Effect on U937 Differentiation Co-cultured with Nasopharyngeal Cancer Cell

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Abstract **Objective** To study the effect of nasopharyngeal cancer cells on U937 differentiation. **Methods** U937 was co-cultured with CNE-2, and then our research adopted microscopes to observe the change of cell shape and used flow cytometry to examine the expression of CD36. **Results** Part of the U937 cells adhere to the surface of CNE-2 cells after co-culture and some of these cells protruded protuberance. There are more CD36 expressed on U937 after co-cultured than normal. **Conclusion** co-culture can promote the differentiation of U937

Key words U937; CD36; Nasopharyngeal cancer cell

INTRODUTION

U937 is a kind of promonocyte, which grows mainly in the state of suspension. Shape of U937 is round, and its surface is smooth. When stimulated with PMA, LPS or growth factor, the state of cell changed from suspension wall sticking, shape turned from round to polygonal. Furthermore, there are projectings protrude. CNE-2 is a kind of nasopharyngeal cancer cell, which grows in the state of wall sticking. According to the reports, nasopharyngeal cancer cell can promote the development of B lymphocyte, but there is still no report about the study on differentiation of promonocyte. Our researches adopt flow cytometry and microscope to observe the expression of CD36 and the transformation of U937. CD36 is mainly expressed on monocyte and macrophage, so it can be act as a marker in differentiation of promonocyte.

MATERIALS AND METHODS

Materials

Clinical Research Center of Singapore National University offers both human promonocyte cell line U937 and nasopharyngeal cancer cell cell line CNE-2. Cells are cultured at 37℃ in RPMI1640 culture medium containing 10% calf serum, 100u/ml penicillin, 100ng/ml streptomycin and 5% CO_2. The activity of cells were more than 95% tested by 0.4% trypan blue..

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Goat antihuman FITC marked by monoclone antibody was purchased from Sigma Company. Cell separate fluid also purchased from Sigma Company.

Methods

The observe of cell shape

Put 2×105 CNE-2 cell in 6-well plate, added 2×105 U937 cell when CNE-2 adhered to wall totally. Then cultured 24h with 5% CO2 at 37℃. Moved the cells did not adhere before observed under cofocal microscope.

Examine the expression of CD36 by flow cytometry

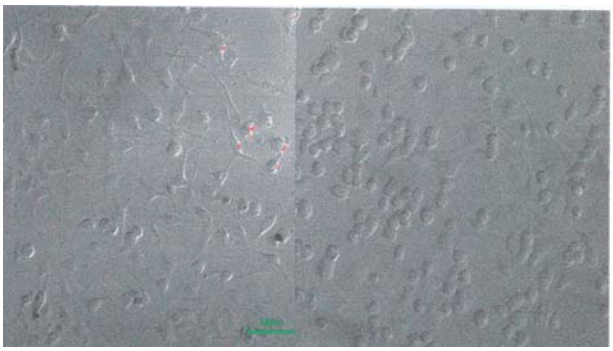
Wish down the adhered U937 cells with cell separate fluid did not containing enzyme. However, CNE-2 adhered very tight and not easy to isolate. Gather the cells and wished by 1 ×PBS, then centrifugated at 1000rpm for 5 minutes. Fixed by 4% formalin for 15 minutes, wished by 1 ×PBS and centrifugated at 1000rpm for 5 minutes. Blocked by 1:100 goat serum for 30 minutes. Put CD36 1:200 at room temperature, wished by 1 ×PBS. Examined on flow cytometry for three times.

RESULTS

The change of U937 cell shape

U937 grows mainly in the state of suspension, but part of the U937 cells adhere to the surface of CNE-2 cells after co-culture and some of these cells protruded protuberance. CNE-2 cells grow with adhesion to the plate wall (Fig.1).

