Cell Differentiation, Cell Proliferation and Cell Cycle

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1 Introduction

In developmental biology, **celll differentiation** is tkhe process by which a less specialized cell becomes a more specialized cell type. Cell differentiation produces variety of cells rooted from fertilized egg. These difference are largely due to highly-controlled modifications in gene expression, which almost never change DNA sequence itself. In mammals, only the zygote and early embronic cells are able differentiate into all cell types, which is hard to isolate. Adult stem cells only partially differentiated. We will induce U-937 cell to adhension cell or macrophage by PMA as following.

The U-937 cell line is a human hematopietic cell line from a 39 years odl man with histiocytic lymphoma, which is an oncogenic human monocyte cell line. Phorbol 12-Myristate 13-Acetate(PMA) is a phorbol ester used to convert monocytes into macrophages.With addition of the PMA, the non-adherent U-937 cell up-regulate their adhesion molecules to attach to the flask surface and differentiate. PMA enhace the expression of CD11b and PKC, while increase $\beta - 2$ integrins to help monocytes to attach to flask surface. Then PMA activates calcium and phospholipid dependent isoforms of PKC and stimulates cyclic AMP metabolism, causing maturation into a macrophage-like morphlogy. We will observe morhology change during different differentiatal time.

Not only cell differentiation , but also cell proliferation is the funcdamental of living organisms. Cell cycle is the series of events that take place in a cell leading to its division and dulication of its DNA to produce two daughter cells.[2] Normally, cell cycle could by seperated into 3 stage:

- Interphase: the cell grows, proteins are made, the number of organelles increase, DNA replication. This stage contain 3 phage: G_1 phase, S phase, G_2 phase. The amount of genetic information(in human) will double to 4n from 2n, but keep same chromsome number(2n). G_1 cells have half of DNA content of the G_2 cell.

- Division of the nucleus(mitosis or meiosis): In mitosis, this stage called M phase, when chromsome separated. M phase can be sperate to 4 subphase.The amout of genetic information(in human) will change to 2n from 4n after M phase and cytokinesis.

- Cytokinesis: the cytoplasm divides between the new daughter nuclei.

So, G_1, S, and G2/M cells can be distinguished based on their DNA content. In this Lab, we will use the flow cytometry to character cell cycle based on the propidium iodide(PI) statin for nucleic acids. PI bind to DNA and RNA by interaction with base.

Flow cytometry can handle larger population of cells for obtaining more data than other method, which has 3 mean components in the sytem to operate cell and collete data:

1. Fluidics System:Hyrodynamic focusing

2. Optics System: produce excited light, collect emission fluorescen(also called side-scattered light) and forward-scattered light.

3. Electronics System: generate electral pulse, transform light signal to electral signal and store data.

Flow cytometry data could be analysised in 3 features: A(area), W(width),H(height). We apply width and height to deduce cell cycle as following:

- If compared to normal cells(2n), area is twice more, width doesn't chagne, then it should be a 4n cell

- If compared to normal cells(2n), both area and witdth are twice more, then its may be a double 2n cell together.

2 Materials &Methods

U-937 cells, PI, Flow cytometry(Beckman Conlter Cytoflex), PBS(phosphate buffer saline), PMA(Santa Cruz, SC-3576, MV6161.83g/mol, dissolved DMSO, 10mM,stock solution), Accutase^{TM}(sigma A6964), 70% alcohol(-20 precold), PI staining solution(50μg/ml PI, 100 μg/ml RnaA, 02%TrionX-100 in PBS), RMI1640 supplied with 10%FBS(cell culture medium)

Centrifuge, Inverted Microscopy, CO_2 incubator, hood, piptles and tip, cell density counter, 60mm tissue culture dish, 1.5mL centerifuge tube, 15ml centrifuge tube, Pasteurs pipette, Flow cyometer, refrigerator, 5mL FCM tube

2.1 Cell difference

Transfter cell suspension to 15ml tube centrifuge at 120xg for 3min $\longrightarrow$Discard supernant, resuspend the cells with 1mL RPMI 1640 $\longrightarrow$ counter cell and take 10^6

cell to 60mm dish with 4mL 1640 $\longrightarrow$ add $4\mu L$ PMA toexperimental group + add $4\mu L$ DMSO to control group $\longrightarrow$ culture in CO_2 incubator for 24hr, 48hr, 72hr.

