Identification of antigens recognized by T cells

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Disclosures

Agenus Inc.
Consultant
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Characteristics of identified tumor antigens

- Tissue-specific, cancer-testis antigens
- Patient-specific neoantigens
- Phosphopeptides

General approaches to antigen discovery

• Direct methodology
  – Isolate cancer reactive T cells from patients
  – Identify MHC and peptide that comprise the antigen recognized

• Reverse methodology
  – Identify genes, proteins, or peptides of interest expressed in/on cancer cells
  – Establish whether there are T cells in patients that recognize them
**cDNA library approach**

T. Boon/S. Rosenberg

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**Identify peptide using truncation cDNAs, synthetic peptides**
Mass spectrometry approach

V. Engelhard/D. Hunt

10^8 - 10^{11} cultured cells

Detergent lysis

Immunoaffinity Purification of MHC

20,000 peptides

HPLC

T cell assay using MHC matched APC

Active fraction
Mass spectrometry approach

V. Engelhard/D. Hunt

Micro-Capillary Column

Online Effluent Splitter Device

Nano-Electrospray Injection

Fourier Transform Mass Spectrometer

Candidate Peptide Masses and Charge States

CTL Epitope Reconstitution Assay to Identify Plate Wells Containing Active Peptide & Use Fluorescence to Precisely Align Activity in Wells with Scans Collected by Mass Spectrometer

Active Fraction

Spike in Fluorescent Peptides

Fluorescent Plate Reader
Antigens identified among naturally processed melanoma peptides
Approach to identification of antigens recognized by “orphan TCR” from TIL

Indirect Methods

Every method needs a hook.....
SEREX

L. J. Old

- Establish whether transfectant is recognized by T cells
- Identify MHC and peptide

Success!: NY-ESO1

Mass spectrometry for general MHC peptide analysis and potential antigen identification

1. Tumor tissue
2. Immunoaffinity purification of HLA complexes
3. Recovery of HLA peptides
4. High pressure liquid chromatography
5. Quadrupole Orbitrap mass spectrometer
6. Customized database search and peptides identification
7. Personalized immunotherapy
Targeted mass spectrometry: Finding what you are looking for
iTRAQ enables quantitative comparison of the same peptide in different samples

Fuller & Morris in Integrative Proteomics (2012)
Rapid neoantigen identification enabled by NGS
Sources of NeoAgs

Missense

Frameshift

Splice variants

Analysis of factors predicting display of identified MHC-I associated peptides

Software tools to predict whether point mutations will lead to MHC-I presented peptides

Fleri et al Front Immunol 8 278 (2017)
Proteasomal splicing during protein degradation creates new antigens

Post-translational modifications of proteins can survive to be presented on MHC peptides

MHC-associated phosphorylated peptides

*a better class of tumor antigen*

**Normal Cell**

**Cancer Cell**

![Diagram of normal cell showing kinase, proteasome, and MHC-associated phosphorylated peptides]

![Diagram of cancer cell showing overactive kinase, inhibited phosphatase, and proteasome]

- Kinase
- Proteasome
- MHC-associated phosphorylated peptides