Integration of activating and inhibitory receptor signaling by regulated Vav1 phosphorylation

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***Short Abstract* — Natural Killer (NK) cell cytotoxicity is regulated by the interplay of activating and inhibitory receptor signals. Triggering activating receptors leads to Src-family kinase activation and Vav1 phosphorylation, whereas inhibitory receptors dephosphorylate Vav1 via SHP-1. Combining predictive mathematical modeling and experimental verification, we investigate the integration of these opposing signals on a molecular level. Our data show a switch-like regulation of Vav1 phosphorylation, correlating with NK cell cytotoxic activity. Using a novel approach we identify essential components of the signal transduction network. Our data indicate a key role of Vav1 in the decision making process of NK cells and provide novel insights into the integration of positive and negative signals during lymphocyte activation.**

I. Introduction

Activation of cytotoxic natural killer (NK) cells is dependent on positive and negative signals transmitted by cell surface receptors. Activating NK cell receptors include NKG2D/DAP10 and 2B4[1]. Recruitment of these receptors to the immunological synapse initiates the hyperphosphorylation of tyrosine residues in their cytoplasmic tail by Src-family kinases (SFKs), leading to the relocalization and phosphorylation of the guanine-nucleotide exchange factor Vav1. pVav1 initiates complex cytoskeletal rearrangements which eventually trigger target cell killing[2]. These stimulatory signals are modulated by inhibitory receptors like CD94 and KIR2DL1 which can be tyrosine-phosphorylated by SFKs to recruit the essential protein tyrosine phosphatases SHP-1 and SHP-2[3]. One of the first targets of SHP-1 is pVav1[4], thereby counteracting the activity of stimulatory receptors. Here, we use mathematical modeling of the complex interaction of inhibitory and activating signaling pathways in combination with experimental validation of model predictions to investigate the crosstalk between the opposing signals.

II. Results

A. Modeling the NK cell signal transduction network

To investigate the response of Vav1 to inhibitory and activating signals, a two-compartment ODE model describing the signaling events inside the immunological synapse and the cytoplasm was set up. To incorporate ambiguity about the network topology, an ensemble of 48 alternative models were constructed and parameterized with kinetic rates from the literature and with initial conditions measured by qifi-FACS and quantitative western blots.

B. pVav1 shows a 2D-sigmoidal dose-response behavior

Titration of NKL cells with increasing concentrations of crosslinked activating and inhibiting antibodies results in a 2D-sigmoidal dose-response behavior of pVav1: an increase in activator concentration leads to a rapid increase in pVav1 levels, which is effectively and dominantly blocked by adding even low amounts of inhibitor. Comparison of this behavior with the results from a novel qualitative parameter scan method indicates that, out of the 48 models, only those including the association of SFKs with the phosphorylated receptors can create this behavior. This novel predicted interaction was subsequently experimentally confirmed.

C. Vav1 phosphorylation correlates with target killing

The ultimate result of NK cell activation is the killing of a target cell. As the dose-response behavior of pVav1 shows the hallmarks of a decision making point, we investigated the dose-killing behavior of NKL and primary cells. The qualitative correlation between perturbed and unperturbed pVav1 levels and NK cell cytotoxicity suggests that pVav1 levels establish a target cell killing check point.

III. Conclusion

This combined theoretical and experimental study shows how activating and inhibitory signals are combined at the Vav1 phosphorylation level to compute a killing decision and establishes ensemble modeling as a promising tool for the identification of necessary and sufficient signal transduction elements.

References

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