High Throughput Technology and Predictive Immune Monitoring

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Cancer & the Immune System

- Cancer can evade and modulate the host immune response
  - Regulatory T cells are increased within the tumor microenvironment, tumor-draining lymph nodes, and blood
  - anti-tumor T cells develop but are dysfunctional
  - Other immune cell types also altered

- Immune status may predict cancer patient prognosis and guide therapy

- Immune markers may serve as surrogates for efficacy of cancer immunotherapy

- High throughput methods to systematically assess host immune function are needed
Some High Throughput Methods for Immune Analysis

- Enumeration of immune cell populations and subtypes: FACS, pMHC tetramers, ELISPot

- Immune cells biology
  - Gene expression, microRNA, epigenetics: microarrays
  - Functional responses: CFC, phosflow, Luminex, qPCR

- Soluble factors: proteins, lipids, small molecules
Gene Expression Profiling of Lymphocytes from Melanoma Patients

12 Melanoma Patients: Stage IV, resected, no recent systemic therapy
12 Healthy donors: age- and gender-matched

PBMCs sorted by FACS into:
CD8 T cells, CD4 T cells, B cells and NK cells (>99%)

Total RNA, amplified (with amino-allyl labeling)

Hybridized onto Agilent Human microarrays (22K) with Total Lymphocyte Reference RNA
<table>
<thead>
<tr>
<th>Entrez Gene Symbol</th>
<th>Entrez Gene Name</th>
<th>Adjusted P value</th>
<th>↑ or ↓ in melanoma</th>
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<td>interferon-induced protein with tetratricopeptide repeats 3</td>
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* Interferon-stimulated gene

Interferon signaling pathways

- STAT1 pY701 common to both type-I and –II IFN signaling pathways

- Anti-STAT1 pY701 validated for Phosflow analysis
Phospho-flow cytometry for high-throughput immune monitoring

- Analysis of signaling capacity of immune cell populations on a single-cell basis
- Intracellular staining of phosphorylated signaling molecules after stimulation with various cytokines
- Example: pSTAT1 after IFN-α or IFN-γ stimulation
IFN-α-induced pSTAT1-Y701 is reduced in T cells, B cells and NK cells from cancer patients

- Lymphocytes were stimulated with 1000 IU/mL IFN-α and pSTAT1 was measured in T cells, B cells and NK cells
- Fold change in pSTAT1: MFI pSTAT1 in IFN-stimulated cells/MFI pSTAT1 in unstimulated cells

* p-value < 0.05

Critchley-Thorne et al. PNAS 2009 Jun 2;106(22):9010-5
IFN-γ-induced pSTAT1-Y701 is reduced in B cells from cancer patients

- Lymphocytes were stimulated with 1000 IU/mL IFN-α and pSTAT1 was measured in T cells, B cells and NK cells
- T cells and NK cells from healthy donors and cancer patients show minimal phosphorylation of STAT1 in response to IFN-γ

Critchley-Thorne et al. PNAS 2009 Jun 2;106(22):9010-5
ISG Expression is Reduced in Lymphocytes from Breast Cancer Patients

ISGs measured directly *ex vivo* by rQ-PCR in unstimulated lymphocytes

Critchley-Thorne et al. PNAS 2009 Jun 2;106(22):9010-5
Other cytokine signaling pathways

Invitrogen

Expanded Phosflow Panels

• Examine multiple immune cell types: CD4 T, CD8 T, B, NK, monocytes

• Assess additional cytokine signaling pathways beyond IFN: IL-2, IL-4, etc.

• Measure multiple signaling molecules: JAK, STAT, etc.

• Limited by available phospho antibodies and overlapping spectra of fluorophores
Luminex for Multiplex Analysis of Phospho-Proteins

• Multiplex analysis of up to 100 analytes from a single sample

• Allows comprehensive analysis of entire signaling networks

• High sensitivity allows signaling analysis from small sample sizes (<10 ug of cell lysate)

• Limited by inability to analyze cells on single-cell level
  – First need to separate different immune cell populations
Phosflow vs. Luminex
Defects in downstream IFN functional responses in T cells from breast cancer patients

Multivariate analysis of CD25, HLA—DR, CD54 and CD95: the expression levels of these activation markers were significantly reduced in T cells stimulated with anti-CD3/CD28 alone (p=0.021) and in combination with IFN-α (p=0.038) in breast cancer patients vs. healthy controls.
Summary

• IFN signaling defects develop in lymphocytes from patients with three major cancers: melanoma, breast, and GI
  – IFN-α in T, B, and NK cells
  – IFN-γ in B cells

• Downstream functional defects include reduced activation, proliferation, and increased apoptosis

• Signaling in other cytokine pathways in different immune cell types being assessed via expanded phosflow and Luminex

• Each high throughput method has advantages and limitations
Current and future directions

• Understanding global cytokine signaling patterns in different immune cell populations at different times (pre-tx, remission, relapse) will provide snapshots of immune function in cancer

• Specific immune defects may provide prognostic information or predict relapse

• Strategies to correct specific cytokine signaling defects may be useful as standalone or adjuvant therapy for cancer
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