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TCR fingerprinting and off-target peptide identification

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Background

Adoptive T cell therapy using patient T cells redirected to recognize tumor-specific antigens by expressing genetically engineered high-affinity T-cell receptors (TCRs) has therapeutic potential for melanoma and other solid tumors. Clinical trials implementing genetically modified TCRs in melanoma patients have raised concerns regarding off-target toxicities resulting in lethal destruction of healthy tissue, highlighting the urgency of assessing which off-target peptides can be recognized by a TCR.

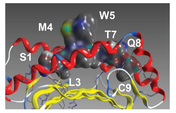
Methods

As a model system we used the clinically efficacious NY-ESO-1-specific TCR C259, which recognizes the peptide epitope SLLMWITQC presented by HLA-A*02:01. We investigated which amino acids at each position enable a TCR interaction by sequentially replacing every amino acid position outside of anchor positions 2 and 9 with all 19 possible alternative amino acids, resulting in 134 peptides (133 altered peptides plus epitope peptide). Each peptide was individually evaluated using three different in vitro assays: binding of the NY-ESOc259 TCR to the peptide, peptide-dependent activation of TCR-expressing cells, and killing of peptide-presenting target cells. To represent the TCR recognition kernel, we defined Position Weight Matrices (PWMs) for each assay by assigning normalized measurements to each of the 20 amino acids in each position. To predict potential off-target peptides, we applied a novel algorithm projecting the PWM-defined kernel into the human proteome, scoring NY-ESOc259 TCR recognition of 336,921 predicted human HLA-A*02:01 binding 9-mer peptides.

Results

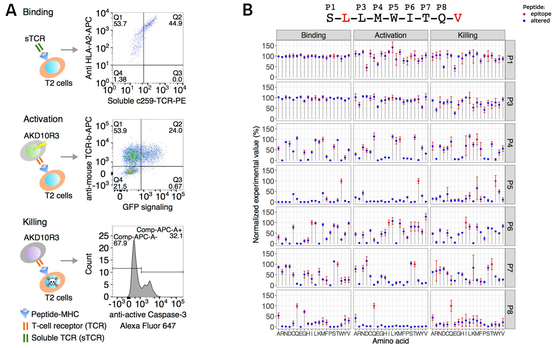
Of the 12 peptides with high predicted score, we confirmed 7 (including NY-ESO-1 antigen SLLMWITQC) strongly activate human primary NY-ESOc259-expressing T cells. These off-target peptides include peptides with up to 7 amino acid changes (of 9 possible), which could not be predicted using the recognition motif as determined by alanine scans.

Figure 1. The NY-ESO peptide



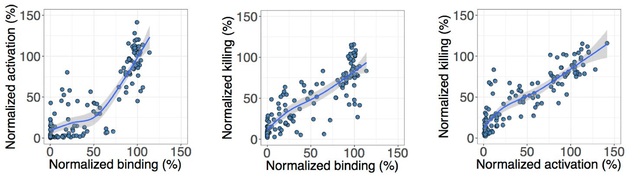
*The NY-ESO-1-derived SLLMWITQC peptide in complex with HLA-A*02:01.*

Figure 2. Experimental validation of TCR NY-ESOc259 specificity



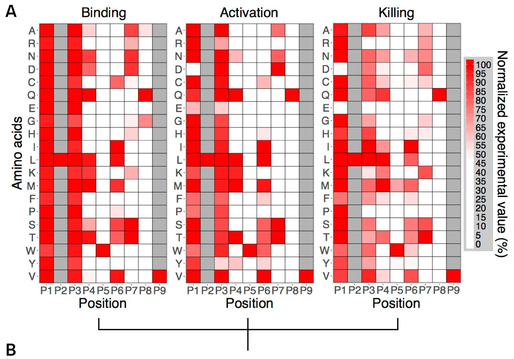
(A) An overview of the assays used for TCR specificity assessment analyzing binding of the TCR to the peptide-MHC complex on antigen-presenting T2 cells, activation of TCR-expressing cells by co-culturing with T2 cells and final killing of the target T2 cells. (B) The effect of amino acid substitution in the epitope peptide on TCR binding/activation/killing. Blue dots depict the average signal of at least two independent experimental replicates (binding n=3, activation n=3, killing n=2).

