Characterization of T/B cell antigen specific-engineered tumors for studying T cell and GC B cell function in a murine model of NSCLC

Armando J. Ruiz-Justiz‡, Mona Yazdani‡, Xin Sun‡, Lili Chen§, Michael Wang§, John Le†, Haiping Guo‡, Can Cui‡, Kelli Cannelly§, Aya Tai†, Wei Hu MD, PhD§, Nikhil Joshi PhD, and Tina Cascone MD, PhD‡*

1University of Puerto Rico – Medical Sciences Campus, San Juan, PR. 2Thomas Jefferson University, Philadelphia, PA. 3Thoracic/Head and Neck Oncology Department, The University of Texas MD Anderson Cancer Center, Houston, TX. 4Department of Immunobiology, Yale University School of Medicine, New Haven, CT. *Corresponding author: cascone@mdanderson.org

Background

• Therapy with immune checkpoint inhibitors (ICIs) has changed the treatment landscape of Non-small Cell Lung Cancer (NSCLC), yet only ~20-40% of patients benefit from it [1].
• Tertiary Lymphoid structures (TLSs) have been shown to be associated with response to ICIs in cancers [2].
• TLS formation and maturation correlate with higher intratumoral T/B cell function, suggesting that understanding the mechanisms by which T/B cell interact within TLSs in NSCLC tumors could improve the efficacy of ICI treatment [3].
• NINJA- and HELLO antigen-expressing cancer cells may drive tumor-specific T, B and TFH cell responses which allow to study their interaction in tumors [4].

Hypothesis

We hypothesized that HELLO and NINJA-antigen-expressing NSCLC cells will be useful models to study T/B cell interaction within the tumor microenvironment.

Methods

Cell transfection

We performed cell transfection for the generation of NINJA and HELLO cell lines. These cells were engineered with a lentiviral vector expressing the Flag-tagged HELLO or NINJA proteins.

ANOVA

Statistical analysis was performed using ANOVA to determine significance levels.

Results

344SQ-HELLO and 344SQ-NINJA cells expressed HELLO and NINJA neoantigens

Figure 1. Diagram representing the experimental design.

• 344SQ cells were transfected with a lentiviruses containing HELLO (mSarlet HELLO; Flag/GFP) or NINJA (GFP-GP33; Flag/GFP neoantigens) transcript.
• EVOX microscopy was used to evaluate HELLO and NINJA expression.
• 129e/6 mice were injected subcutaneously in the flank with 106 HELLO or NINJA cells and spleens were harvested.
• The tumor size was measured using a digital caliper 3 days per week up to day 23. Tumor volume was calculated using the formula: (length x width x 1/2).

Tumor Growth Curve and Tumor Volume

Figure 3. Tumor growth curve and tumor volume of HELLO and NINJA tumors was measured from day 0 to day 23. On day 24 mice were euthanized and tumors were harvested. Tumor growth curves were calculated using the formula: (tumor volume on day 23 - tumor volume on day 24) / tumor volume on day 24 x 100. The results were statistically analyzed using the Student's t-test.

Significant increase of activated Tetramer+ CD4+ and CD8+ T cells, and cytotoxic CD8+ T cells in NINJA tumors

Figure 4. Flow cytometry analysis was performed on tumors and splenocytes from HELLO and NINJA mice. The results were graphed using Prism 9 and One-way ANOVA test was performed for statistical purposes.

Tumors of HELLO mice exhibited greater amounts of activated antigen-specific CD8+ T cells in dLNs

Figure 5. The frequency of CD4+ T cells (A) and CD8+ T cells (B) in HELLO tumors was measured by flow cytometry.

Conclusions

• Tumor growth was significantly decreased in the 344SQ-NINJA model, compared to 344SQ-HELLO and 344SQ-LentIT models.
• Infiltration of antigen-specific CD4, CD8 T cells and cytotoxic CD8 T cells was increased in NINJA tumors compared to LentIT tumors.
• NINJA tumors presented higher infiltration of CD4 T cells, which may suggest a role of this subpopulation in the antitumor response.
• HELLO tumors presented higher infiltration of Tregs, which may play a role in the reduced antitumor effect of HELLO vs NINJA antigens.
• The higher amount of activated Tet+ CD8+ T cells in dLNs of HELLO vs that of LentIT and NINJA tumor-bearing mice suggests potential distinct tumor antigen-specific immune responses in these models.

Future directions

• In vitro functional study of CD4 and CD8 T cells and B cells isolated from the HELLO, NINJA and LentIT tumors.
• Evaluate T and B cell infiltration in HELLO and NINJA tumors by IHC.

References


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