Tumor Microenvironment:

Immune Responses Associated with the Progression of Cancer

Poster Discussion Session

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Tumor Microenvironment

Immune Regulation and Tumor Propagation

- Goals:
  - Immune Recognition of Cancer
  - Elimination of Cancer

- Obstacles:
  - Immunoregulatory Cells
  - Link of chronic inflammation and cancer development

Why the Immune System Fails?

- Defects in the Priming Phase
- Defects in the Effector Phase
- Immune Cell Trafficking
- Immune Regulatory Mechanisms: Treg
- Immune Inhibition: PD-1, iNOS, cytokines
- Tumor Anti-Apoptotic Mechanisms
- Dysregulated Immune Responses

Immune Response Contributes to Tumor Propagation

- Cancer Induces Immune Regulatory Responses
- Immunoediting
- Chronic Inflammation

Tumor Microenvironment of Head and Neck Squamous Cell Carcinoma Promotes Induction and Expansion of a CD4^{+} T regulatory Cell Type 1

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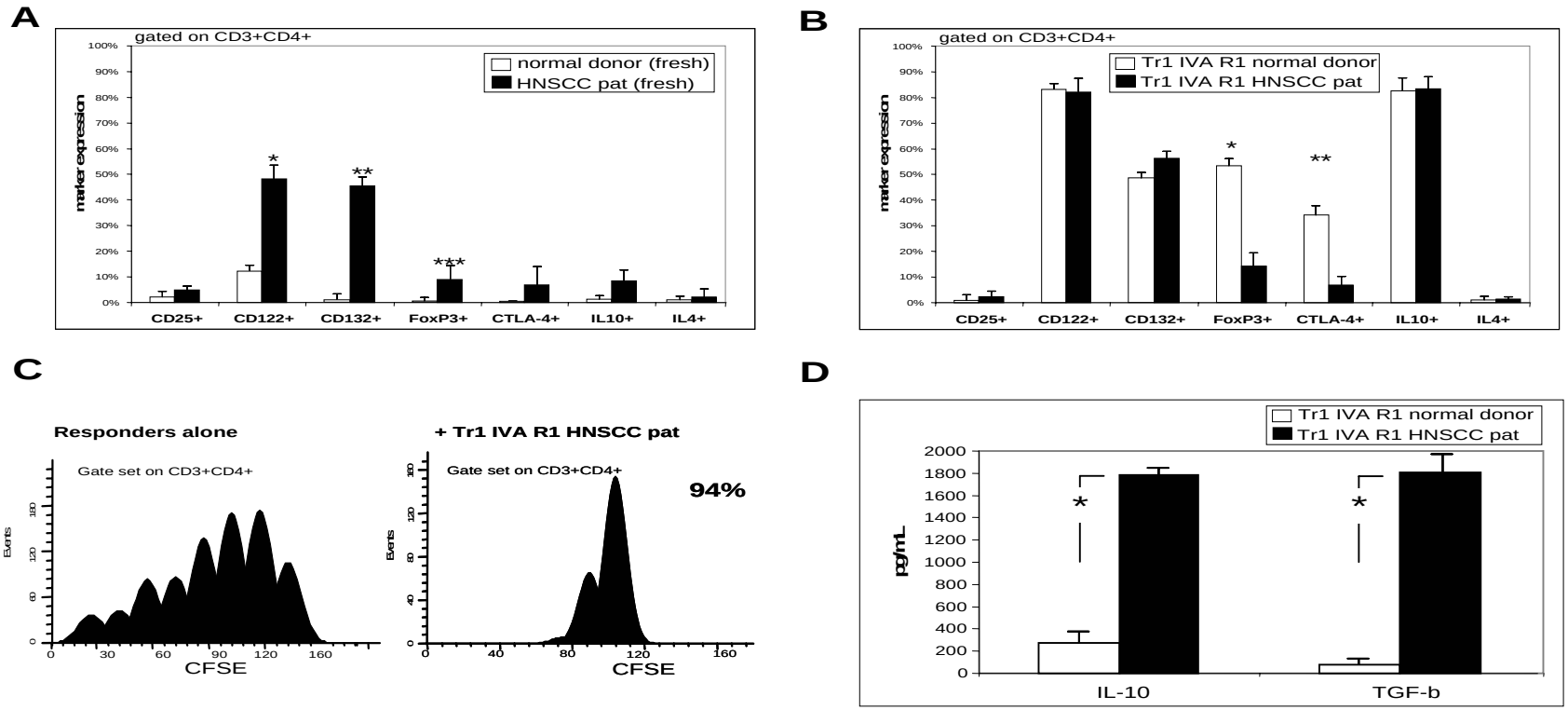
Treg in Tumor Cell Escape from Immunosurveillance

