Natural Killer Cells Activated By Oncolytic Reovirus E Antibody Dependent Cellular Cytotoxicity in an in Vi Colorectal Cancer

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Abstract

The naturally occurring oncolytic virus, reovirus, exhibits cytotoxic effects on cancer cells. Reovirus is currently being tested in multiple clinical trials for the treatment of different cancers. In addition, they also activate the innate
and adaptive immune responses targeting immune cells like dendritic cells and macrophages. In this study we investigated the direct effect of reovirus on Natural Killer cells (NK cells) and its effect on NK cell mediated antibody dependent cellular cytotoxicity (ADCC) against the EGFR (Epidermal Growth Factor) positive colorectal cancer cell line: DLD-1 (KRAS mutant). NK cells isolated from human PBMCs were cultured with 1pfu of reovirus for 12 hrs and subsequently co-cultured with DLD-1 cells coated with increasing concentrations anti-EGFR antibody cetuximab. ADCC was measured after 4 hrs using a lactate dehydrogenase (LDH) based cytotoxicity assay. We observed that the reovirus treated NK cells (Reo-NK cells) exhibited a ~16-fold increase in cytotoxicity against DLD-1 (16.3% ±1.5, n=3) compared to untreated NK cells (NK), even in the absence of any cetuximab. In the presence of cetuximab, NK cells showed a dose dependent increase in ADCC, with maximum ADCC, observed at 0.1 µg/ml of cetuximab (DLD-1+NK: 33.4%± 7.1, n=3). Interestingly, Reo-NK cells showed maximum ADCC even at 0.01 µg /ml of cetuximab (DLD-1+Reo-NK: 39.1±7.4, DLD-1+NK: 26.7±2.4%, n=3). Reo-NK cells also exhibited an increased expression of activation marker CD69 (Reo-NK: 70.4%, NK: 35.2%) and degranulation maker CD107a (Reo-NK: 14.6%; NK: 4.45%) compared to the untreated NK cells. We further characterized the Reo-NK cells by using the HIMChip microarray platform; a custom Agilent SurePrint HD 8x15k format array containing over 7,000 unique probes for over 4,274 human immune-related genes. In ingenuity pathway analysis, we observed that the Interferon pathway (2.13E-20) and pathway controlling activation of IRF by cytosolic pattern recognition receptors (1.27E-11) were the predominant pathways observed in the Reo-NK cells. These results suggest an interferon-mediated response could be contributing to the increased cytotoxicity of the NK cells. In an in vivo study, DLD-1 cells were grown subcutaneously in athymic nude mice and injected intravenously with reovirus (5x 10^8 pfu), followed by intraperitoneal injection of Cetuximab (200 ug/mice) every week. We observed a significant regression of tumors in the Reovirus+Cetuximab combination group compared to the Reovirus treated (Reovirus+Cet: 349.9 mm^3, Reovirus: 623.8 mm^3; n=9; P=0.0028) or Cetuximab treated (Reovirus+Cet: 349.9 mm^3, Cet: 730.5 mm^3;n=9; P= 0.030) groups on day 28 post treatment. Thus, in this study our results demonstrated that human NK cells when treated with reovirus show increases in activation, degranulation and cytotoxicity when
compared to untreated NK cells. Further, in the in vivo model we observed increased tumor regression in mice treated with reovirus in combination with cetuximab. We propose that reovirus activated NK cells are a potential candidate for cell based immunotherapy in combination with FDA approved tumor targeting antibodies to treat malignancies, including lymphomas. Further studies are ongoing to investigate the underlying mechanisms that contribute to the increase in cytotoxicity by NK cells treated with reovirus.

**Disclosures** No relevant conflicts of interest to declare.

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