A novel auto-adhere method for the capture and analysis of live circulation tumor cells

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Abstract: - A novel auto-adhere method was proposed based on auto-adhesion process driven by chemokines for the screening of circulating tumor cells within the whole blood samples. Initial fluorescent assay tests showed that when combined with inflammation induced from IL-1, live cancer cell line Hep G2 would attach to the surface of epithelium cells, but not the dead ones after the surface was washed with PBS. Therefore, it is concluded that this auto-adhere CTCs diagnosis method may serve as a possible platform for future development for cancer early detection and treatment stage assessment.

Key-Words: - Cancer, Epithelium cell, IL-1, TNF-a, Inflammation, Cell adhesion, Neutrophil, Hep G2cell

1 Introduction

The detection of live circulating tumor cells (CTCs) in the peripheral blood would offer several advantages in early diagnosis. With disadvantage of loss of morphological information and high false-positive rates, CTC detection method such as RT-PCR [1], and flow cytometry [2] would also need specific markers in micro metastases detection, for example, prostate-specific antigen for prostate cancer [3-4], CK- 19, CK-20, for colon cancer [5, 6] and tyrosinase for the melanoma [7-8]. Moreover, cell-separation technique based on large magnetic beads that have several hundreds or thousands of antibodies on the bead surface was proposed subsequently for the isolation of viable hematoprogenitor cells and specific immune cells [12-14]. However, its detection liability is often compromised by the fact that not all CTCs would present expression of epithelial-specific antigens [9-11].

Several recent studies have implicated multifunctional cytokines, such as interleukin-1 beta (IL-1beta) and tumor necrosis factor-alpha (TNF-alpha) in the activation of epithelium and vascular endothelium. Particularly relevant to the presence of ICAMs and VCAMs were the observed mediators that act directly on monolayer to increase the adhesion of blood leukocytes and cancer cells [15]. Nevertheless, discoveries in the mechanisms and modulators of direct adhesive interactions between tumor cells and endothelial cells, inflammation treated epithelium cells are also shown to play a key role in the formation of micro metastases [16]. Therefore, base on the effect mediated through the synthesis and expression of CTCs attractant chemokines, it is the purpose of this
study to used inflammation treated epithelium cells as immobilization substrate, along with intrinsic cancer cell self-adhering property, to provide not only a new method to capture the live cancer cells but also a high sensitivity protocol for early cancer diagnosis.

2 Problem Formulation

2.1 Cell culture

All the cell lines, including Human liver cancer cell line (Hep G2, B16-F0, B16-F10), HL-60, U937, HEK 293T, HUVEC were provided from BCRC in Taiwan. Human umbilical vein endothelial cells (HUVEC) were serially passaged using Medium 199 (Invitrogen, USA) supplemented with 20% fetal calf serum (FCS, Gibco Laboratories, Grand Island, NY), endothelial cell growth factor (ECGF, 50 jig/ml, Biomedical Technologies, Inc., Stoughton, MA), and porcine intestinal heparin (50-100 ,g/ml, Sigma Chemical Co., St. Louis, MO). Human liver cancer cell and human embryo kidney cell were cultured in DMEM medium (Invitrogen, USA) with 10% FBS (Fermentas, USA). For experiments use, the cell were incubated in 10 cm petri dish with 10 ml medium. The WBC and RBC samples were separated by ficoll from BCRC in Taiwan. The neutrophil were induced from HL-60 with IL-2. And the blood cells were cultured in RPMI 1640 (Fermentas, USA). All the cell samples were incubated in 37°C with 5% CO2.

2.2 Cancer cell adhesion assay

Assay of neutrophil and Cancer cell adherence to epithelium cell monolayers. Epithelium cells were plated in DMEM-10% FBS on tissue culture-treated 96-well microtiter plates and grown to confluence at 37°C with 5% CO2. After stimulation with IL-1β and TNF-α (100 μg/ml) in DMEM-10% FBS for 24 hours, the cells were washed with DMEM. One hundred thousand neutrophils were added to each well, and the microtiter plates were incubated for 30 min at 37°C with 5% CO2. The nonadherent cells were removed by carefully washing the cells three times with PBS. All the Hep G2 cells were transfected the green fluorescence protein (GFP) with green fluorescence labeling. Then the fifty thousand Hep G2 cells were added into the plate. Finally, the number of adherent cells was determined by counting them in a defined area. In this experiment, the neutrophils were preincubated at 37°C for 15 min with RPMI 1640 medium. Before being added into the HEK 293T monolayers, the Hep G2 cancer cells were washed once with PBS solution. And the living cells were labeled the florescence stain by DAPI in the injected sample. In the optical microscope counting, cancer cells were stained by wright’s stain.

3 Problem Solution

In figure 1, the adhesion assay it is observed that the target cancer cell line Hep G2 cells are attached to HEK293T epithelium cell substrate after the inflammation induced by IL-1beta and TNF-alpha. However, at the control without treatment, cancer cells were mostly being sorted out and collected from the epithelium cell monolayer. In addition, from DAPI staining results, it is showed that the living Hep G2 cells attached on the epithelium cell surface. They were presented the blue florescence when laser with 325nm wavelength excited them. And the adhesion rate of control group were apparent smaller than of the testing group with inflammation treatment.
Fig 1, Microscope images of cancer cell in Wright’s stain or DAPI (blue light), and epithelium cell with transparent in (A) the control group for Hep G2 cells with Wright’s stain; (B) the microscope observation; (C) Hep G2 cells with DAPI stain; (D) Hep G2 cells of the test group with Wright’s stain; (E) merged picture of Hep G2 with DAPI staining; (F) the test group.

In order to test if the dead cancer cell can also be captured by the inflammation induced auto cell adhesion, dead Hep G2 cells were applied into the epithelium system. After frozen in 4°C in 40 minute, it is shown in Fig 2 that out of 5000 there were less than 20 dead cells on the epithelium cell surface after the PBS wash for 3 times. Meanwhile, there are more than 60% of live cells that are attached to the epithelium cell surface after the washing process. Therefore, the result showed that this system could avoid the dead debrid noise without self-adhesion ability.

Subsequently, the adhesion rate of cancer cell though inflammation treated was evaluated in use of fluorescence microscope. The \( t \)-test result showed that the counts of dead and live fluorescent-labeled Hep G2 cells under fluorescence microscope are highly correlated with 95% confidence level. When comparing the quantitative results of testing group (see fig 3), it showed that the adhesion rate was larger than control group. After the IL-1beta and TNF-alpha treatment, the cell adhesion rate was calculated by the cell number which didn’t wash out per seeding number (50000 cells). At result, the adhesion rate of control was 17.34% and the adhesion rate of testing group was 72.16%.

Fig 2, Cell adhesion assay for dead and live cells.
Form (A) was the dead cells with Wright’s stain. Form (B) was the live cells with Wright’s stain.

Fig 3, the bar chart of cell adhesion rate between control and testing group.

4 Conclusion
A cell auto-adhesion method has been reported for the screening of circulation tumor cells. Our initial results support the possibility that target cancer cells can be separated and captured live from blood samples, but not with debris after the washed-out. Therefore, with the use of specific fluorescence marker, similar concept can be used for other examinations on clinical diagnosis by simply changing its trigger cytokines to fit the microenvironment of the target cancers. Furthermore, this method may
also provide platform for the screening of living primary cancer cells that can be used for subsequent diagnosis and clinical cancer drug treatment.

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