Research Article

Dendrimer-Trastuzumab Contrast Agent Improves Imaging of Magnetic Resonance Imaging (MRI) On Her2 Positive Breast Cancer Cell Line HCC-1954 in Apple Scaffold

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I

Magnetic Resonance Imaging (MRI) is a radiological imaging examinations that exploits proton elements (hydrogen ion) residing in human tissue. e advantage of MRI imaging compared to CT scan are a non-ionizing radiation imaging, multiple image/multiplanar drawing images, good spatial resolution, no bone artifact, noninvasive and superior able to assess so tissues such as breast cancer [1]. e general approach to MRI data analysis involves the extraction of signals from a given area. e use of ROI analysis becomes standard practice across all MRI imaging [1,2].

One way to improve imaging quality of MRI is administration of contrast agent. MRI contrast agent is a paramagnetic compound having considerable moments and able to shorten the relaxation time in T1 and T2 weighing mode, which will result in a more vivid and clearly discernible tissue image [3,4].

Gadolinium chelate is a widely used as MRI micromolecular contrast agent, acting on extracellular and non-speci c. To overcome non-speci c trait to become speci c agent, another compound is needed. e target is that the contrast compound would be able to be retained in intracellular and a clearer image. Hardiani et al. have successfully performed the synthesis and stability test of Radiogadolinium (III) Gadolinium-DOTA-PAMAM (Poliamidoamine) Generation 3.0-Trastuzumab as SPECT contrast agent (Single Photon Emission Computed Tomography) - MRI contrast agent for breast cancer diagnostics [5-7]. Previous result successfully demonstrated a strong signal intensity increase on MRI examination with the Gd-DOTA-Dendrimer-Trastuzumab contrast compound against the SKOV3 cell line. SKOV3 is a positive HER-2 cell line in ovarian cancer that is sensitive to trastuzumab.

HCC cell line 1954 breast cancer which is a cell line of breast cancer of HER-2 positive expression resistant to Trastuzumab. Approximately 25-30% of breast cancers express HER-2 (Human epidermal growth factor receptor 2), although the incidence is low but has poor biological behavior in the context of aggressiveness, thus a ecting its prognosis [8-10].

Cell culture is a technique associated with a complex process of cell isolation from the original environment (*in vivo*) as well as in controlled and aseptic (*in vitro*) environmental conditions. e cell is taken from the original tissue, primary culture or cell line in the form of suspension of cells isolated for days to weeks in sterile conditions with

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an environment adapted to the *in vivo* environment of the cell so as to survive and controlled proliferation occurs [11-15].

Cell cultures can be planted in apple scaffolds. Characteristics of apples used are apples of fuji type. The apple scaffold after peeling is frozen in temperature -80°C, and then incorporated into the methanol solution resulting in deselularization of the organelles within the apple cytoplasm. Then cell line culture is planted in the framework of apples that have experienced the deselularization [16-20].

The purpose of this study was to evaluate Dendrimer-Trastuzumab in enhancing of imaging using MRI in cell line HCC 1954 on breast cancer HER-2 positive expressions.

Materials and Methods

Chemicals

Roswell Park Memorial Institute (RPMI) 1640 medium, fetal bovine serum (FBS), and penicillin streptomycin were purchased from GIBCO, USA. Dimethylsulfoxide (DMSO), and 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) were purchased from Sigma-Aldrich, USA. All the other chemicals were of analytical grade purchased from Merck, USA.

Gd-DOTA-Dendrimer-Trastuzumab was developed in-house by

Apple scaffold 3D

Apple scaffold was made and modified according to previous study (Figure 1). Briefly, Fuji apple was put in a -20°C freezer for 5 minutes before punched with micro-centrifuge tube 2 ml and cut to a uniform tube form with 1 cm height. Tissue was decellularized from apple scaffold using 0.5% sodium dodecyl sulphate (SDS) (Sigma-Aldrich) solution for 24 hours then washed and incubated with PBS (GIBCO) for 24 hours.

For culture, apple scaffold was centrifuged at 1000 rpm for 10 seconds to drain remaining PBS then placed on 24-well-plate followed by 0.5 ml cell suspension of 1 x 10^6 breast cancer cells. Sample was then incubated in temperature of 37°C and 5% CO_2 for an hour before added 1.5 ml complete medium.

Experiments were conducted in triplicate and two repeats. Breast cancer cell line was cultured in apple scaffold for 8 weeks inside a 12 well plate with RPMI medium. Culture condition was monitored and medium was changed twice a week.

HE

Fixation of cell inside apple scaffolding was done using cold methanol 100% for 10 minutes. The sample were then washed with pH balanced PBS 3x and then stored for one day before embedded in paraffin with standard method. Paraffin block then sliced into 5 µm slice using microtome and mounted on glass slide. Hematoxylin and Eosin stain was done to visualize structure of interest.

Image was acquired using Olympus CX-51 inverted microscope with mounted digital camera.

GD-DOTA

To evaluate enhancing MRI imaging of GD-dota-dendrimer trastuzumab in HER2+ breast cancer cells, HCC-1954 cells in apple scaffolds were treated with 0.5 nM of Gd-DOTA-Dendrimer-Trastuzumab or Gd-DOTA for 24 hour prior to scanning with MRI.

MRI (Magnetic Resonance Imaging)

MRI is an imaging modality that has the magnetic properties that each proton has in producing the image. Every human body is made up of atoms. These atoms will form a water molecule consisting of 2

Figure 4: There was a trend in increase of MRI signal intensity of cultured apple scaffold as shown with white spheres using contrast of (a) Gd-D or (b) Gd-D-T, and (c) ROI data, although not signifcantly signifcant (p=0.05).

Although the results of statistical analysis with p value 0.06 (p>0.05) but not necessarily reduce the bene ts of clinical aspects [22]. HCC cell line 1954 cancer of HER-2 positive expression is a cell line that is resistant to trastuzumab, so at some internalization does not occur endocytosis.

e intensity of the signal resulting from the bonding of the Gd-DOTA and dendrimer contrast compounds forms the macromolecules to enhance the imaging.

is study has several limitations among others; no trials of trastuzumab in HER-2 cell line HCC 1954 cancer of positive HER-2 breast cancer were performed.

C

There is a trend of signal intensity increase between apple scaffold and apple scaffold with cell line; i.e. there was a binding of contrast compound Gd-DOTA-Dendrimer-Trastuzumab in cell line HCC 1954, although the increase of signal intensity on the average MRI examination of the Gd-Dotrim-Dendrimer-Trastuzumab contrast group was not significantly significant (p 0.06). Further tests are needed to prove trastuzumab attached to the HER-2 receptor of breast cancer.

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