Aponetics - new standards in rapid and efficient cancer drug discovery

The field of apoptosis is the subject of intensive investigation in the search for novel therapeutic compounds especially for cancer therapy. Aponetics has developed a simple multi-stage screening system which identifies such compounds and clearly discriminates between apoptosis and necrosis, no matter what the mechanism of action of a given compound is. The validated system has already identified a number of lead series which are currently under active development.

Text: JOST HARR, FELIX BACHMANN, ALESSANDRO STREBEL AND MARTIN EBERLE[1]

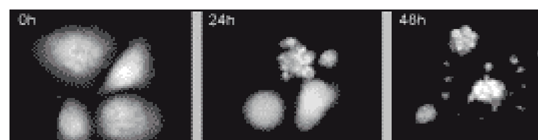

Fig. 1: Apoptosis in NCI-H460 non small lung cancer cells

Apoptosis (often referred to as «programmed cell death») is the regulated elimination of surplus or unwanted cells. Apoptosis occurs naturally in tissue homeostasis and during the course of development, as well as in many pathological circumstances that require cell death for the benefit of the organism. The process is initiated by a number of death signals (e.g. UV-light, TNF-α, TGF-β, toxins, absence of growth factors and/or natural death ligands). The genetically programmed, highly orchestrated process of apoptosis is characterized by a number of distinct morphological changes. Initially, the apoptotic cell starts to shrink. The nucleus condenses before fragmentation of the cell into apoptotic bodies starts (illustrated in Fig 1). These apoptotic bodies are finally taken up and digested by surrounding cells or macrophages without harming the surrounding tissue.

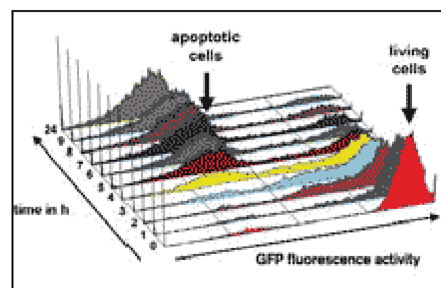

Fig. 3: Time course of apoptosis as measured by the GFP method upon treatment of Jurkat cells with recombinant mFasL.

Assay Principle

Aponetics´ assay system is based on the use of living cells. It depends on linking the fundamental biological event of apoptosis with a change in physical property, i.e. the intensity of a fluorescent signal. This change occurs in the target cell itself as a result of modification of the cell to express green fluorescent protein (GFP) in a stable manner (for a review on GFP see reference 1). It has been shown that the incorporation of this GFP reporter has no influence on any of the cellular properties. However, if activation of the cell by either endogenous or exogenous stimuli results in cell death through apoptosis or necrosis, the cellular fluorescence is reduced (Reference 2, 3). Thus the fluorescence read-out allows precise monitoring of the status of cell populations.

This assay is non-destructive, allowing time-laps measurements for the early assessment of kinetic and dynamic responses already at the screening level (Fig 3). This leads to predictive results allowing subsequent in vivo studies to be tailored in a so far unparalleled manner.

Resistance to apoptosis has been postulated as one of the many potential mechanisms by which tumor cells evade immune-mediated and cytotoxic drug-induced destruction.

Therefore, selective induction of apoptosis for treatment of cancer appears to be a valid concept – the basis of Aponetics' business idea.

Screening platform and its validation

A tiered high throughput screening system serves to detect and characterize apoptosis-inducing compounds irrespective of their mode of action.

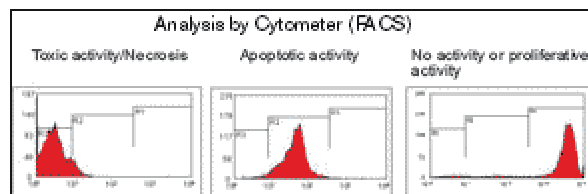

Fig. 2: Fluorescence profiles of cells in different stages (FACS analysis)

In Aponetics' primary screen compounds are tested in a 96- and 384-well format for their ability to induce apoptosis and/or necrosis on a panel of 12 to 15 cancer cell lines. In the secondary screen, single cells are measured using a flow cytometer (FACS) which allows an unequivocal differentiation between apoptotic and necrotic cells (Fig 2). Finally, in a third tier, hits are profiled by golden standard assays such as nuclear fragmentation, measurement of mitochondrial membrane potential, Annexin V binding analysis, cell cycle analysis

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