSSION

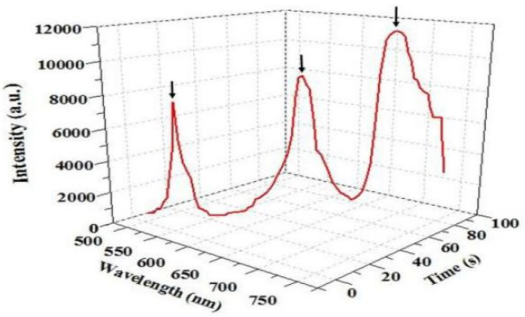


Figure 1. A spectrum of a mixture solution containing quantum dots used for the conjugation to CD133, CD44, and aldehyde dehydrogenase1.

Fig.1 clearly represents how high-content detection of hepatocellular CSCs can be achieved without the crosstalk among three different CD133, CD44, ALDH1 biomarkers labelled with Qdot-antibody conjugates. The emission spectrum of a mixture solution composed of CD133, CD44, and ALDH1 Qdot-antibody conjugates was obtained as a function of wavelength from 500 nm to 748 nm with the interval of 3 nm by the constructed multicolor cellular imaging system. The CCD detector was operated coincidentally as the AOTF was scanned as a function of wavelength. The emission intensities were obtained at every 1 second.

Fig. 2 clearly shows that the number of hepatocellular CSC increases from HepG2 cells treated with aflatoxin B_1 as the treatment concentration of aflatoxin B_1 increases from 5 μM to 20 μM. Biomarkers used for the detection of hepatocellular CSCs were CD133, CD44, and ALDH1. Hepatocellular CSC has subtype of CD133-positive, CD44-positive, and ALDH1-positive. HepG2 cells treated with aflatoxin B_1 was sorted using the CD133 microbeads. The collected CD133-positive cells were immune-stained with CD133 antibody-QD705, CD44 antibody-QD525, and ALDH1 antibody-QD625. The percentages of CSCs were determined to be 9.7%, 10.9%, 11.4% and 12.8%, respectively when the treatment concentrations of aflatoxin B_1 were control, 5 μM, 10 μM, and 20 μM.

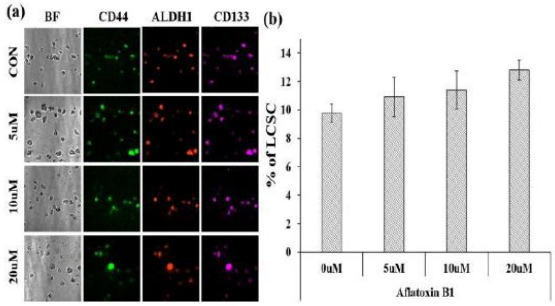


Figure 2. (a) High-content detection of hepatocellular cancer stem cells isolated through the bead sorting from HepG2 cancer cells treated with aflatoxin B_1 as a function of its concentration. CD133, CD44, and aldehyde dehydrogenase1 markers specific to hepatocellular cancer stem cells 1 were concurrently detected using the antibody-quantum dot conjugates. (b) Quantitative determination of hepatocellular cancer stem cells as a function of aflatoxin B_1 treatment concentration.

Flow cytometric detection of hepatocellular CSC was executed to compare its result with that obtained by the multicolor cellular imaging, and to verify accuracy of the multicolor cellular imaging as a tool for the detection of hepatocellular CSC. As shown in Fig. 3(a), the hepatocellular CSC proportions were found to be 8.62%, 11.1%, 15.5%, and 16.3%, at control, 5 μM, 10 μM and 20 μM aflatoxin B_1 treatment concentration, respectively. These values were plotted as a bar graph in Fig. 3(e). Compared to the control, the increase of aflatoxin B_1-induced CSC ratio was determined to be 2.48%, 6.88% and 7.68%, at 5 μM, 10 μM, and 20 μM, respectively.

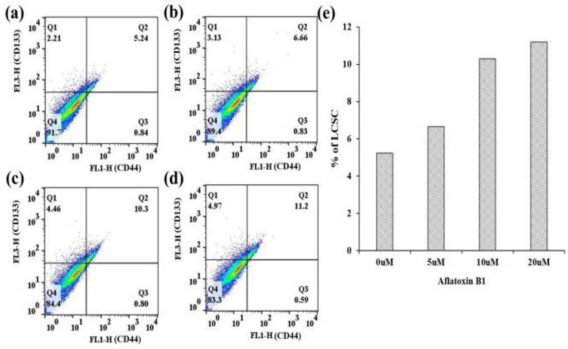


Figure 3. (a)-(d) Flow cytometric detections of hepatocellular cancer stem cells acquired from HepG2 cancer cells as a function of aflatoxin B_1 treatment concentration. The upper right section corresponds to the hepatocellular cancer stem cell. (e) Quantitative determination of hepatocellular cancer stem cells obtained using flow cytometry.

CONCLUSION

This work demonstrated quantitative Qdot-based multicolor cellular imaging of hepatocellular CSCs produced by the treatment of HepG2 cells with aflatoxin B_1. Aflatoxin B_1 was metabolized to aflatoxin B_1-8,9-epoxide that is highly instable and an active carcinogen in HepG2 cells and formed aflatoxin B_1-8,9-epoxide-DNA adducts. As the concentration of aflatoxin B_1 increased, larger amount of aflatoxin B_1-8,9-epoxide-DNA adducts was found. In the identical concentration range of aflatoxin B_1, the hepatocellular CSCs were formed and their number increased as a function of aflatoxin B_1 concentration. The formed hepatocellular CSCs could be successfully detected based on the simultaneous monitoring of CD133, CD44 and ALDH1 without the crosstalk among them.

REFERENCES

[1] Chawanthayatham, S., Thiantanawat, A., Egner, P.A., Groopman, J.D., Wogan, G.N., Croy, R.G., Essigmann, J.M., 2015. Int. J. Cancer 136, 1254–1262

[2] Hashimoto, N., Tsunedomi, R., Yoshimura, K., Watanabe, Y., Hazama, S., Oka, M., 2014. BMC Cancer 14, 722

[3] Chittmittrapap, S., Chieochansin, T., Chaiteerakij, R., Treeprasertsuk, S., Klaikaew, N., Tangkijvanich, P., Komolmit, P., Poovorawan, Y., 2013. Asian Pac. J. Cancer Prev. 14, 7675–7679

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