Cancer Neoepitope Identification in Soluble HLA from Patient Plasma

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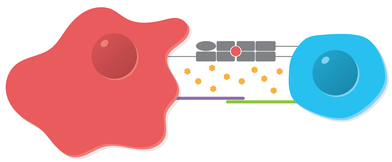
KEY FINDING

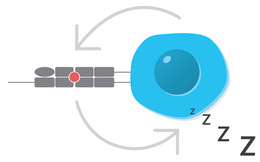
Soluble HLA can be immunoprecipitated from patient plasma for immunopeptidome analyses.

Abstract

The roles of the major histocompatibility complexes (MHCs) in anticancer immunity are well established. Cytotoxic immune cells can recognize MHC presentation of unique peptides from altered proteins on the surface of tumor cells (tumor neoantigens) or the downregulation of MHC itself and kill the affected cells. One of the hallmarks of cancer progression is the evasion of antitumor immunity by various mechanisms, including the upregulation of checkpoint proteins. Checkpoint inhibitors, which have recently seen dramatic success in the clinic in some cancers, act by inducing potent immune responses to tumor neoantigens. In addition, the promise of personalized medicine lies in the theoretical ability to teach the immune system through vaccination to recognize and kill tumor cells bearing MHC-presented neoantigens. Aside from MHC on cell surfaces, the presence of soluble MHC (sMHC) has been shown in a variety of bodily fluids, including blood. sMHC may be an important component of the tumor arsenal for inhibiting antitumor immune responses. The precise function of sMHC may be disease-dependent, but it has been postulated to play an inhibitory role, presumably through signaling cognate T cell receptors in the absence of a cellular signal (signal 2). Presence of sMHC carrying cancer-associated neoantigens could predict success of vaccination therapy or checkpoint therapy. Furthermore, sMHC-associated peptides could help to identify neoantigens that have already induced T cell anergy and would hence not be good targets. Here, data are presented describing the plasma immunopeptidome of advanced non-small cell lung cancer subjects under a variety of treatment paradigms.

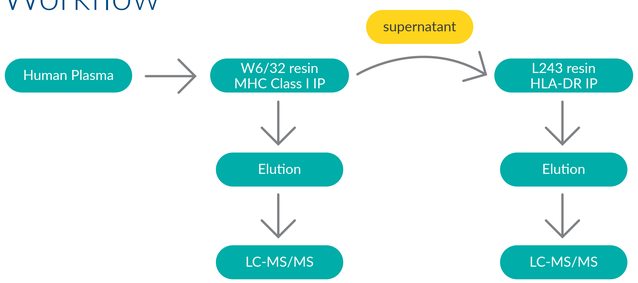


Cancer cell displaying neoantigen in the presence of co-stimulation results in T cell-mediated killing of tumor.



T cell encounters sMHC displaying neoantigen with no co-stimulation and becomes tolerant to that antigen.

Workflow

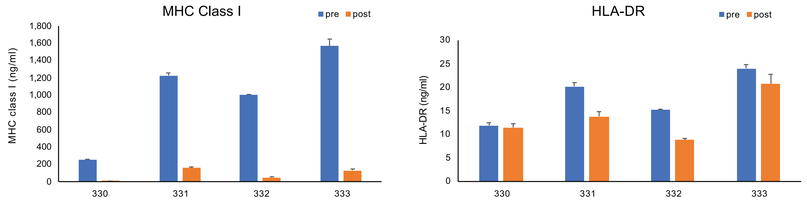


Demographics

	Gender	Age	Race	Diagnosis	Medication
330	F	76	Caucasian	NSCLC	Alimta®, Keytruda®, Carboplatin
331	M	71	Caucasian	Adenocarcinoma, lung	None
332	M	70	Caucasian	Squamous cell carcinoma, lung	Taxol®, Carboplatin
333	F	85	Caucasian	NSCLC	EGFR inhibitor

Samples consisting of 3 ml of plasma collected into cell-free BCT tubes from the above subjects were obtained from a biobank.

MHC ELISA for Quality Control



ELISA is used to compare levels of MHC molecules in plasma before and after immunoprecipitation. On the left, pan-MHC class I and β2-M antibodies are used to detect total MHC class I. Post-IP levels are dramatically lower, suggesting effective capture of MHC by the W6/32 resin. On the right, antibodies specific to HLA-DR form a sandwich and show that very little HLA-DR was found in the plasma of the subjects included in this study, limiting the ability of L243 resin to capture.

