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Quantum dot nanoprobe-based high-content monitoring of notch pathway inhibition of breast cancer stem cell by capsaicin

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ABSTRACT

Breast cancer is the major cause of cancer death for women worldwide. Breast cancer patients are treated with chemotherapy and radiotherapy. Although chemotherapy and radiotherapy are applied, some cancer cells still survive. These cells, called cancer stem cell (CSC), exhibit special capabilities, such as drug and radio resistance. The remaining CSC can trigger cancer recurrence. Thus, it is critical to find an effective way to target CSC. Capsaicin has been reported to affect anticancer activity in many cancers. It also has been shown that capsaicin induces apoptosis in the MCF-7 breast cancer cell line. In this study, we demonstrate that capsaicin causes dose-dependent growth disruption in breast CSC and inhibits translocation of notch intracellular membrane domain (NICD) into the nucleus. MCF-7 cells were treated with capsaicin at various concentrations (5 μM, 10 μM, and 20 μM) for 24 h. After capsaicin treatment, it was found that the number of breast CSC (%) decreased as the treatment concentration of capsaicin increased. This result was also confirmed with FACS. NICD translocation to the nucleus and apoptotic cell death of breast CSC were concurrently observed at the single breast CSC level using highly sensitive quantum dot (Qdot)-antibody nanoprobes. The control breast CSCs without the capsaicin treatment were able to translocate NICD into the nucleus. On the other hand, translocation of NICD into the nucleus was not observed in capsaicin-treated cells. In addition, apoptotic cell death was caused when the breast CSC were treated with capsaicin at more than 10 μM. Although many studies have shown that capsaicin produces anticancer activity in cancer cell lines, the present result is the first report to demonstrate that capsaicin is capable of causing breast CSC apoptotic cell death via inhibiting its notch signaling pathway.

1. Introduction

In recent years, cancer stem cell (CSC) has received great attention as a key factor in the treatment of cancer. In CSC theory, it is thought that only a small subset of cells in the tumor is capable of initiating and maintaining tumor growth. Those cells are considered to be CSC. Particularly, CSC is known to be responsible for the multiple drug resistance (MDR) of cancer. Conventional chemotherapy has been developed mainly against bulk cells of tumor. Although existing therapies obviously contribute to the shrinkage of tumor, regression of tumors can be observed frequently due to the MDR of CSCs to permit regrowth of tumors [1,2]. Accordingly, chemotherapy specifically against CSCs should constitute a more durable therapeutic strategy for the cure of tumors. Notch signaling pathway is involved in embryonic development and the proliferation, differentiation, and apoptosis of stem cells. In most human cancer, notch performs a function as an oncogenic protein. G-secretase inhibitors have been reported to cause inhibition of the notch pathway in glioblastoma [3,4]. As a result of blocking the notch pathway, the reduction of tumor growth, as well as expression level of CSC markers, was observed. In addition to the notch signaling pathway, the Hedgehog (Hh) and Wnt signaling pathway have received attention as main cellular signal transductions to be deactivated for eradicating CSCs.

High-content cell-based assay offers a promising intracellular observation to verify whether a particular cellular signal transduction is inhibited or activated by the drug being tested. Especially, high-content monitoring is quite appropriate for simultaneous monitoring of CSC markers, which usually consists of more than three biomarkers. Based on excellent brightness and strong photostability, Quantum dot (Qdot)-antibody nanoprobe is capable of playing a very efficient role as a nanoprobe to maximize

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imaging excellence inherent to high-content assay that clearly shows activation of cellular signal transduction or biomarkers related to CSC without the need of cell lysis [5–10]. In this work, for the first time, it is demonstrated that capsaicin disrupts the notch signaling pathway of breast CSC and reduces the number of breast CSCs using Qdot-based high-content monitoring. Translocation of notch intracellular membrane domain to CSC nucleus and apoptotic CSC death were concurrently observed as a function of capsaicin concentration. The present study proves that capsaicin can target the notch pathway of CSC and contribute to therapy specifically against CSC, which is considered to be the main reason for MDR of cancer.

2. Experiment

2.1. Cell culture

MCF-7 was obtained from the Korean Cell Line Bank. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM; 11995–073, Gibco) supplemented with 10% heat-inactivated fetal bovine serum (FBS; 16000–044, Gibco), 5 mg/ml penicillin, 5 mg/ml streptomycin, and 10 mg/ml neomycin (PSN; 15640–055, Gibco) at 37 °C under 5% CO_2.

2.2. Capsaicin treatment

MCF-7 cells (1.8×10^7 cell) were cultured in T125-cell culture flasks and kept overnight at 37 °C in 5% CO_2 atmosphere to maintain the cells in a log phase, followed by the cells were kept in FBS and antibiotic-free DMEM for 4 h at 37 °C in 5% CO_2 atmosphere. Then, cells were treated with various concentrations of capsaicin (Capsaicin; M2028, SIGMA) dissolved in DMSO (5 μM, 10 μM, and 20 μM) for 24 h at 37 °C in 5% CO_2 atmosphere. The final DMSO concentration was maintained under 0.1% and served as the control.

