Leveraging Cancer-Testis Antigen Expression in Metastasis: A Precision TCR-Engineered Approach for Head and Neck Cancer

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Background

- HNSCC is aggressive with a critical need for novel therapies.
- CTAs represent promising precision immuno-oncologic targets given their restricted expression to immune-privileged tissues (testis) and aberrant expression in tumors.
- We previously demonstrated tumor-specific transcriptional and translational expression of certain CTAs in untreated (de novo) and recurrent HNSCCs, with absence in benign oral mucosa.
- For effective cytotoxic therapy, CTAs must be recognized by T cells and exhibit consistent expression across disease settings.
- To address this, we performed bulk and single-cell RNA sequencing on patient-matched primary tumor, regional metastatic node, and peripheral blood (PBMCs), with immunohistochemistry (IHC) validation.
- Separately, we used *in silico* prediction of peptide:HLA binding to identify complexes from CTA-derived peptides. These peptides represent potential TCR-engineered T cell therapy targets.

Methods

1. Bulk and single-cell RNA sequencing was performed on patient-matched tumor, node, and PBMCs from an advanced HNSCC (P120).
2. Tumor, node, skin, and testes were stained for *MAGEA3* (ab223162, Abcam) and *MAGEA6* (14602-1-AP, Proteintech).
3. In another HNSCC (P110), 8-11mer peptides were generated for *MAGEA1* and *MAGEA4* using custom code. HLA subtypes were determined with HLAscan, and netMHCpan predicted high-affinity peptide binding to patient-specific HLA alleles.

Results

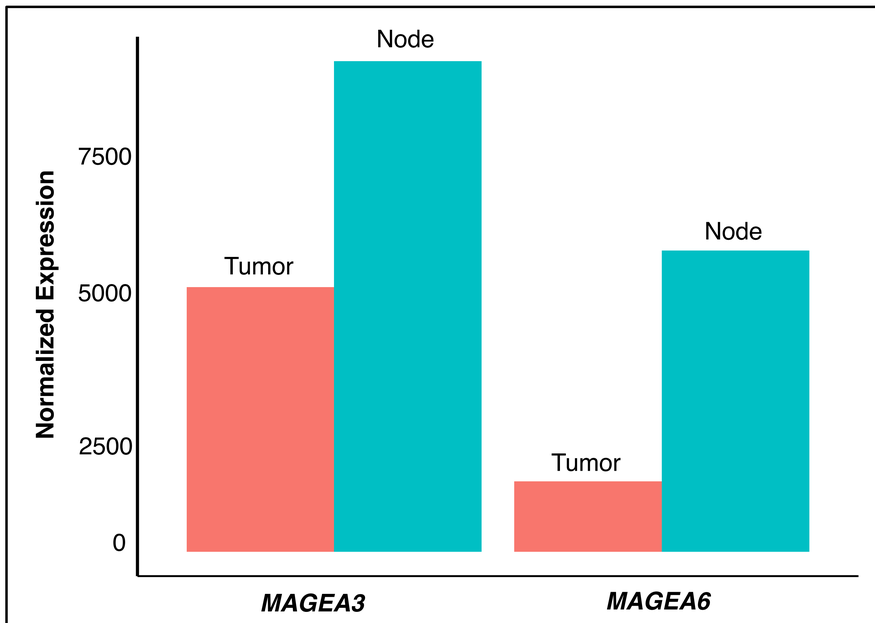


Figure 1 Bulk RNA sequencing: normalized expression of *MAGEA3* and *MAGEA6* between patient-matched primary tumor and metastatic node.

