EVALUATING THE CONTRIBUTION OF EXTRACELLULAR MATRIX INVASION FOR KILLING OF TUMOR CELLS BY NATURAL KILLER CELLS

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Background Extracellular matrix (ECM) is a generic term for the diverse types of acellular structural component in tissues that play an important role in homeostasis. The ECM in solid tumors is greatly altered and can contribute to the modulation of immune cell function in the tumor microenvironment. Lymphocytes such as the cytotoxic natural killer and CD8+ T cells that are harnessed in immunotherapy can be regulated by the ECM. We hypothesized that matrigel layer presents a challenge for the NK cells and increasing the distance for invasion would delay the kinetics of tumor cell killing. Furthermore, invasion and killing of tumor targets would depend on their ability to remodel the matrix with matrix-metalloproteinases.

Methods Briefly, increasing volumes (50–110 μL/well) of matrigel (6mg/mL) representing increasing distance for invasion was layered over MCF7-red target tumor cells expressing nuclear-localized mKate2 (red fluorescent protein). NK92 cells were seeded over the solidified matrigel layer at E:T of 3:1. Agilent eLive Green (1 μL/mL) was added to the wells. NK cell invasion and function was evaluated using impedance-based measurements of immune cell killing and live cell imaging data collected on xCELLigence RTCA eSight.

Results Impedance increases with time as MCF7 target cells adhere and proliferate. The addition of NK92 cells results in a drop in impedance due to killing of the target cells. Total time taken for impedance levels to fall to levels at the time of NK92 addition increased progressively from 46h for 50 μL/well to 72 h for 110 μL/well. The eLive green stained NK cells and dead cells, but not healthy MCF7 targets. Consistent with impedance data, the loss of red fluorescence from target cells and increase in green fluorescence was progressively delayed with increasing volume of matrigel. A similar delay in kinetics was achieved with the broad spectrum MMP inhibitor GM6001 (2 μM and 10 μM), suggesting that MMPs played a role in the NK function. Interestingly, the NK-92 cells induce significant morphological changes in MCF7-red target cells prior to invading all the way through the Matrigel, suggesting an early distal effect.

Conclusions The results suggest a role for effector functions of NK cells potentially involving cytokines, that is independent from invasion and/or ability to degrade the matrix, for the killing of susceptible target cells. The assay also demonstrates the potential for adapting the RTCA eSight platform to study various ECM interactions with immune cells.