Single-Cell Heterogeneity and Transient Resistance in the Apoptotic Response to TRAIL

Deborah A. Flusberg[1,2], Sabrina L. Spencer[1,2], and Peter K. Sorger[1,2,3]

***Short Abstract* —In response to treatment with TRAIL (TNF-Related Apoptosis Inducing Ligand), individual cells within a clonal population exhibit extensive heterogeneity: some cells undergo apoptosis, while others escape death and survive. To understand this divergence in cell fate, and how it relates to TRAIL sensitivity, we explore the induction of death and survival pathways by TRAIL at the single cell level. We show that cells treated with TRAIL undergo a life-death decision based on the initial state of the cell and stochastic induction of death/survival pathways; once cells enter the survival state, they exhibit transient TRAIL resistance which eventually mixes back to the original population sensitivity profile.**

***Keywords* — TRAIL, apoptosis, cancer, cell-to-cell variability, NFκB, persistence, phenotypic switching**

I. Introduction

TRAIL holds promise as an anti-cancer agent due to its relative toxicity toward tumor cells compared with normal tissue, but many tumors are only partially sensitive to TRAIL [1]. Partial sensitivity to chemotherapy has often been ascribed to genetically distinct subpopulations or to cell cycle effects. Nongenetic variability and its effects on cell behavior have received recent attention in diverse systems from bacteria to mammalian cells [2]; while much literature exists on the determinants of TRAIL sensitivity in different cancers, the contribution of nongenetic variability to partial TRAIL sensitivity has not been explored.

TRAIL induces apoptosis via the extrinsic, or receptor-based, cell death pathway. However, in certain contexts TRAIL can also activate pro-survival pathways, such as the transcription factor NFkB [3]. Preferential activation of death vs. survival signaling has been noted in different cell types treated with TRAIL, but it is not known how differential activation of these pathways manifests at the single-cell level, creating heterogeneity among individual cells within a population.

Acknowledgements: This work was funded by NIH Grant P50-GM68762.

[1]Department of Systems Biology, Harvard Medical School, USA E-mail: dflusberg@hms.harvard.edu

[2]Center for Cell Decision Processes, Massachusetts Institute of Technology, USA., spencers@mit.edu

[3] Bioengineering Dpt, MIT., peter_sorger@hms.harvard.edu

II. Results

We and others have observed cell-to-cell variability, both in timing of cell death and in ultimate cell fate, in clonal populations of cells treated with TRAIL [4]. Using live cell imaging and model simulations, we showed that variability in timing of cell death in HeLa cells treated with TRAIL could be attributed both to the initial state of the cell at the time of treatment and to stochastic divergence from that state after treatment [5].

To examine the nature of the life-death decision in response to TRAIL, we treated cells with TRAIL and measured the responsiveness of survivors to a second TRAIL treatment. We found that survivor cells were transiently resistant to a second stimulus one day after the first treatment, but sensitivity reset over the course of several days. Resistance could be sustained when cells were treated daily with TRAIL, suggesting induction of a survival response. Immunostaining revealed heterogeneous activation of the NFkB pathway in cells treated with TRAIL; targeting this pathway suggested that it contributes to the survival state observed in individual cells.

III. Conclusions

The life-death decision of TRAIL-treated cells may be mediated by competition between induced death and survival pathways, leading to phenotypic switching between states of sensitivity and resistance. This phenomenon is reminiscent of bacterial persistence in response to antibiotic treatment [6]. Our data has implications for the effectiveness of co-drugging and dosing strategies, and suggests that heterogeneity may be beneficial for tumor cells adapting to a changing microenvironment.

References

[1] Ashkenazi A (2008) Targeting the extrinsic apoptosis pathway in cancer. *Cytokine Growth Factor Rev* **19**, 325-331.

[2] Losick, R, Desplan C (2008) Stochasticity and cell fate. *Science* **320**, 65-68.

[3] Ehrhardt H, et al. (2003) TRAIL induced survival and proliferation in cancer cells resistant towards TRAIL-induced apoptosis mediated by NF-kappaB. *Oncogene* **22**, 3842-3852.

[4] Albeck JG, et al. Quantitative analysis of pathways controlling extrinsic apoptosis in single cells. *Mol Cell* **30**, 11-25.

[5] Spencer SL, et al. (in press) Nongenetic origins of cell-to-cell variability in TRAIL-induced apoptosis. *Nature*.

[6] Balaban NQ, et al. (2004) Bacterial persistence as a phenotypic switch. *Science* **305**, 1622-1625.

[7] Big MR, Rocky Mountain Town, Santa Fe, NM, private communication.