- Chronic inflammation and HNSCC: immune dysregulation
- *In vitro* co-culture: HNSCC cell line (PCI-13), $CD4^{+}CD25^{-}$ T cells, and iDC
- IL-2, IL-10, IL-15 +/- rapamycin
- Flow cytometry and proliferation assays for Tr1 function using CD4+CD25- autologous cells as responders
- Inhibition of proliferation to OKT3, anti-CD28 by comparing +/- Tr1 cells
- Compared normal PBMC vs. 15 HNSCC patients

Bergmann et al.: Tr1 Cells

- Defined Tr1 culture conditions: IL-2, IL-10, IL-15 combined with 1nM rapamycin
- Tr1 Cells Phenotype: CD3+CD4+CD25-IL-10+IL2Rβ+IL2Rγ+FoxP3+CTLA-4+
- Absence of CD25 upregulation upon antigen or polyclonal stimulation in Tr1 cells distinguish Tr1 cells and nTreg
- Tr1: suppressed autologous T cell proliferation upon polyclonal activation; correlate IL-10 expression and suppressor function
- High levels of IL-10 and TGF-β1 (no IL-4, IL-2 or INF-γ): Tr1

In vitro expansion of Tr1 cells in PBMC from HNSCC patients



A. PBMC HNSCC compared to normals: higher percentages Tr1 – FoxP3 and IL-10

B. 10d Tr1 cytokines and rapamycin: normal cultures enriched in FoxP3 and CTLA4 while HNSCC were not

C. Tr1 lymhs co-cultured with CD4+CD25- and anti-CD28 – suppression 94%

D. Increase 60xIL-10, 180x TGF-beta compared to normals ($p<0.001$)

CONCLUSIONS

- HNSCC patients already acquired a Tr1 marker expression profile readily expandable with Tr1 cytokines and rapamycin

- No iDC or tumor cells needed for proliferation of Tr1 cells with high suppressor activity

- *in vitro* system for Tr1 generation: cytokine milieu and the tumor microenvironment

COX-2 Overexpression in Head and Neck Squamous Cell Carcinoma is directly associated with induction of $CD4^+$ T Regulatory Cell Type 1

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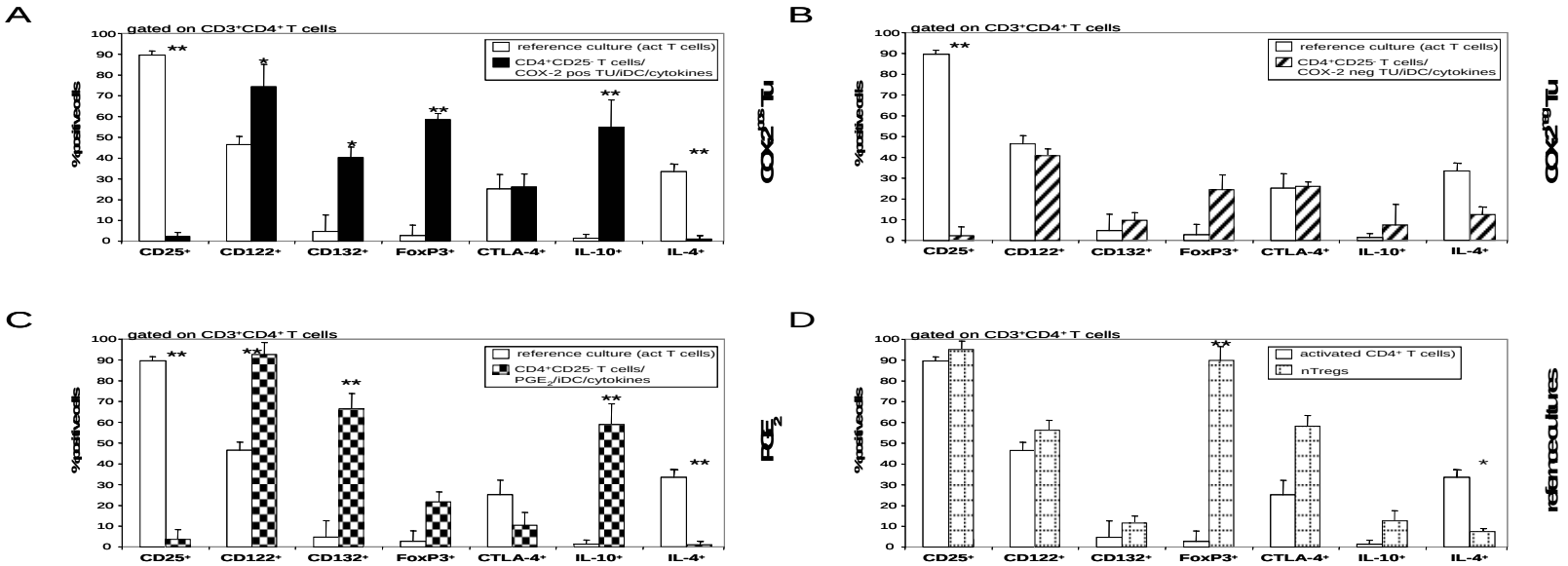
Bergmann et al.

