Automatic Counting of Apoptosis in HCT116 human colon adenocarcinoma cell

Liu Hai-ling

Shin Young-suk

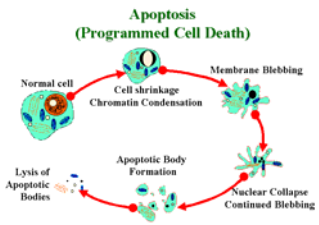
CHOSUN UNIVERSITY 1946

Background

Apoptosis is a complex,tightly regulated and active physiological process whereby individual cells are triggered to undergo self-destruction. As cancer cells frequently have acquired the ability to avoid apoptosis and continue to multiply in an unregulated manner, induction of apoptosis is an important factor in cancer therapy. Many anticancer drugs attempt to achieve the effect by inducing the cancer cell to apoptosis. So the quantitative analysis of apoptosis is required not only to understand the mechanism of this esoteric, quaint process but also to quantificationally evaluate the effect of anticancer drugs. Currently counting apoptosis in vivo is generally carried out manually and may consist of estimates of number of cells stained with a particular cell marker. But this method is extremely time-consuming and labor-intensive. Also manually counting is subjective and difficult to achieve high-throughput. There is a compelling necessity for developing an automatic cell counting method.

Process of Apoptosis

Apoptosis is a cellular response to a cellular "insult" such as UV light, chemical or physical damage or a viral infection. This insult starts a cascade of events which lead to the destruction of the cell. This mechanism is often called "programmed cell death" as it is an innate response of the cell which protects the rest of the organism from a potentially harmful agent. During the early process of apoptosis, cell shrinkage and pyknosis are visible by light microscope. With cell shrinkage, the cells are smaller in size, the cytoplasm is dense and the organelles are more tightly packed. Pyknosis is the result of chromatin condensation and this is the most characteristic feature of apoptosis. Extensive plasma membrane blebbing occurs followed by karyorrhexis and separation of cell fragments into apoptotic bodies during a process called "budding". Apoptotic bodies consist of cytoplasm with tightly packed organelles with or without a nuclear fragment.



Cell Culture and Drug Treatment

❖ HCT116 human colon adenocarcinoma cell line obtained from American Type Culture Collection was grown in RPMI 1640 medium (Invitrogen, USA) supplemented with 10% (v/v) fetal bovine serum (FBS; Invitrogen) and 1% penicillin-streptomycin in a 37° C humidified incubator in an atmosphere of 5% CO_2. Drug treatment of cells was performed by adding 50 μM oxaliplatin and incubating for 48 h.

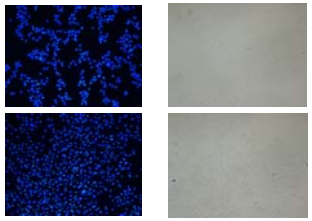
❖ The cells were incubated with 1 μg/ml Hoechst 33342(HO)for the final 10 min of drug treatment in the 37°C incubator and then, both floating and attached cells were collected by centrifugation. The pooled cell pellets were washed with ice-cold phophate-buffered saline (PBS), fixed in 3.7% formaldehyde on ice, washed again with PBS, and a fraction of the suspension was centrifuged in a cytospinner (Thermo Shandon, Pittsburgh, PA).

Image Acquisition

The slides were air dried, mounted in an anti-fade solution, and images were analyzed using a DM5000 fluorescence microscope (Leica, Germany) at excitation/emission wavelengths of 340/425 nm. For images capture, microscope connected Leica DFC480 camera and Application Suite software were used. The image size is 2560 ×1920

Sample Image

❖ Before adding anticancer drug
con-H: Nuclei stained image (Upper Left)
con-L: Optical image of con-H (Upper Right)
❖ After adding anticancer drug (Bottom Left)
L-OHP-H: Nuclei stained image (Bottom Right)
L-OHP-light : Optical image of L-OHP-H



System Framework

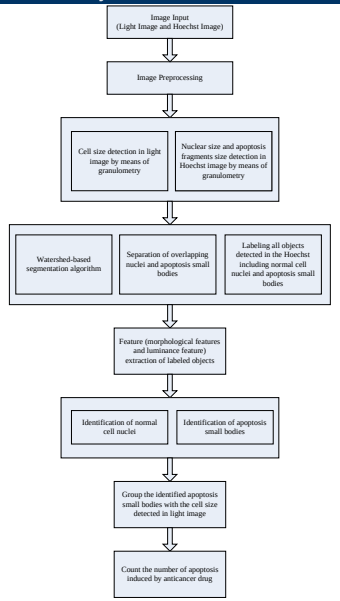
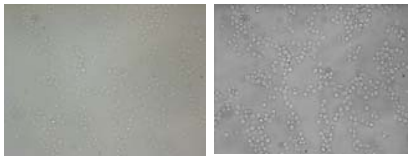


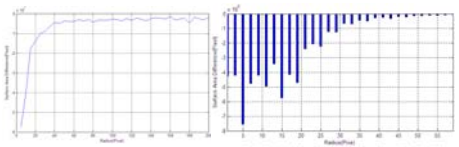
Image Preprocessing

Noise reduction: Median filter
Contrast enhancement: contrast-limited adaptive histogram equalization
Image before preprocessing (Left) and after preprocessing (Right)



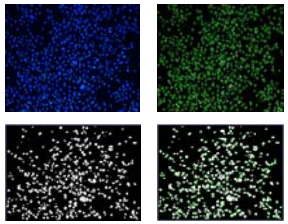
Size Distribution Estimation

Method: **Grey scale granulometries**
Normal cell size (Left) and nuclei & apoptotic bodies size (Right)



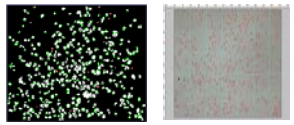
Apoptosis Identification

❖Target Objects identification. This step includes single objects identification and clumped objects separation. Single objects can be identified by thresholding. Clumped objects is firstly identified by the size distribution of nuclei and apoptotic bodies and then separated by region growing method and watershed algorithm. L-OHP-H (Upper Left) and objects identified in L-OHP-H (Upper Right)
❖ Feature extraction. This step extracts six features as the basis of identification of apoptosis.
❖ Apoptosis identification. This step uses neural networks classifier to identify apoptotic bodies. (Bottom Left)
❖ Grouping apoptosis: This step group apoptotic bodies into an intact cell with the normal cell size obtained before.(Bottom Right)



Results and Conclusions

❖The apoptosis number identified by our methods is 359 and we also compare the results with four biomedical expert and their mean number is 364. The precision is about 98.63% and our method shows great performance.
❖ Although improvements are need in some aspects as to the identification of apoptosis or not for a specific cell, our methods provide a accurate and operable method for the automatic counting of apoptosis



Contact Information

375 Seosuk-dong, Dong-gu.
Gwangju 501-759, Korea

Tel: +82-62-230-7011
Fax: +82-62-230-7011
Email: allen1979_12@hotmail.com
ysshin@chosun.ac.kr