2.2 Charastic cell cycle with FACS

Induce U-937 cell with PMA(final soulution 10mM) for 72hr $\longrightarrow$ harvest cell. For control group, directly transfer to 15ml tube. For experiment group cell: wash dish with 2ml PBS, transfer to tub; digest residual cell with 1ml Accutase solution in 37°C incubator; terminate 2ml complte medium; finally transfer to 15ml tube. $\longrightarrow$ Spin down cell, centrifuge 120xg, 5min $\longrightarrow$ discard supernatant $\longrightarrow$ Flick the tube $\longrightarrow$ wash cell with 3ml cold PBS $\longrightarrow$ centrifuge 120Xg, 5min , 4°C $\longrightarrow$ Discard supernatant, flick tube $\longrightarrow$ Add 2ml cold 70% ethanol **slowly** $\longrightarrow$ Fix cells at -20°C for 1 hr.

Centrifuge 250xg, 5min $\longrightarrow$ Discard enthnal $\longrightarrow$ Wash with 3mml of cold PBS $\longrightarrow$ Flick the tube, avoid clumping of cells $\longrightarrow$ Suspend with 3ml PBS, add PI staining solution to work concentration. Stain at dark, RT, 30min $\longrightarrow$ Transfer cells though the cell strainor to 5ml FCM tube. $\longrightarrow$ Run flow cytometry

3 Result

- PMA succefully induce the differentiation of U-937 cell. Differential U-937 tread to attach to surface of dish.(Figure 3,4)

- The longer U-937 was treated, more cell differentiated.(Figure 1,3 and Figure 2,4)

- 52.6% no treated U-937 cell is in G_1 phase, 16% no treated cell is in G_2 or M phase(Figure 7). Almost all PMA treated cell stayed on G_1 phase.(Figure 8)

4 Discussion

How PMA effect cell cycle?

As we known, protein kinase C (PKC) play a import role in vascular endothelial cell proliferation. We found following phenonmen from our and other data:

1. when added to G2 cells, PMA inhibited subsequent cell division; (our flow cytometer data Figure 7, 8)

2. these growth-arrested cells did not show morphological features of mitotic cells[1]

3. PMA did not interrupt mitosis in cells released from nocodazole-induced M phase arrest.[1]

To solve these problem, other expriment showed that,

1. 1-Oleoyl-2-acetyl-sn-glycerol (OAG) added repeatedly from G2 also inhibited mitosis.The activation of cdc2 kinase around the G2/M transition was suppressed by PMA and OAG.

2. Although cdc2 was expressed in the presence of PMA, dephosphorylation of its tyrosine residue was inhibited by PMA.

3. In parallel, the expression of cdc25B was suppressed by PMA. The total and the cdc2-associated amount of cyclin B were both reduced by PMA.

Sumary up, experiment data suggested that the PKC pathway negatively regulates the G2/M transition and that the inhibition of cdc2 kinase by the reduction in the levels of cdc25B and cyclin B may contribute to this effect.[1]

5 Figures

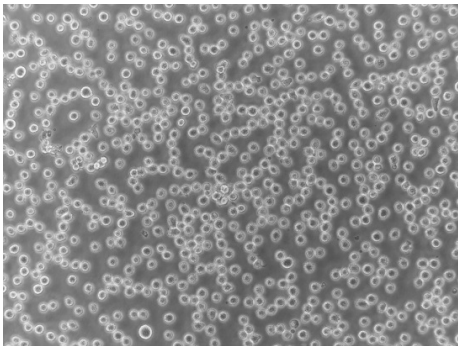

Figure 1. No treated U-937 after 24hr(20x)

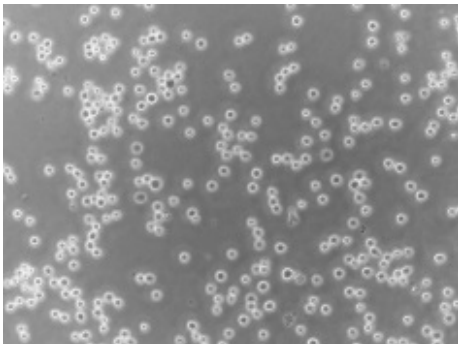

Figure 2. PMA treated U-937 after 24hr

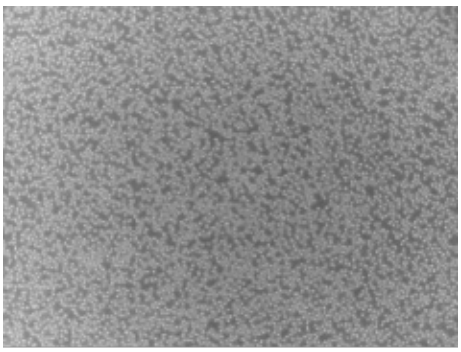

Figure 3. No Treated U-937 after 72hr(20x)