- HNSCC cells engineered to COX-2
- +/- Diclofenac or synthetic PGE_2

RESULTS

Phenotypic analysis of $CD4^+CD25^-$ T cells after 10d culture: effects of COX-2 or PGE_2 on Tr1 cells



A. COX-2 positive
B. COX-2 negative
C. PGE-2
D. nTreg reference: IL-2, anti-CD28, rapamycin

COX-2 and Tr1 Cells

- Presence of COX-2 has impact of suppressor function of TR1 cells
- COX-2 inhibitor abrogates differentiation to Tr1 cells

Foxp3-positive regulatory T cells are associated with nodal status in breast cancer.

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Foxp3-positive regulatory T cells are associated with nodal status in breast cancer.

Aim of Study:

- To determine if accumulation of regulatory T cells and dendritic cells in sentinel lymph nodes is associated with nodal dissemination in breast cancer

Foxp3-positive regulatory T cells are associated with nodal status in breast cancer.

Study Population and Methods:

- 40 breast cancer patients:
  - SLN tumor negative (n=11)
  - micrometastases (n=16)
  - macrometastases (n=13)
  - 10 control patients false-positive core needle biopsy

- Stained for:
  - CD1a (immature DC)
  - DC-LAMP (mature DC)
  - Foxp3 (regulatory T cells)

- Percentages of positively stained cells were quantified

Foxp3-positive regulatory T cells are associated with nodal status in breast cancer.

Results:

Medians and group analysis as compared by the Kruskal-Wallis test

	DC-LAMP (p=0.37)	**CD1a** (p=0.17)	**Foxp3** (p<0.001)
Macrometastasis	0.1	12	19
Micrometastasis	0.3	14	20
Tumor-free	0.3	11	14
Controls	0.1	10	10

Foxp3-positive regulatory T cells associated with nodal status in breast cancer: Conclusions

- Foxp3-positive cells associated with size of SLN metastases
- Treg may have a role in nodal dissemination of breast cancer
- Potential arrest of DC maturation in SLN with macrometastases: lower percentage of mDC despite similar populations of iDC

Many Types of Regulatory Cells

- CD4:
  - Tr1: IL-10, IFN-γ; no IL-2 or IL-4
  - Th2: IL-4
  - Th3: TGF-β
  - Induced Treg
  - Natural Treg ($CD25^{hi}$)
  - FoxP3 and cell contact important for induced and natural Tregs
- Invariant Natural Killer T cells
- Immature Myeloid and Immature Dendritic Cells
  - VEGF, M-CSF, GM-CSF, IL-6, IL-10

Cancer Induces Immune Regulatory Responses in Tumor Microenvironment

- Dysregulated Immune Responses to Cancer
  - Regulatory Cell Development in Cancer Patients
  - Immune Defect in Antigen Presentation
  - Role of Chronic Inflammation
  - Initiated at the Sites of Draining Lymph Nodes
- Potential Therapeutic Interventions
  - Lymphodepletion; Chemotherapy
  - Anti-CD25
  - CTLA-4 blockade; PD-1 blockade; costimulatory molecules
  - *Cytokines/Chemokines in the Microenvironment*

Gene Profiling of Sentinel Lymph Nodes in Melanoma Predicts Inflammatory Patterns Associated with Metastases