We can observe CNE-2 cells with protuberance and the adhesion to CNE-2 of some U937 after co-cultured



CNE-2 CNE-2+U937 24h

Fig.1 U937 cells were Co-cultured

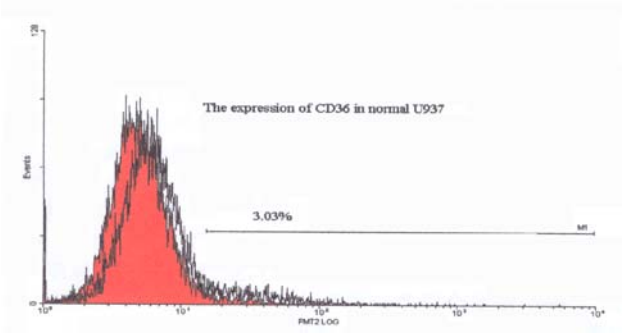


Fig.2 The Hormal expression of U937 and CD36

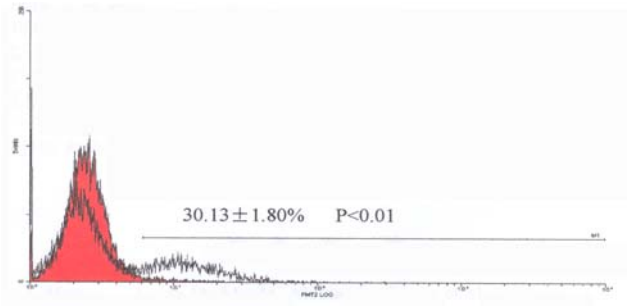


Fig.3 The expression of U937 and CD36 after Co-cultured

under cofocal microscope. Many U937 cells had changed to monocyte.

Examine the expression of CD36 on U937

There are more CD36 expressed on U937 after co-cultured, which show the differentiation of U937 to monocyte. However, there is less CD36 expression on normal U937 cells (Fig.2, 3).

DISCUSSION

CD36 is a kind of glycoprotein on membrane, which mainly expressed on erythrocyte, platelet and monocyte. In monocyte system, CD36 are mainly expressed on marrow of anaphase, monocyte in circulation and macrophage in tissue. CD36 interpose the lick up of death cell and decorated lipoprotein. Some study also say that TSP-1 enhances IL-6 release from macrophages by interaction with CD36 [1]. In general, there are low-level expression of CD36 on promonocyte, but the stimulate of PMA can promote the expression of CD36 [2]. So CD36 and CD38 can be seen as the marker of the differentiation from promonocyte to monocyte[3,4]. Our research use CD36 as guideline of U937 differentiation.

Our research co-culture promonocyte U937 with nasopharyngeal cancer cell CNE-2. Nasopharyngeal cancer is a kind of malignancy with has relation with EBV virus, which also relate to Burkitt's lymphoma and Hodykin's disease[5]. The tumor cells in the phgase of rapid growth can promote the mature of monocyte.

Cultured monocyte with human navel endothelium cells, the expression of CD36 will be promoted on monocyte[6]. When block E-selectin by its antibody, the expression of CD36 will not be promoted. So E-selectin plays a key role in the expression of CD36.

Liu also report there are more E-selectin expression on nasopharyngeal cancer[7]. Our research shows the co-culture of promonocyte and nasopharyngeal cancer cell can promote the differentiation of promonocyte. But whether E-selectin plays a role in this process need further study.

REFERENCES

1. Yamauchi Y, Kuroki M, Imakiire T, et al. Throm-bospondin-1 differentially regulates release of IL-6 and IL-10 by human monocytic cell line U937. Biochem Biophys Res Commun 2002, 290(5): 1551.
2. Massimo Alessio, Lucia De Monte, Alessandra Scirea, et al. Synthesis, Processing, and Intracellular Transport of CD36 during Monocytic Differentiation. J. Biochem. 1996, 271:1770.
3. Finstad HS, Drevon CA, et al. Cell proliferation, apoptosis and accumulation of lipid droplets in U937-1 cells incubated with eicosapentaenoic acid. Biochem J. 1998, 336:451.
4. Prieto J, Eklund A, Patarroyo M, et al. Regulated expression of integrins and other adhesion molecules during differentiation of monocytes into macrophages. Cell Immunol, 1994, 156(1): 191.
5. Rabb-Traub, Flynn N K, Pearson G, et al. The differentiated form of nasopharyngeal carcinoma contains EBV virus DNA. Int. J. Cancer, 1987, 39(1):25-29.
6. Hub HY, Lo SK, Yesner LM, et al. CD36 induction on human monocytes upon adhesion to tumor necrosis factor-activated en dothelial cells. J. Biol Chem, 1995, 270(11): 6267.
7. Liu CM, Sheen TS. Circulationg intercellular adhesion molecule-1(ICAM-1), E-Selectin and vascular cell adhesion molecule-1(VCAM-1) in head and neck cancer. Ko JY Br J Cancer, 1999; 79(2): 360.