222

GENETIC REPROGRAMMING OF MERKEL CELL CARCINOMA AND MELANOMA LEADS TO INCREASED MHC-I EXPRESSION AND ANTITUMOR IMMUNE ACTIVATION IN VITRO AND IN VIVO

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Background Merkel cell carcinoma (MCC) is a rare skin cancer with 46% disease-associated mortality and half of patients unresponsive to immune checkpoint inhibitors.^{1 2} MCC and melanomas often display decreased MHC class I (MHC-I) expression on the surface of cells, which prevents antigen recognition by T cells ("signal 1") and hampers immune activation. We therefore sought to genetically reprogram cells to express their own costimulatory molecules ("signal 2") and immunostimulatory cytokines ("signal 3") to increase MHC-I expression and drive a targeted immune response.

Methods We used biodegradable poly(beta-amino ester) nanoparticles (NPs) to co-deliver plasmids encoding a signal 2 molecule (4-1BBL) and two signal 3 molecules (IL-12 and IFN γ) to cancer cells. For in vitro evaluation of NPs we used two patient-derived MCC cell lines with low baseline MHC-I expression; MCC13 and UIISO. Co-culture experiments were performed with human PBMCs or primary human natural killer (NK) cells. All in vitro analysis was performed 7 days following PBMC or NK cell addition. For in vivo evaluation, subcutaneous B16F10 mouse melanoma tumors were implanted in C57BL/6J mice and NPs were administered by direct injection into the tumor with and without intraperitoneal injection of α PD1. Tumors were harvested for analysis on day 16.

Abstract 222 Figure 1 Administration of signal 2/3 NPs to MCC13 and UIISO cells led to increases in MHC-I and MHC-II expression after 7 days. MHC-I expression in transfected cells (red) and MHC-II expression in transfected cells (blue) compared to untreated control (black)

Abstract 222 Figure 2 Co-culture of transfected MCC cells with human PBMCs led to increases in CD45+ cells and reduced MCC cell viability after 7 days

Abstract 222 Figure 3 Co-culture of 4-1BBL/IL-12 transfected MCC13 cells with isolated CD56+ NK cells demonstrated robust NK-cell expansion and low MCC cell viability after 7 days

Abstract 222 Figure 4 Direct intratumoral injection with signal 2 and 3 NPs led to increases in MHC-I and MHC-II in cancer cells in vivo.

Abstract 222 Figure 5 NPs were administered intratumorally $\pm$ intraperitoneal α PD1 on day 9, 11, and 13 following B16F10 melanoma tumor implantation. 4-1BBL/IL12 particles in combination with α PD1 demonstrated a significant improvement in survival compared to control particles (Luc) with α PD1 ($p=0.0010$)

Results Transfection with particles delivering the three plasmids to MCC13 and UI50 increased MHC-I expression (mean fluorescence intensity) 1.6- and 5.0-fold, respectively, and MHC-II expression increased 1.6- and 6.3-fold, respectively (figure 1). In co-culture with human PBMCs, signal 2/3 particles resulted in increased leukocyte proliferation (4.6- and 6.1-fold increase, respectively) and led to significantly reduced MCC viability (10.6 and 1.6% vs control particles)(figure 2). When MCC13 cells were co-cultured with primary human NK cells, NK cell expansion increased 355-fold with 4-1BBL/IL-12 particles compared to control particles and was accompanied by 2.5% MCC13 cell viability, indicating a potent innate immune response with signal 2/3 NP administration in vitro (figure 3). Following evaluation of NPs in vivo, assessment of MHC-I and MHC-II expression in the melanoma tumors found increased expression with signal 2/3 NPs compared to control NPs (figure 4). When signal 2/3 NPs were administered in combination with α PD1 treatment, 4-1BBL/IL-12 NPs with α PD1 demonstrated improved survival compared to α PD1 treatment with control NPs ($p=0.0010$) (figure 5).

Conclusions Together, these results show the ability of signal 2/3 NPs to reprogram MCC and melanoma cells, leading to increased MHC-I expression in vitro and in vivo, eliciting a productive immune response against cancer cells.

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