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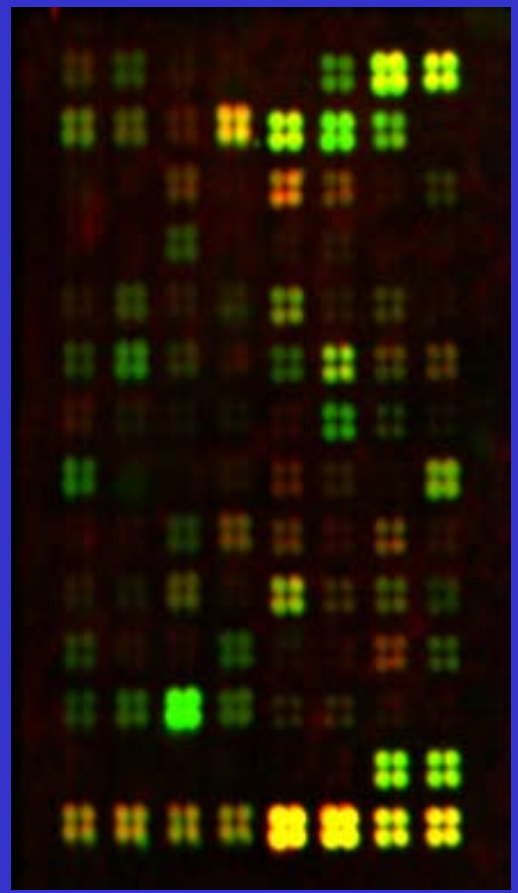
Hypothesis

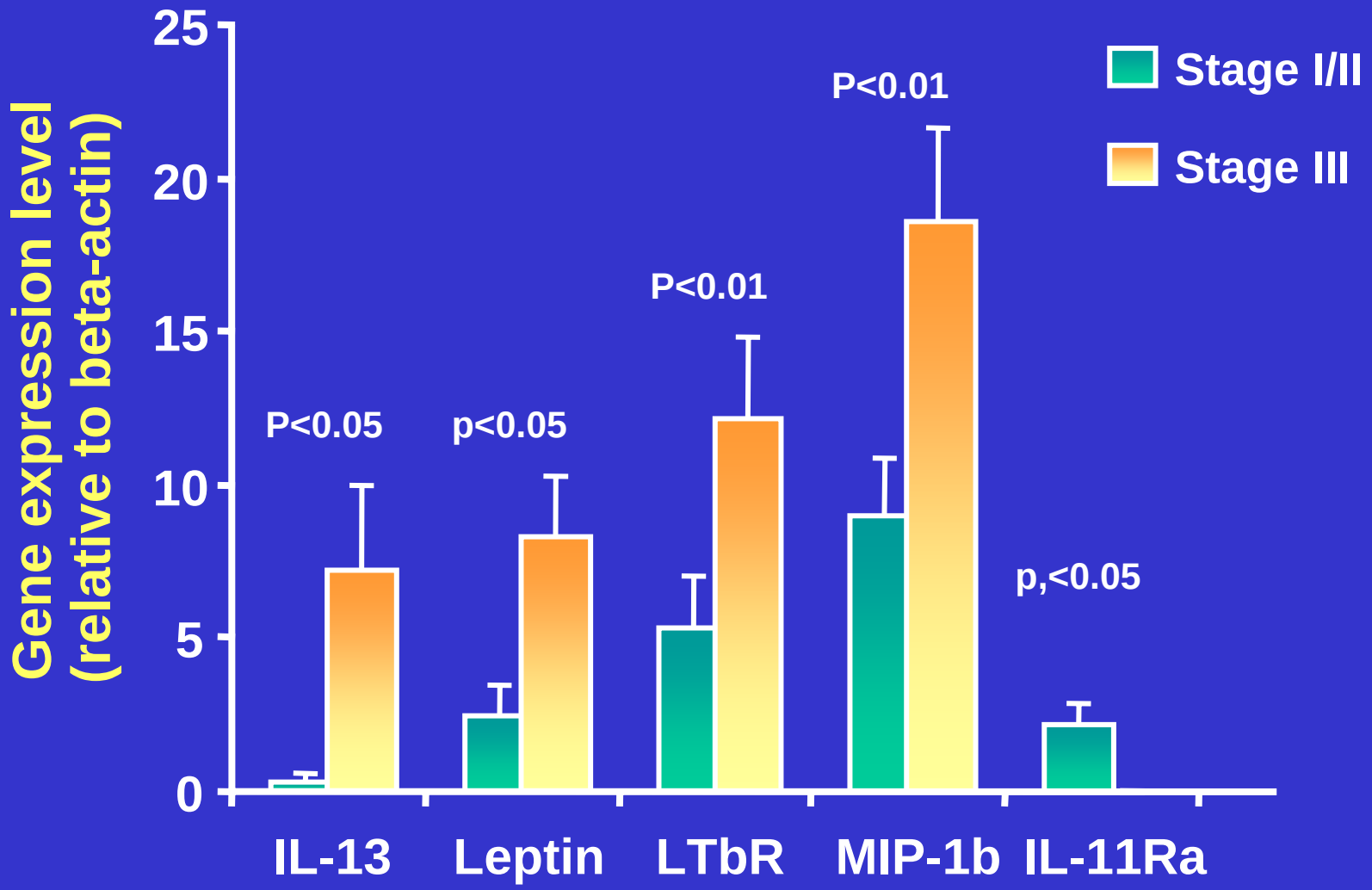
- Tumor-positive SNs have different immunoregulatory cytokine profiles
- Changes in cytokine and gene expression may be molecular markers of occult metastasis early-stage melanoma



