Identification of an intratumoral immune gene signature associated with tumor regression in an axalimogene filitolisbac-treated murine HPV+ tumor model

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INTRODUCTION

Axalimogene filitolisbac (AXAL) is a live attenuated Listeria monocytogenes (Lm) bacterium that mediates tumor regression by expressing the large T antigen of human papilloma virus type 16 (HPV-16).

AXAL is currently being evaluated in clinical trials as a monotherapy and in combination with checkpoint inhibitors to treat patients with HPV-associated cancers, such as cervical cancers, head and neck cancers, and anal cancer.

Axalimogene filitolisbac therapy is delivered intravenously using multiple mechanisms including the SLING pathway, by reducing the frequency of immune-privileged cells in the tumor microenvironment, and by inducing the generation of tumor antigen-specific T cells that infiltrate and destroy cancer cells.

To gain a better understanding of the molecular mechanisms of action of AXAL and to identify immune gene signatures that correlate with AXAL-mediated tumor regression, we performed intratumoral gene expression profiling of tumors in an AXAL-treated murine HPV+ tumor model.

OBJECTIVES

To evaluate the intratumoral gene expression profiles of regressing and progressing tumors in a murine HPV+ tumor model.

To identify immune gene signatures associated with AXAL-mediated tumor regression.

MATERIALS AND METHODS

1 TC-1 tumor cells were derived from a C57BL/6 J lymphadenoma cell line that was immortalized with HPV-16 and transformed with an engineered virus. To establish primary tumors, 1.5 × 10^5 TC-1 cells were injected subcutaneously in the flank of C57BL/6 J mice to generate tumors for 8 days prior to the start of treatment.

2 Total RNA was extracted from tumors harvested on day 19 post implantation of the first tumor cell line, at the end of the treatment (Figure 1A). We also included groups for analysis of the following tumor subpopulations: 1. Tumor cells from day 8 tumors were harvested for intratumoral gene expression analysis.

Differential expression analysis was conducted on normalized handling assay data by identifying upregulated genes that were differentially expressed (as adjusted FDR < 0.05 and log fold change > 2) between the 2 groups.

The Gene Set Enrichment Analysis (GSEA) software was used to identify signaling pathways and networks that are activated in AXAL-treated murine tumors. For this analysis, the threshold for significance was set at a false discovery rate of 0.1.

To validate the immune cell types identified in regressing tumors using the GSEA software, we performed flow cytometric analysis on the following immune cell types harvested at day 19, as the same day tumors were harvested for analysis of intratumoral gene expression data.

Statistical analysis was performed using GraphPad Prism software. The applied Student's t-test was used for all pairwise comparisons. The p-values were adjusted using the Benjamini-Hochberg procedure to account for multiple comparisons.

RESULTS

1 To establish the molecular mechanisms of action of AXAL in mediating tumor regression, we sought to determine the optimal time point to evaluate tumors for intratumoral gene expression analysis.

2 To this end, C57BL/6 J mice were injected subcutaneously with 1.5 × 10^5 TC-1 tumor cells and were then treated on days 8, 15, and 22 with PBS (1× 10^6 CFU AXAL), or 1× 10^6 CFU AXAL (Figure 1A).

3 Analysis of tumor growth curves revealed that AXAL-mediated tumor growth inhibition began on day 19 post implantation (Figure 1B). Consequently, we chose day 19 post implantation to evaluate tumors for intratumoral gene expression and for validation of immune cell composition.

CONCLUSIONS

This study was identified an intratumoral immune gene signature that highlights the importance of cytokines and cytokine-producing effector lymphocytes, reactive antigen-presenting cells, and intratumoral immunity in AXAL-mediated tumor regression.

The intratumoral immune gene signature may serve as a guide to identify molecular diagnostic and therapeutic approaches for patients with HPV-associated cancer receiving AXAL immunotherapy.

REFERENCES


Figure 1A. AXAL-mediated tumor growth inhibition began on day 19 post tumor implantation.

Figure 2. Stratification based on tumor growth.

Figure 3. Increased expression levels of gene signatures identifying major effector cell subsets in regressed tumors.

Figure 4. Flow cytometric analysis of day 19 tumor-infiltrating immune cells.

Figure 5. Differential expression of immune-related genes in tumor regression vs tumor progression.

Figure 6. Functional categories of the upregulated genes in tumor regression.

Figure 7. Upregulated genes in the Th1 pathway.

Figure 8. Upregulated genes in the cross-talk between dendritic cells and NK cells pathway.