

# Activation of MDA5 improves anti-tumor therapy and reduces suppressive capacity of myeloid-derived suppressor cells

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**Objective:** Pancreatic cancer is infiltrated by stromal cell and suppressive immune cells, including myeloid-derived suppressor cells (MDSC). MDSC are key determinants for maintaining the immunosuppressive tumor microenvironment. Both, monocytic MDSC (M-MDSC) and polymorphonuclear MDSC (PMN-MDSC), are highly T cell suppressive. Stimulation of RIG-I-like helicases (RLH), such as melanoma differentiation antigen 5 (MDA5), induces a type I interferon (IFN)-driven immune response and induces an immunogenic form of tumor cell death in pancreatic cancer. The aim of the study is to assess the effects of RLH treatment, using the synthetic MDA5 ligand polyinosinic:polycytidylic acid (poly(I:C)), on the MDSC phenotype and function.

**Methods:** C57BL/6 and IFNAR<sup>-/-</sup> mice were orthotopically injected with KPC-derived T110299 pancreatic cancer cells and treated i.v. with poly(I:C), complexed with PEI. After 21 days, mice were sacrificed and immune cells from tumors and spleens were analyzed by flow cytometry. PMN-MDSC (CD11b<sup>+</sup> Ly6G<sup>+</sup> Ly6Cint) and M-MDSC (CD11b<sup>+</sup> Ly6G<sup>-</sup> Ly6C<sup>+</sup>) as well as bulk tumor biopsies were isolated for RNA-sequencing as well as for functional assays. RNAseq data were subjected to KEGG pathway analysis, Gene Set Enrichment Analysis.

**Results:** Survival of poly(I:C)-PEI-treated mice with orthotropic pancreatic tumors was significantly prolonged, and a reduction of tumor size was observed after only two injections of poly(I:C)-PEI. The suppressive capacity of both, PMN- and M-MDSC populations was significantly reduced after poly(I:C)-PEI treatment. KEGG pathway analysis of RNA sequencing data revealed an enrichment of type I IFN signaling, virus-associated as well as antigen processing- and presentation-associated gene signature upon poly(I:C)-PEI treatment. In addition, flow cytometry confirmed the upregulation of MHC-I and CD86

surface molecules in MDSC-subpopulations. In IFNAR<sup>-/-</sup> mice, expression levels were unaltered pointing towards a type I IFN-mediated effect. CIBERSORT analysis of bulk tumor tissue pointed towards a reduction of the M2 phenotype, which was paralleled by increased expression levels of M1-related genes, after therapy.

**Conclusion:** The survival benefit of RLH ligand-treated mice with pancreatic cancer correlated with the reduction in tumor size, reduced T cell suppressive capacity of MDSC and functional reprogramming towards a M1/G1 phenotype. Type I IFN signaling induced by RLH-based immunotherapy has the potential to revert tumor induced immune suppression.

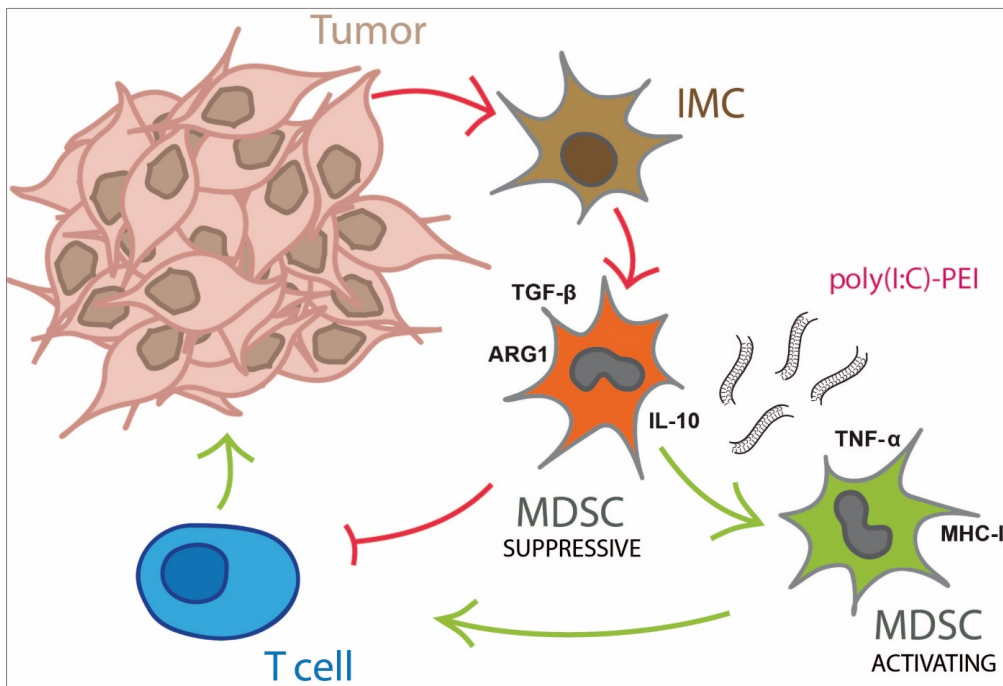


Figure 1: Rlg-like helicase treatment induced reprogramming of MDSC