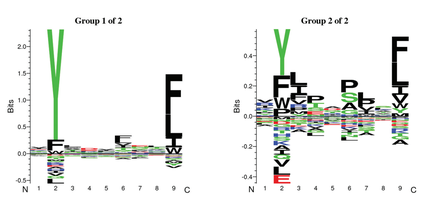
Summary of Peptides Identified by Mass Spec

	Class I Peptides	Class II Peptides
330	161	208
331	827	178
332	206	91
333	408	66
Mutant	6	5
TOTAL	1,820	457

Peptides were gently eluted from immunoprecipitated MHC and analyzed by nano LC-MS/MS using a Waters nanoACQUITY UPLC system interfaced to a Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid™ mass spectrometer. A 2-hour gradient was employed. The mass spectrometer was operated using a custom data-dependent method, with MS performed in the Orbitrap at 60,000 FWHM resolution and sequential MS/MS performed using high-resolution CID and EThcD in the Orbitrap at 15,000 FWHM resolution. Raw data was searched against Swiss-Prot Human using PEAKS with a 1% PSM FDR. Single amino acid substitutions using SPIDER were allowed to generate the "mutant" line above.

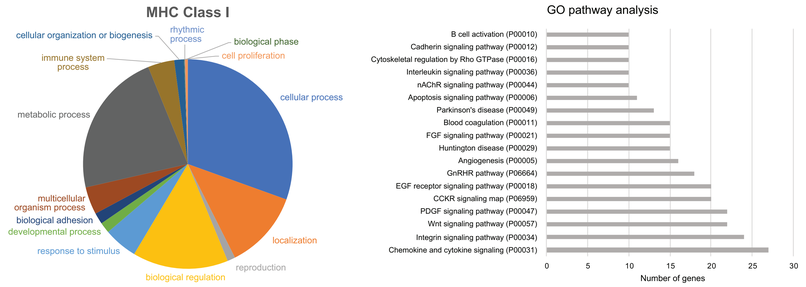
Raw data were additionally searched against published neoantigens[1-4] with no hits detected.

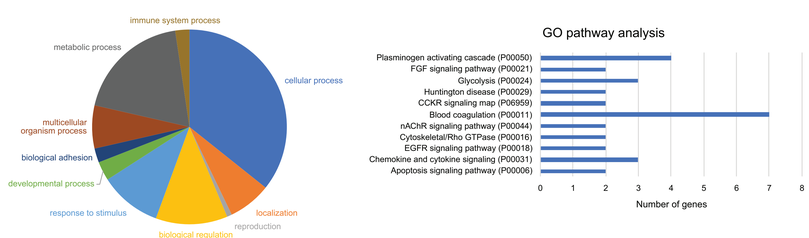
Example Motif Analysis



As HLA haplotypes for subjects were unknown, the free online tool GibbsCluster (https://services.healthtech.dtu.dk/service.php?GibbsCluster-2.0) was used to identify motifs from each subject. MHC class I peptide sequences from subject 331 were clustered into 2 motifs, shown above. Cluster 1 is likely the HLA-A*24 motif.

Gene Ontology (GO) Analysis of Identified Peptides





Panther Classification System (http://pantherdb.org/) was used to classify the proteins, which originated the peptides identified in the sMHC class I (top) and sMHC class II (bottom)-associated immunopeptidomes from the lung cancer subjects. The pie charts above show the biological processes represented by the peptides, while the GO pathway analyses on the right show pathways associated with these peptides. Notably, the sMHC class I immunopeptidome from a single healthy control subject (not shown) was enriched for the same pathways as the lung cancer subjects.

Conclusions

- Soluble MHC is identifiable in plasma samples.
- Peptides associated with soluble MHC (soluble immunopeptidome) can be enriched by immunoprecipitation and identified by LC-MS/MS.
- The majority of these peptides seem to be derived from common plasma and liver proteins.
- True neoantigen identification would benefit greatly from tumor transcriptome data.

Learn more about our Immunopeptidome Profiling services and download this poster by scanning the code

References

1. Chen, F., Zou, Z., Du, J., *et al.* Neoantigen identification strategies enable personalized immunotherapy in refractory solid tumors. *J. Clin. Invest.* **129(5)**, 2056-2070 (2019).
2. Fehlings, M., Jhunjhunwala, S., Kowanetz, M., *et al.* Late-differentiated effector neoantigen-specific CD8+ T cells are enriched in peripheral blood of non-small cell lung carcinoma patients responding to atezolizumab treatment. *J. Immunother. Cancer* **7(1)**, 249 (2019).
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