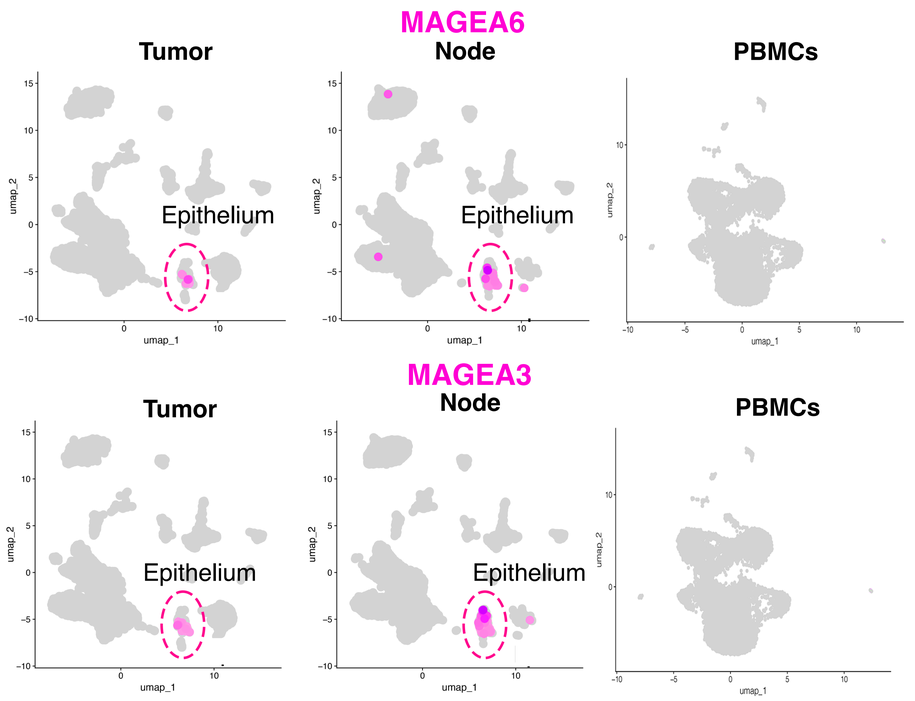


Figure 2 Single-cell RNA sequencing: overlapping epithelial expression of *MAGEA3* and *MAGEA6* between patient-matched primary tumor and metastatic node, with absence in PBMCs.