2.3. Cell sorting using microbead

One of the CSC markers is CD44. MCF-7 cells were sorted to select CSC. The sorting buffer was composed of 47.3 mL 1xPBS, 2.5 mL 10% BSA, and 200 μL 0.5 M EDTA. Capsaicin treated MCF-7 cells were washed with 1xPBS and treated with accutase enzyme for 20 min at room temperature. Then, the attached cells were detached from the surface of the cell culture plate, collected, and centrifuged at 230 rcf for 3 min. The supernatant was discarded, and the pellet was resuspended in 80 μL sorting buffer. The resuspended cells were then centrifuged at 300 g for 10 min. After centrifugation, the supernatant was discarded, the pellet was resuspended in 20 μL CD44 microbead solution (CD44 microbead kit human; 130-095-194, MACS Miltenyi Biotec), and it was incubated for 15 min at 4 °C. The cells were resuspended in 1 mL sorting buffer and centrifuged at 300 g for 10 min. The supernatant was discarded, and the pellet was resuspended in 500 μL sorting buffer. The LS column (LS column; 130-042-401, MACS Miltenyi Biotec) was placed in the magnetic field of the MACS separator. Pre-separation filter (Pre-separation filter; 130-041-407, MACS Miltenyi Biotec) was put on the LS column. Next, the column and filter were rinsed with 1 mL sorting buffer three times. The resuspended cells were applied on the pre-separated filter. Then, the column was rinsed with 1 mL sorting buffer three times. The columns were removed and placed in a 15 mL tube. The column was then washed with 5 mL sorting buffer. The flushed out fraction was composed of CD44 magnetically labeled cell.

2.4. Qdot-antibody conjugation and imaging cytometry

CD44, CD24, and ALDH1 antibodies were conjugated to Qdot525, Qdot565, and Qdot625 (Qdot Conjugation Kit; Invitrogen, Carlsbad, CA, USA), respectively. The antibodies were treated with dithiothreitol (DTT) for antibody reduction to break the disulfide bond. Then, the antibodies were conjugated to maleimide-functionalized Qdot. The Qdot-antibody conjugations were incubated at room temperature for 1 h. Then, the conjugations were treated with 2-mercaptoethanol for removing the maleimide group. The Qdot-antibody conjugations were applied over the column for elimination of unconjugated Qdot. The Qdot-antibody conjugation was diluted at 1:200 with 1% BAS. The sorted MCF-7 cells were then treated with 4% formaldehyde for 10 min at room temperature. Next, the cells were washed with 1 mL 1xPBS and centrifuged at 230 g for 3 min. Then, the supernatant was discarded, and the pellet was resuspended in 1 ml 1xPBS. The cells were then washed again, and the pellet was treated with 0.2% saponin for 10 min at room temperature. Then, the cells were washed with 1xPBS twice. The washed cells were incubated with the diluted Qdot-antibody conjugations at room temperature for 1 h. After 1 h, the MCF-7 cells were washed with 1 mL 1xPBS and centrifuged at 230 g for 3 min. After centrifugation, the supernatant was discarded and washed again. The pellet was then resuspended in 50 μL 1xPBS. Next, taking 10 μL cells, it was placed in the 1.5μ-slide vi (1.5μ-slide vi; ibide GmbH, Am Klopferspitz 19, 82152 Martinsried, Germany). Qdot has a specific emission wavelength, so that the Qdot-antibody conjugations were detected at their own emission wavelengths. For the simultaneous detection of biomarkers, the acousto-optical tunable filter (AOTF) was scanned as a function of wavelength, and the transmitted emissions of biomarkers were detected on the CCD.

2.5. Flow cytometry

Flow cytometry was used as a complementary technique to compare with imaging cytometry. CD44 and ALDH1 antibody-Qdot conjugations were employed to detect CSC. MCF-7 cells were treated with various concentrations of capsaicin (5 μM, 10 μM, and 20 μM) for 24 h at 37 °C. Then, the MCF-7 cells were treated with accutase for 20 min at 37 °C. The attached cells were treated with 4% formaldehyde and 0.2% saponin in the same conditions used in image cytometry. The cells were treated with 1:200 diluted CD44 antibody-Qdot conjugation and ALDH antibody-Qdot conjugation for 1 h at room temperature. After 1 h, the cells were washed with 1 mL 1xPBS and centrifuged at 230 g for 3 min twice. The supernatant was discarded, and the pellet was resuspended with 2 ml 1xPBS. Then, the resuspended cells were subjected to flow cytometric analysis using FACS Calibur apparatus (FACS Calibur; BD Bioscience).

2.6. Confocal microscopic study

Notch pathway signal was observed in MCF-7 cells. MCF-7 cells were sorted with CD44 microbead. Sorted cells were grown in a glass slide for 24 h at 36 °C. The cells were treated with various concentrations of capsaicin (5 μM, 10 μM, and 20 μM) for 24 h. Afterwards, the cells were treated with 4% formaldehyde and 0.2% saponin in the same conditions used in image cytometry. The cells were treated with 1:200 diluted NICD antibody-Qdot conjugation in 1% BSA for 1 h (cleaved Notch 1; m1711, Santa Cruz), 200 μL of Hoechst 200 ng/mL for 20 min at room temperature, and 5 μL of FITC-Annexin V for 15 min at room temperature. (FITC-Annexin V Apoptosis Detection Kit; 51-6710AK, BD Pharmigen). Then, the cells were washed with 1 mL 1xPBS twice carefully. The intracellular

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