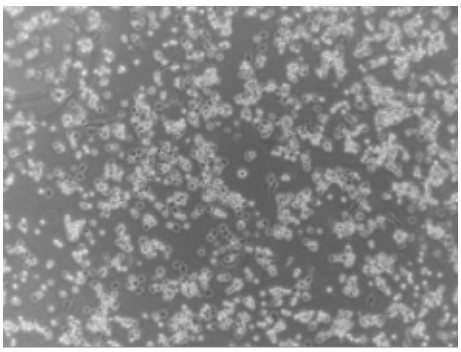

Figure 4. PMA treated U-937 after 72hr

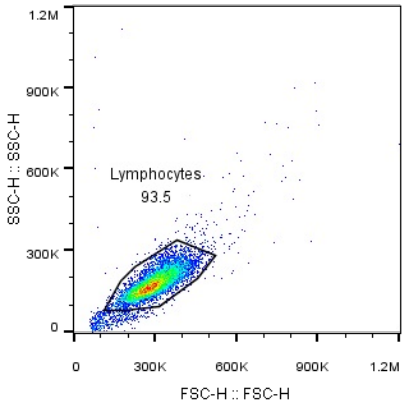

Figure 5. No treated U-937 cell data(all cell)

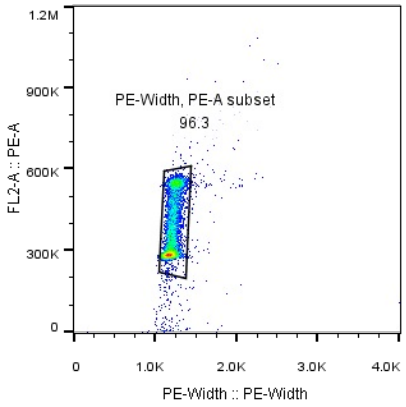

Figure 6. No treated U-937 cell data(gated)

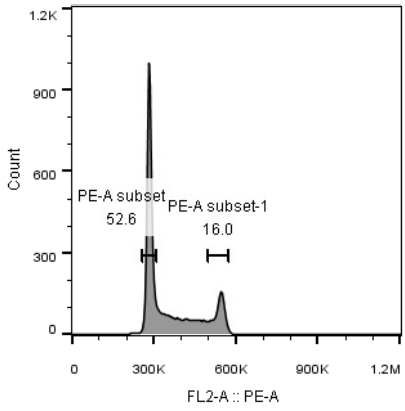

Figure 7. No treated U-937 cell fluorescence distribution

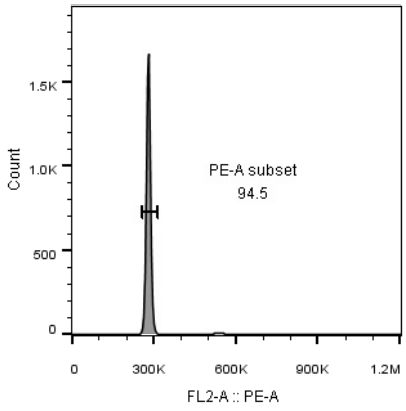

Figure 8. PMA treated U-937 cell fluorescence distribution

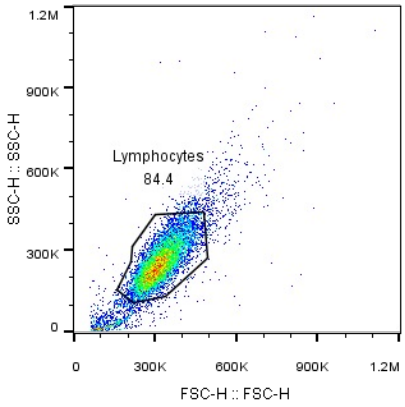

Figure 9. PMA treated U-937 data

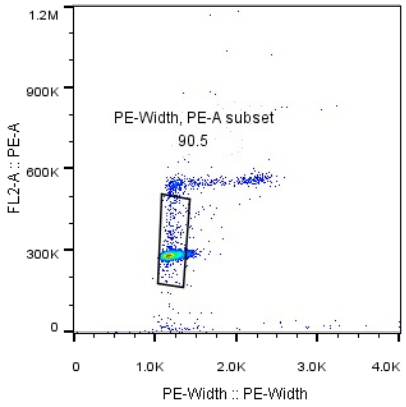

Figure 10. (gated)PMA treated U-937 data

6 Reference

1. Cell cycle arrest in the G2 phase induced by phorbol ester and diacylglycerol in vascular endothelial cells C. Kosaka, T. Sasaguri, A. Ishida, and J. Ogata. American Journal of Physiology-Cell Physiology 1996 270:1, C170-C178

2. https://en.wikipedia.org/wiki/Cell_cycle

7 Contribution

Wenhao zhang & I finished these work together. Thanks to Teacher Jia and Zhao, who parepare materials and teach the experiment pricinple