Figure 3. Pairwise comparisons of the normalized experimental values among the three assays



Activation and killing assays yielded a strong linear relationship indicating a strong dependence between T cell activation level and killing efficiency. Comparing binding with activation and killing assays resulted in a less linear relationship, and in many cases at least 75% binding efficiency is required to trigger T cell activation and killing mechanisms.

Figure 4. Predicting TCR NY-ESOc259 cross-reactive antigens



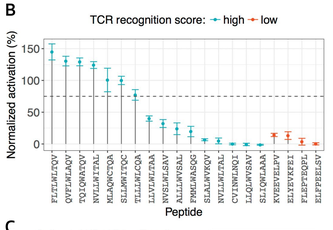
	Peptide (9-mer)	Predicted Binding	TCR recognition score Activation	Killing
1	SLLMWITQC	1.000	1.000	1.000
2	FLTLWLTQV	0.979	0.954	0.890
3	MLAQWCTQA	0.965	0.946	0.896
4	NVLLWITAL	0.927	0.917	0.920
*N	...	...	...	...

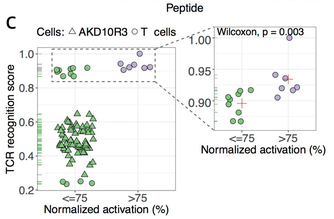
*(A) Position Weight Matrices (PWMs) are constructed by assigning a normalized experimental value to each of the 20 amino acids (rows) at each position (columns). The generated PWMs represent the TCR NY-ESOc259 fingerprint profile: a unique pattern for TCR recognition of the peptide-MHC complex. (B) Scoring 336,921 HLA*A02:01 binding 9-mer peptides with PWMs. Binding affinity of individual peptides to HLA*A02:01 determined by software package netMHCpan v3.0 and defined as IC50 score <= 500 nM.*

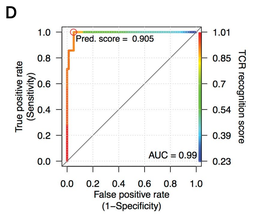
Figure 5. Validation of NY-ESOc259 TCR predicted peptides in primary T cells and AK-D10R3 cells

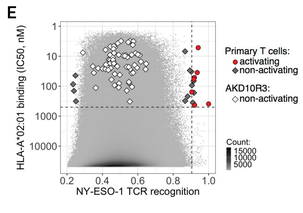
A

Group	Peptide	Gene	Edit distance	HLA binding (IC50)	TCR recognition score
high	SLLMWITQC	CTAG1B CTAG1A CTAG2	0	390	1.000
high	FLTLWLTQV	MKI67	5	5	0.941
high	MLAQWCTQA	FBXO41	5	35	0.936
high	NVLLWITAL	NLGN4Y	5	429	0.921
high	QVFLWLAQV	SH3TC2	7	51	0.920
high	CVINWLNQI	DNAH10	7	166	0.917
high	TLLLWLCQA	FGFRL1	5	59	0.917
high	SLALWVKQV	TECTA	5	67	0.916
high	NVLLWLTAL	NLGN4X	6	354	0.911
high	LLVLWLTAA	LRRC28	6	94	0.906
high	TQIQWATQV	COG7	6	157	0.905
high	ALLLWVSAL	CXorf36	6	33	0.902
high	LLQLWVSAV	ACAP1	7	30	0.894
high	NVSLWLSAV	DIP2A	8	218	0.881
high	FMMLWASQG	CYP8B1	6	307	0.868
high	SLIQWLAAA	MDN1	6	7	0.867
low	KVEVVELPV	PRMT1 PRMT8	9	318	0.250
low	FLEPTEQPL	NRG2	8	44	0.248
low	ELFPEELSV	PRSS55	8	143	0.239
low	ELYEVKFEI	WHAMM	8	86	0.234









(A) An overview of the peptides selected for validation in primary T cells. An edit (Hamming) distance, the minimum number of operations required to transform a peptide sequence into an epitope peptide sequence (SLLMWITQC), is depicted. HLA binding affinity (IC50, nM) was predicted by the netMHCpan v3.0 software package. The TCR recognition score integrates the three assays.