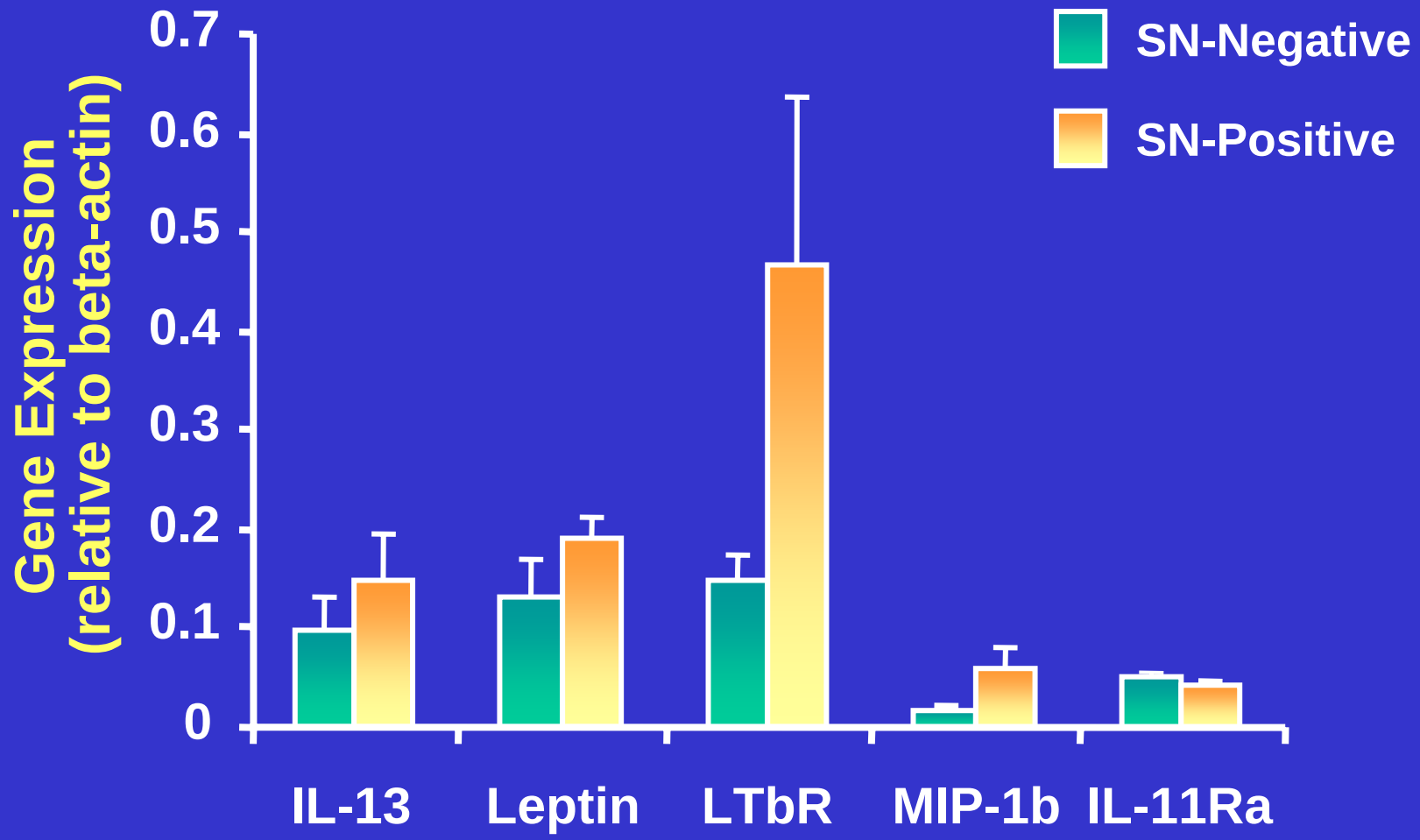
Essner et al.: Cluster Analysis

- 96 Cytokines and Chemokines
  - cDNA Microarray
- Compared using hierarchical clustering
- 57 genes expressed at significantly different levels in SNs and NSNs
  - 4 higher expression
  - 53 lower expression

