Cell fate decisions in response to a short pulse of TNF

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***Short Abstract* — Tumor necrosis factor (TNF) cytokines regulate survival and death signaling pathways in the same cell. Although exposure to TNF can be of short duration *in vivo*, in experiments cells are often exposed to TNF continuously. To understand how survival and death signals respond to transient TNF exposure, we monitored live cells expressing fluorescent reporter proteins in a microfluidic flow device. We find that a TNF pulse of the order of seconds can provoke both pathways. Strikingly, a short pulse can be more effective at killing than a longer pulse, suggesting that TNF concentration and duration together coordinate cell fate decisions.**

***Keywords* — NF-kB transcription factor, apoptosis, caspase protease, microfluidics, laminar flow**

I. Introduction

TNF is a pro-inflammatory cytokine that modulates cellular behaviors including proliferation, differentiation and apoptotic cell death. While TNF is important for the normal development and function of immune cells, chronically elevated TNF is associated with autoimmune diseases and linked with tumor progression in some cancers.

TNF regulates many cellular behaviors by sequentially activating intracellular signals [1]. Binding of TNF to its receptor at the plasma membrane rapidly induces nuclear accumulation of the NF-κB transcription factor, driving transcription of anti-apoptotic genes to promote cell survival. This is followed by internalization of TNF-bound receptors, a process that initiates signals for caspase protease-dependent apoptotic death in the same cell.

To regulate diverse cell fates in a healthy tissue, TNF exposure is strictly controlled and likely to be transient. However, little is known about the duration of TNF exposure required to activate NF-κB–driven transcription or induce apoptosis. Here, we set out to determine the minimum TNF pulse duration required for activation of NF-κB, and to study TNF-induced apoptosis, comparing cell fates in continuous versus transient TNF exposures.

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II. Results

We designed and built a microfluidic system that uses laminar fluid streams to provide spatiotemporal control over TNF delivery to cell cultures. The device was seeded with HeLa cells stably expressing EGFP-fused NF-κB, and its nuclear fluorescence was quantified from time-lapse microscopy. Using our recently collected dataset of same-cell NF-κB localization dynamics and target transcript numbers [2], we established the threshold of nuclear NF-κB accumulation required to induce gene transcription in single cells. For a high TNF concentration, a short pulse of 10s elicits significant NF-κB translocation in a fraction of cells, although a 30s pulse or longer is required to approximate continuous exposure. We also find that the minimal TNF pulse is dose dependent, with lower concentrations requiring a longer pulse for comparable NF-κB activation.

To monitor caspase activity in single cells exposed to a pulse of TNF, we imaged HeLa cells stably expressing a FRET-based initiator caspase reporter (IC-RP; [3]) by time-lapse microscopy and quantified IC-RP cleavage in single cells. Consistently we observed a non-monotonic relationship between TNF pulse duration and both the timing and extent of caspase-dependent cell death. There is a relative maximum of cell killing in response to a 1-minute pulse when compared to a pulse of shorter or longer duration.

III. Conclusion

Our data show that a short pulse of TNF is sufficient to induce substantial activation of pro-survival and pro-death signaling, and that the pulse duration in turn affects efficacy and timing of cell death. These data also suggest that the condition for highest fractional kill may not require sustained exposure to the pro-death stimulus. Overall, our study complements a growing body of work showing that signaling dynamics as well as the timing and sequence of drug addition together influence cell fate decisions.

References

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