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Methods for the Detection of Apoptosis in Cultured Mammalian Cells: A Critique

哺乳動物細胞凋亡檢測方法：評論

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Abstract

Apoptosis is an essential physiological process, instrumental to the maintenance homeostasis in multicellular organisms. Deregulations of apoptosis lead to a diverse spectrum of pathological conditions, including autoimmune, neurodegenerative diseases, and cancers. In the past decades, huge progress has been made in the understanding of the molecular mechanisms that regulate apoptosis, and in the identification of mediators that prevent or trigger this process. Pathways connected to apoptosis emerged as key therapeutic targets, and cell-based cytotoxicity assays are commonly used for testing the apoptosis-inducing properties of small molecules. The compatibility of these assays with high-throughput screening allows the rapid discovery of potential apoptotic compounds. However, very few of these compounds have progressed to successful clinical application. The relatively poor rate of clinical translation is in part attributed to the intrinsic shortcomings of these assays.

This PhD work contains four loosely connected studies, each addressing a previously uncharacterized aspect of the existing in vitro apoptosis detection methods. First, in order to assess the general quality of these assays, I have conducted a meta-analysis on the published cytotoxicity data of well-established drugs, namely etoposide (ETP), stauroporine (STS), and TRAIL, on two commonly used cell lines, HeLa and MCF 7 cells. I discovered that an unexpected level of inconsistency in the data among publications.

Based on published information, experimental parameters including apoptosis detection methods, cell culture media, glutamine concentration used, and source of drugs have been examined as potential sources of such data inconsistency, and found that none can be attributed to the discrepancy. In many cases, the experimental details of cell culture experiments are not completely described, making it difficult to reproduce the published results.

In the second part of this thesis, I address how cell culture conditions can affect the measurement of cytotoxicity events. As a case in point, I studied the effect of the culture density on the phosphorylation of H2AX, a widely used DNA damage marker, in a variety of human cell lines. I observed that higher levels of γH2AX in densely cultured cells in the absence of any cytotoxic treatment. Thus, culture density of cells in routine cell-based assays can dramatically affect the baseline level of γH2AX expression, leading to aberrant measurement endpoints.

In the third part of the thesis, I examined a common phenomenon in which a single cytotoxic compound can exert distinct growth inhibitory effects on different cell types, depending on their physiological background and histological origins. Andrographolide (ANDRO), a small molecule previously shown to induce apoptosis in numerous cancer cell lines, was used in this study. Two common cell lines, HepG2 and HeLa cells, were treated and the responses were compared, as judged by time-lapse imaging, and flow

cytometry. ANDRO induced DNA damage as indicated by the expression of phospho-H2AX in both cell lines, but it uniquely induces G2/M cell cycle arrest in HepG2, a hepatocellular cell line. This cell cycle arrest phenotype on liver cancer cells would have been missed if simple apoptotic assays were used in high throughput screening.

In the final chapter, I addressed to one of the limitations of existing apoptosis detection methods: the lack of speed. I report the development of a novel approach for the specific detection of apoptosis in cultured mammalian cells, based on the Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF-MS) of intact cells. Buffer washed cells were directly mixed with a matrix solution and directly deposited onto the stainless steel target for MALDI analysis. I discovered that the resulting MS profiles were highly reproducible and can be used to reflect cell viability. The MS profiles generated from apoptotic cells were distinct from those from either normal or necrotic cells. The apoptosis-specific features of the mass spectra were proportional to the percentage of apoptotic cells in the culture, but are independent of the drugs used to stimulate apoptosis. The speed and throughput of this method is superior to the existing apoptosis method. This is the first report on the utilization of intact cell MALDI mass spectrometry in detecting mammalian cell apoptosis.

In summary, the study reported in this thesis provides a systematic critique on the quality

of the published data and the technical limitations of existing in vitro cytotoxicity assays. These shortcomings provide a conceptual framework for the better design and reporting of cytotoxicity experiments. I have also explored a new approach in apoptosis detection, which can be used as a basis for the development of a reliable, rapid, label-free, and high throughput method for high demand of drug screening.

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