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

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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 (TLS) 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 TLS 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

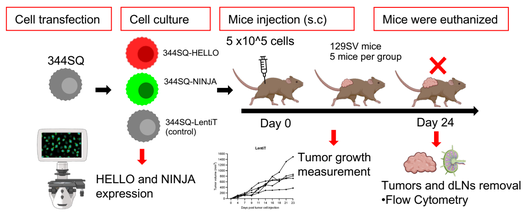

Figure 1. Diagram representing the experimental design.

- 344SQ cells were transfected with a lentivirus containing HELLO (mSarlet-HEL-GP_{33-43}/FLAG/GP_{6180}) or NINJA (GFP-GP_{33-43} /FLAG/$GP_{61–80}$) transcript.
- EVOS microscope was used to evaluate HELLO and NINJA expression.
- 129sv mice were injected subcutaneously in the flank with a 100uL preparation of 5x10^5 cells of the corresponding group suspended in PBS and Matrigel (1:1).
- 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 ^2)/2.
- On day 24 mice were euthanized. Tumors, draining lymph nodes (dLNs) and spleens were harvested.
- Flow cytometry analysis was performed on tumors and dLNs.
- Results were graphed using Prism 9 and One-way ANOVA test was performed for statistical purposes.

Results

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

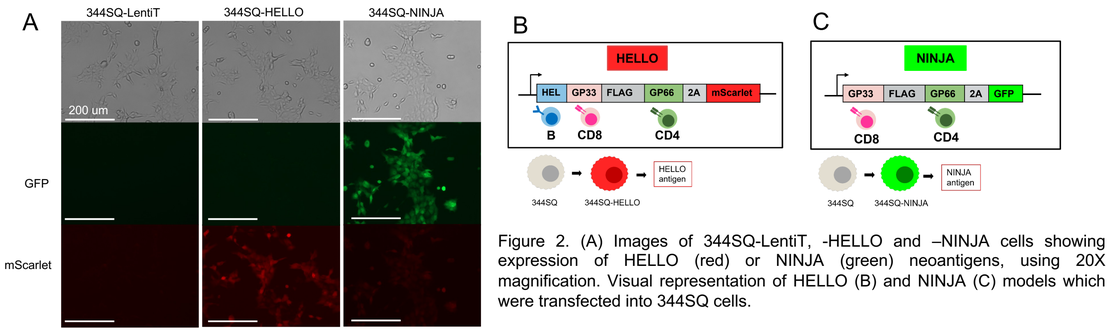

Figure 2. (A) Images of 344SQ-LentiT, -HELLO and –NINJA cells showing expression of HELLO (red) or NINJA (green) neoantigens, using 20X magnification. Visual representation of HELLO (B) and NINJA (C) models which were transfected into 344SQ cells.

NINJA tumors exhibited the greatest tumor growth reduction compared to HELLO and LentiT tumors

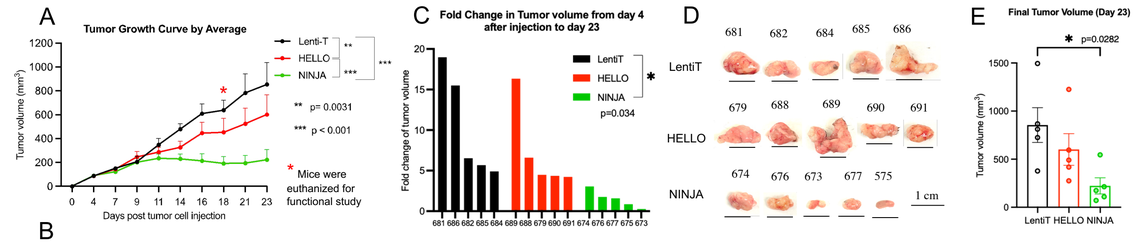

Figure 3. Tumor growth was measured from day 0 to day 23. On day 24 mice were euthanized and tumors were harvested. Tumor growth curves were graphed by using the average of each group (A) and each group (LentiT, HELLO or NINJA) individually (B). (C) The fold change in tumor volume was determined for each mouse from day 4 to day 23 using the formula: (tumor volume on day 23 - day 4) / day 4. (D) Representative images of harvested tumors on day 24. (E) Comparison of final tumor volume by average among groups.

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

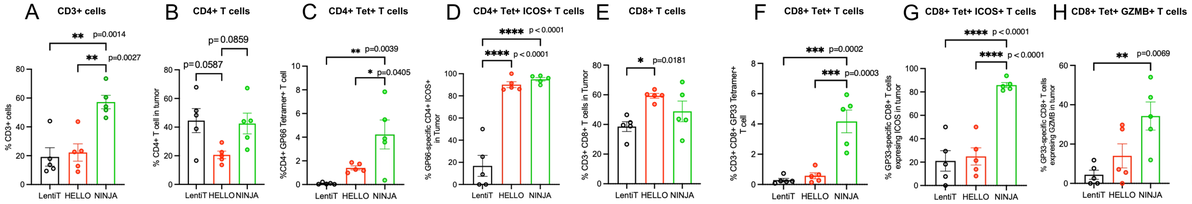

Figure 4. Flow cytometry of tumors was performed using a T cell panel for determining the frequency of CD3+ cells (A), CD4+ T cells (B) and GP66-specific CD4+ T cells (C) in tumors. Activation of GP66-specific CD4+ T cells (D) was measured by ICOS expression. Frequency of CD8+ T cells (E), GP33-specific CD8+ T cells (F), activated GP33-specific CD8+ T cells (G) and cytotoxic GP33-specific CD8+ T cells (H) in tumors.

Greater infiltration of CD4 TFH cells was observed in NINJA tumors whereas Treg infiltration was increased in HELLO tumors

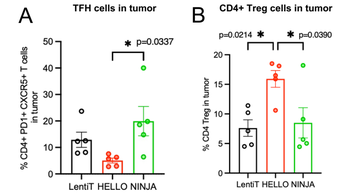

Figure 5. The frequency of CD4 TFH cells (A) and Tregs (B) in tumors was measured by flow cytometry.

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

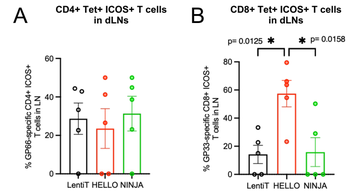

Figure 6. The frequency of activated CD4+ (A) and CD8+ (B) T cells in dLN was measured by flow cytometry.

Tumors of HELLO mice exhibited numerically higher frequencies of GC B cells in dLNs

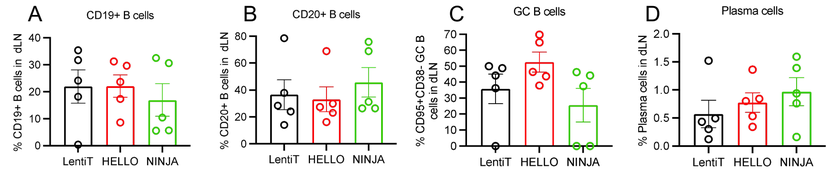

Figure 7. The frequency of CD19+ (A), CD20+ (B), GC B cells (C) and plasma cells (D) in dLNs was measured by flow cytometry.

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 Lenti-T tumors.
- NINJA tumors presented higher infiltration of CD4 TFH 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 NINJA and LentiT 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 and dLNs.
- Evaluate T and B cell infiltration in HELLO and NINJA tumors by IHC.
- *In vivo* efficacy study of ICI therapy in orthotopic murine models of 344SQ NSCLC expressing NINJA and HELLO antigens.

References

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