Melanoma gene expression profiles to identify mechanisms of tumor resistance

Helena Harlin
Human Immunologic Monitoring Facility
University of Chicago
Introduction

• High frequencies of melanoma antigen-specific CD8\(^+\) T cells induced by vaccination or adoptive transfer do not always translate into tumor regression
• This observation has prompted investigation into the tumor microenvironment to identify potential factors that positively or negatively regulate the effector phase of an anti-tumor immune response
• One approach has been to characterize gene expression profiles from metastatic melanoma tumors (core biopsies or resected specimens)
Methods

• RNA was extracted from tumor biopsies of patients with metastatic melanoma (n=33) as well as from melanoma cell lines (n=5) and primary melanocyte cell lines (n=3)
• Gene array analysis was performed (Affymetrix chip U133A)
• Data was analyzed using dChip software, filtering for genes present in >10% of samples and with variation of std. dev./mean between 1 and 10. 735 genes with variable gene expression were identified.
• Genes and samples were clustered using hierarchical cluster analysis.
• In some cases, gene expression was analyzed using real time RT-PCR, both to confirm gene array data and to look for expression of genes not detected by the chip.
The gene array samples cluster into 3 main groups.
Tumor group 1 expresses immunologically relevant transcripts

Putative positive factors:
- Cytokines (IL-18)
- Chemokine (CCL27)
- Cytokine receptor (IL-1R)

Putative negative factors:
- Arginase
- IL-1R antagonist
Tumor group 2 expresses T cell, B cell, and other immune cell transcripts

**Putative positive factors:**
- B cell markers (IgH, Igκ, Igλ)
- T cell markers (TcRα, β, γ)
- MΦ markers (MΦ scavenger receptor 1)
- Complement components
- Granzyme A, K
- MHC class II
- Chemokines (CXCL13, CXCL10, CXCL11, CXCL9, CXCL5, CCL5)

**Putative negative factors:**
- Indoleamine-pyrrole 2,3 dioxygenase (IDO)
- Tryptophan 2,3-dioxygenase
- Angiogenesis factors (angiopoietin 2, angiopoietin 1, VEGF)
Tumor group 3 is characterized by a lack of immunologically relevant transcripts and includes melanoma and melanocyte cell lines.

Expression of various tumor markers:
- Cancer/testis Ags
- Melanoma differentiation Ags
- Oncogenes
T cell, B cell and Mφ transcripts are expressed highly in group 2 tumor samples
Representative transcripts encoding "positive" immune factors in tumor groups 1 and 2

**IL-18**

- T 16
- T 9B
- T 2B
- T 3A
- T 3B
- T 6A
- T 6B
- T 15
- T 10
- T 12
- T 2A
- T 8
- T 5
- T 4
- T 11
- T 13

**CD8α**

- T 16
- T 9B
- T 2B
- T 3A
- T 3B
- T 6A
- T 6B
- T 15
- T 10
- T 12
- T 2A
- T 8
- T 5
- T 4
- T 11
- T 13
IL-10 and IFN-γ transcripts are present variably in tumor samples from both group 1 and group 2
Arginase and IDO expression levels show an inverse correlation
Arginase expression validated by real-time RT-PCR is primarily found in group 1 samples
IDO expression validated by real-time RT-PCR is primarily found in group 2 samples.
Regulatory T cell transcripts in tumor groups 1 and 2

**FoxP3**

Expression value

**GITR**

Expression value
Additional transcripts encoding putative negative regulators

PD-L1

Angiopoietin 1
Survivin transcripts are widely expressed, being highest in group 3 tumor cell line samples.
Conclusions

- Metastatic melanoma tumor samples cluster into at least three groups based on gene expression profiling.
- Groups 1 and 2 have distinct gene expression patterns:
  - Both contain distinct sets of immunologically relevant genes.
  - Include putative negative and positive regulators of T cell function.
- Group 3 lacks immune genes and clusters together with melanoma and primary melanocyte cell lines:
  - Suggests defects in inflammatory cell trafficking.
- Group 1 samples express Arginase and group 2 contains all samples with IDO. Arginase and IDO expression are inversely correlated.
- Therefore, all tumors appear to be accounted for by a putative negative immunoregulatory process:
  - Lack of inflammatory cell migration.
  - Expression of inhibitory factors.
- A larger number of samples is currently being analyzed, with planned correlation with clinical outcome.
- Therapeutic strategies to counter inhibitory influences in the tumor microenvironment should be considered for development.
Acknowledgments

University of Chicago
Alpana Sahu
Todd Kuna
Functional Genomics Facility

University of Virginia
Michael Hanshew
Craig Slingluff

Amy Peterson
Mark McKee

Thomas Gajewski
Angiogenic factors are primarily present among group 2 tumor samples
CD8α

CD8β

CD4

B7-1