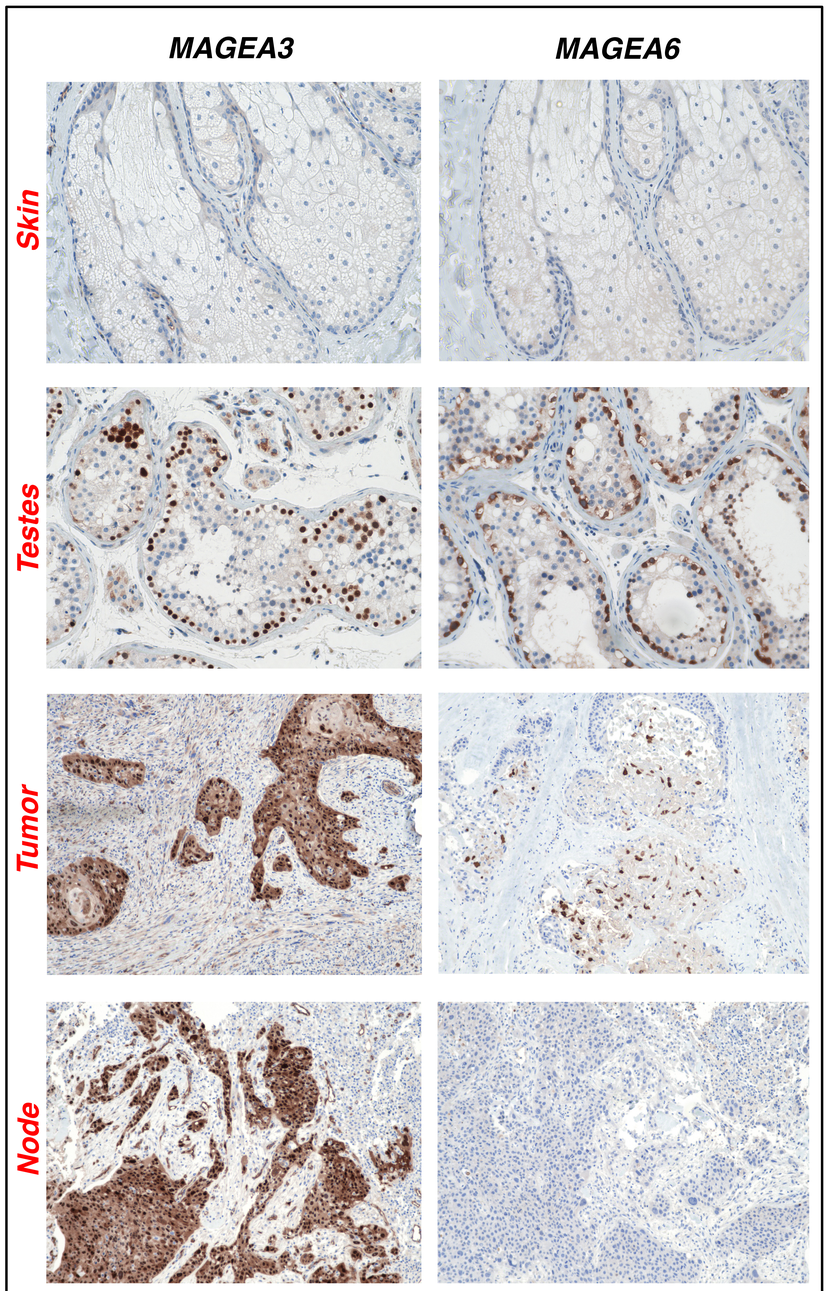


Figure 3 IHC for *MAGEA3* and *MAGEA6* in P120 demonstrates expression in both the primary tumor and matched metastatic node. Positive (testis) and negative (benign skin) controls confirm target specificity. These findings suggest that combinatorial targeting of CTAs could be effective in this patient, reinforcing the rationale for a precision-guided immunotherapeutic strategy.

Discussion, Conclusion and Next Steps

- CTA expression is preserved across metastatic sites in HNSCC, supporting their role as stable, tumor-restricted targets for systemic immunotherapy.
- IHC confirmed protein-level expression, validating transcriptomic findings and confirming antigen availability for targeting.
- *In silico* predicted peptide:HLA complexes show high-affinity binding, supporting the development of HLA-restricted TCR-based therapies.
- These findings will support off-the-shelf TCR approaches, personalized to patient HLA, with broad applicability across tumor sites and patients.
- To advance this strategy, we next aim to validate the CTA-derived peptide:HLA complexes through functional immunogenicity testing, with the end goal of generating CTA-specific TCRs.

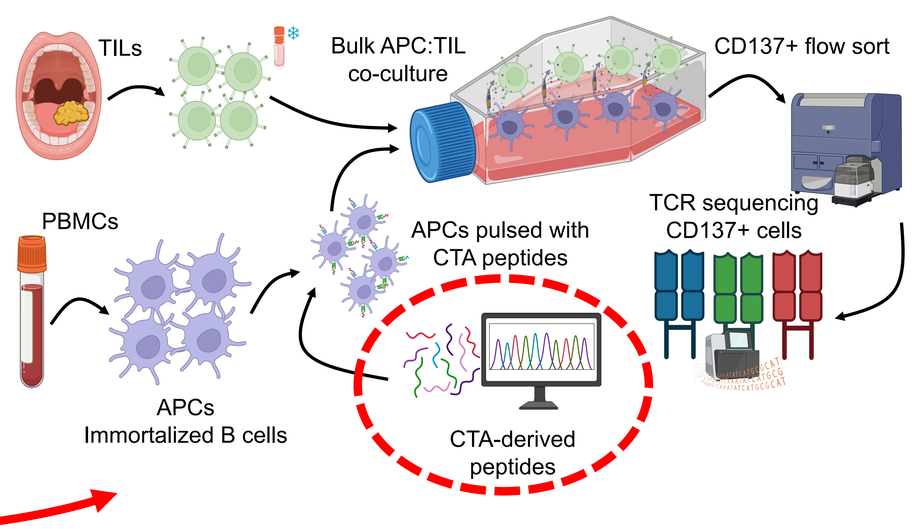
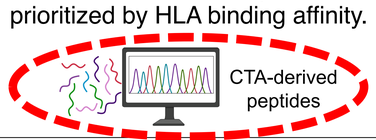


Table 1 *In silico* predicted peptides that *MAGEA1* and *MAGEA4* are processed into, prioritized by HLA binding affinity.



MAGEA1 Peptides	*MAGEA4* Peptides
RQVPDSDPARY	AAVSSSSPL
KEADPTGHSY	EVDPASNTY
SAFPTTINF	TVYGEPRKL
NQIMPKTGF	IAYPSLREA
TQDLVQEKY	GVYDGREHTVY
LVQEKYLEY	RQVPGSNPARY
TSYVKVLEY	ASALPTTISF
VKEADPTGHSY	KEVDPASNTY
ASAFPTTINF	RVNARVRIAY
KASESLQLVF	SALPTTISF
EADPTGHSY	WVQENYLEY
SAYGEPRKL	LAHFLLRKY
TSSSSPLVL	IIVLGTIAM