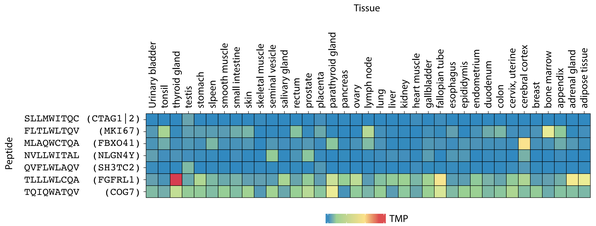
(B) Experimental validation of the predicted peptides in primary T cell activation assay. Peptide pulsed T2 cells were co-cultured with effector T cells expressing TCR NY-ESOc259 and T cell activation was measured by quantifying the percentage of CD25 positive cells. Background noise was subtracted from activation measurements and individual values were normalized to the epitope peptide signal.

(C) Tested peptides: 1) 65 peptides with intermediate TCR recognition scores tested without replicates in AKD10R3 cells and 2) 20 peptides with high and low recognitionscores tested in primary T cells, were separated in two groups based on 75% activating threshold (left panel). Among the peptides with high predicted score (right panel), activating peptides (>75%) on average had a higher predicted score (0.923) than non-activating (0.896) peptides.

(D) Receiver operating characteristic (ROC) curve showing the performance of linear classifier to discriminate between activating (>75% normalized activation) and non-activating (≤75% normalized activation) peptides based on TCR recognition score parameter.

*(E) The distribution of TCR recognition scores (x-axis) and HLA-affinity binding values (IC50, nM) (y-axis) for all human 9-mer peptides (10,470,365). A vertical dashed line is an estimated cutoff of 0.905 for TCR recognition score separating activating peptides from non-activating peptides. A horizontal dashed line depicts the IC50 value of 500 nM separating peptides with high binding affinity to HLA*A02:01 from peptides with low binding affinity.*

Figure 6. Estimating biological relevance of NY-ESOc259 TCR activating peptides



We computationally predicted and experimentally validated seven peptide sequences (including NY-ESO-1 antigen SLLMWITQC) capable of activating T cells through interaction with NY-ESO^{c259} TCR. These peptides, if naturally processed and presented on MHC molecules in normal human tissues, could potentially cause off-target effects in NY-ESO^{c259} TCR based adoptive cell therapies. To verify whether the predicted peptides are indeed presented and constitute a realistic off-target risk, we explored the publicly available gene expression and immunological data.

The expression values of the peptides are obtained from the associated genes. RNA levels in 37 tissues are depicted based on RNA-seq. Expression values are shown in Transcripts Per Million (TPM) units.

Conclusions

This replacement scan assay determines the "TCR fingerprint" and, when coupled with the algorithm applied to the database of human 9-mer peptides binding to HLA-A*02:01, enables identification of potential off-target antigens and the tissues where they are expressed. This platform enables both screening of multiple TCRs to identify the best candidate for clinical development and identification of TCR-specific cross-reactive peptide recognition and constitutes an improved methodology for the identification of potential off-target peptides presented on MHC class I molecules. We have used this platform to screen multiple tumor-targeting TCRs. We continue to improve the platform, including enhancements to the algorithm and internal reference mass-spectrometry HLA ligandome datasets.

1. For references, see original manuscript at https://agenusbio.com/tcr-fingerprint-manuscript/
2. Ligandomics: http://agenusbio.com/wp-content/uploads/2018/11/SITC_CRCPSV_Poster_PTM_version3.pdf

Acknowledgments

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Disclosures

All authors are current or former employees of Agenus Inc. Lexington MA, USA or subsidiaries thereof (including AgenTus Therapeutics, Inc. and subsidiaries thereof).

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