Regulation of the immune synapse and cytotoxic activity of natural killer (NK) cells by nanolithographic ligand patterning

Yossi Keydar[1,2], Guillaume Le Saux[1,2], Avishai Edri[3], Uzi Hadad[2], Angel Porgador[3], Mark Schvartzman[1,2]*

[1] Department of Materials Engineering, [2]Ilse Katz Institute for Nanoscale Science & Technology, [3]The Shraga Segal Department of Microbiology, Immunology and Genetics, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel.

Natural killer (NK) cells are granular lymphocytes able to eliminate cancer viral cells, which play the key role the innate immune system. NK ells express activating receptors that bind their cognate ligands on the target cell surface and regulate the NK cytotoxicity. These ligands are upregulated in cancer and virus-infected cells, yet, they can be also expressed in certain amount by healthy tissues. Importantly. the cytotoxicity of NK cells is managed by the signaling balance of activating, costimulatory, and inhibitory receptors, and the repertoire of different ligands expressed on the membrane of target cell determines whether it will be attacked or tolerated. Yet, given different levels of expression of NK activating ligands on infected, transformed, and activated immune cells, as well as on several healthy cells, it will be reasonable to hypothesize that such level of expression alone might play a role in the cytotoxic activity of NK cells, and that there could be minimal requirements for the expression of activation ligands to stimulate the cytotoxic response of NK cells.

To elucidate this role of composition and spatial distribution of activating ligands in NK cell cytotoxicity, we engineered a nanochip for the controlled activation of human NK. The nanochip is patterned with matrices of MHS class I polypeptide related sequence A (MICA) ligands, that recognize NKG2D activating receptor, and whose nanopattern encodes the receptor clustering in the cell membrane. Each matrix on such a chip was designed to provide an isolated microenvironment for NK cell activation, in which the MICA spatial distribution is regulated independently from other matrices. To enable such delicate positioning of ligands, we immobilized them onto nanopatterned metallic nanodots, thereby creating synthetic vacancies for the recognition by discrete transmembrane receptors. The size of each nanodot is about 10 nm to ensure anchoring of individual receptors.

We fabricated the nanodots by the previously reported by us process based on nanoimprint lithography and angle-evaporation shadow masking, and functionalized them nanodots with thiols terminated with Ni-chelated Nitrilotriacetic acid, following the attachment of histidine-conjugated MICA. We verified the selectivity of our functionalization by immunofluorescent staining of immobilized MICA with fluorophore-conjugated antibody, followed by the fluorescent imaging on the arrays on the chip. Using our biochips, we the monitored the activation of NK cell in several microenvironments, which were different from each other by spatial distribution of MICA. We found that the average area of the spread NK cell was dependent on the density of MICA. Furthermore, we assessed the degree of NK cell activation by fluorescent imaging of lysosomal-associated membrane protein CD107, which is a commonly used functional marker for NK cell cytotoxic activity. We found, that whereas MICA density barely influences the average amount of CD107a per cell, it regulates the average probability of whether a cell will be activated or not.

Our study provided an important insight on the spatial mechanism of the cytotoxic activity of NK cells, by establishing the ligand distribution within the 100 nm length-scale as a critical barrier for the formation of immune synapse and degranulation. This understanding paves the way to the rationally designed immunotherapeutic approaches employing unique NK cytotoxicity against human malignancies.