Single cell analysis reveals a time-dependent threshold for p53 mediated apoptosis

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Short Abstract — **The p53 tumor suppressor responds to multiple types of cellular stress by imposing alternative cellular programs including cell-cycle arrest, senescence and apoptosis. To determine how p53 dictates different cell fates, we measured p53 protein levels and cell fate of individual cells with high temporal resolution after treatment with a chemotherapeutic drug. We found the kinetic behavior of p53, and not its maximum level was predictive of cell fate, with p53 accumulating faster in apoptotic cells. We propose a time-dependent threshold model for p53 mediated apoptosis; cells must reach a critical threshold in a given amount of time to enact apoptosis.**

***Keywords* — p53, apoptosis, senescence, single cell, protein dynamics**

THE tumor suppressor protein, p53 accumulates in response to multiple types of cellular stress and can activate alternative cellular programs such as transient cell cycle arrest, senescence and apoptosis[1,2]. Understanding how p53 dictates these unique outcomes is of great interest as it may provide a way to guide cancer cells into states associated with tumor regression.

Current models suggest that the absolute levels of p53 protein dictate cell fate decisions. These 'threshold' models predict that low levels of p53 predominantly transcribe genes involved in cell cycle arrest resulting in a transient or permanent arrest[3,4]. When the cellular concentration of p53 crosses a critical threshold level, p53 transcribes genes involved in apoptosis leading to cell death. Recent studies have shown that p53 dynamics depend on the stimulus and carries information that control cell fate[5,6]. These studies suggest that a cell's decision to activate a specific outcome might not be solely dependent on the absolute levels of p53 but on how these levels change over time.

One of the main impediments to successful chemotherapy is that often a fraction of tumor cells survive treatment. Given p53's role in cell fate decisions, we aimed to determine whether p53 dynamics can be predictive of the different cellular outcomes observed between cells after chemotherapy. We used fluorescent live cell reporters and time lapse microscopy to track the p53 levels of individual cells after treatment with the chemotherapy drug cisplatin. We used intermediate concentrations of cisplatin that led to apoptosis only in a fraction of the population. Contrary to the threshold model for apoptosis, we found that cells that survived treatment reached similar levels of p53 as the cells that died. Furthermore, increasing concentrations of cisplatin led to an increase in cell death but not to higher levels of p53 in apoptotic cells. Instead we found that apoptosis is linked to the rate at which p53 accumulates; apoptotic cells accumulate p53 faster than surviving cells.

We further determined the effect of p53 rate of accumulation on apoptosis by artificially accelerating p53 induction using a small molecule that inhibits p53 degradation in conjunction with cisplatin. Consistent with our previous results, this perturbation greatly increased the rate of p53 accumulation and the proportion of apoptotic cells.

Conclusion

Quantifying the kinetics of p53 accumulation in single cells allows for a more accurate prediction of apoptosis than the maximum level of p53. Our data suggests a time-dependent threshold for p53 mediated apoptosis, in which a cell's probability to die depends on both the level of p53 and the time of induction. In this model, cells have a window of opportunity to reach a critical threshold of p53 in order for death to occur. We will discuss the various mechanisms which might protect cells from apoptosis if p53 is induced too late and new methods for accelerating p53 and efficiently killing cancer cells.

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