TARGETING THE SECRETORY PATHWAY OF T CELLS TO BOOST THE EFFICACY OF CANCER IMMUNOTHERAPY

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Background The efficiency of adoptive immunotherapy correlates with the ability of the transferred T cells to release effector molecules. Furthermore, T cell memory formation is important to ensure a long lasting anti-tumor effect and thus, to prevent tumor relapse. Here, we optimized the efficacy of adoptive immunotherapy by silencing EBAG9, a negative regulator of cytolytic enzyme release from CD8+ T cells.

Methods Using a retroviral vector that includes an intronically located EBAG9-targeting miRNA, we equipped T cells with defined TCR or CAR specificities and, simultaneously, knocked down EBAG9. In vitro, cytotoxicity as well as cytokine release assays were employed. Kinetics of tumor cell killing and the sensitivity of CAR activation were determined by microscopy-based methods. Knockout mice, cytotoxicity assays and xenotransplantation models served as tools to study EBAG9 functionality in vivo. Furthermore, T cell memory formation, in vivo target cell killing and tumor growth after adoptive transfer of engineered T cells were investigated.

Results EBAG9 knockdown in mouse CTLs resulted in a significant increase of antigen-specific in vivo killing activity. Granzyme A release from engineered human TCR and CAR CD8+ T cells was increased after EBAG9 silencing, while cytokine secretion was not altered. Enhanced granzyme release translated into strongly improved killing capability and kinetics without leading to faster exhaustion. Adoptively transferred EBAG9-silenced BCMA CAR T cells exhibited an extended control of multiple myeloma growth, even at low effector cell numbers. Upon leukemia challenge of Egag9−/− mice, the enhanced CD8+−mediated cytotoxicity was associated with increased commitment to the CD8+ T cell memory lineage. Single cell RNA sequencing revealed a preferential expression of the memory-related transcription factors Zeb1, Id3, and Stat3 as well as anti-apoptotic genes like Traf1 in Egag9−/− CD8+ cells.

Conclusions Targeting EBAG9 has a promising therapeutic potential when applied to adoptive immunotherapy of leukemia and multiple myeloma and represents a platform technology that can be favorably combined with variable CAR or TCR specificities. We suggest a strategy to boost the efficacy of engineered T cells that, concomitantly, facilitates an optimized manufacturing combined with the need for a lower therapeutic dose. From Egag9-KO mice we infer that an enhanced primary immune response leads to improved memory formation and, thus, lowers the risk for tumor relapse. Gain of cytolytic efficacy by EBAG9 silencing does not come at the expense of adverse effects, such as faster exhaustion or release of inflammatory cytokines.