96 Gene Array Expression



qRT-PCR Gene Expression Validation



Cytokine Function

- IL-13 down regulates macrophage activity
- Leptin obesity gene causes immune dysfunction
- LTbR regulates chemokine expression in lymphatic tissue
- MIP-1b major macrophage factor, proinflammatory
- IL-11Ra (CCL27) attracts CD4 T cells
- Interaction between cytokines extremely complex

Conclusions

- Cytokine gene expression in melanoma sentinel lymph nodes varies with stage
- Stage III lymph nodes significantly higher expression of IL-13, leptin, LTbR, and MIP1b, and lower expression of IL-11Ra when compared with Stage I/II
- New molecular surrogates for detecting occult sentinel lymph node metastasis

Tumor Microenvironment: Epithelial-Mesenchymal Transition

- Epithelium:
  - Adherent laterally
  - Cell to cell junctions
  - Polarized
- Mesenchyme:
  - Diffuse
  - Cells have points of contact with other cells
  - Cell migration

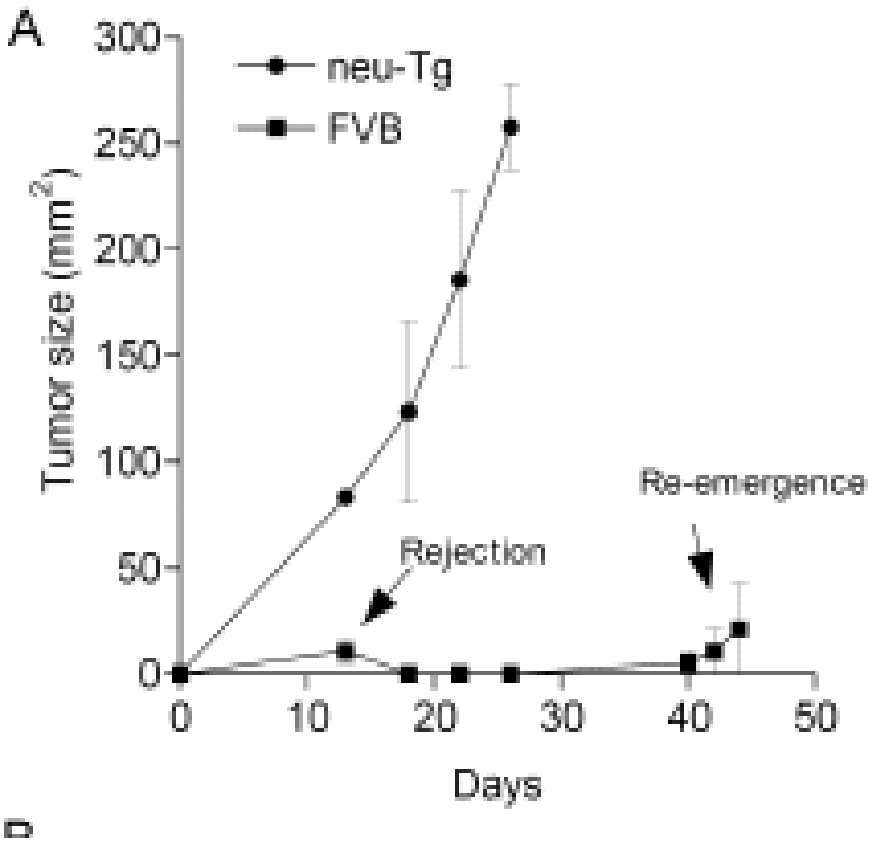
Tumor Microenvironment: Epithelial-Mesenchymal Transition

- Growth Factors:
  - TGF-ß, wnts
- Transcription Factors
  - SMAD, β-catenin
- Adhesion Molecules
  - e.g. E-cadherin to N-cadherin
- Cytoskeleton
- Extracellular Proteases

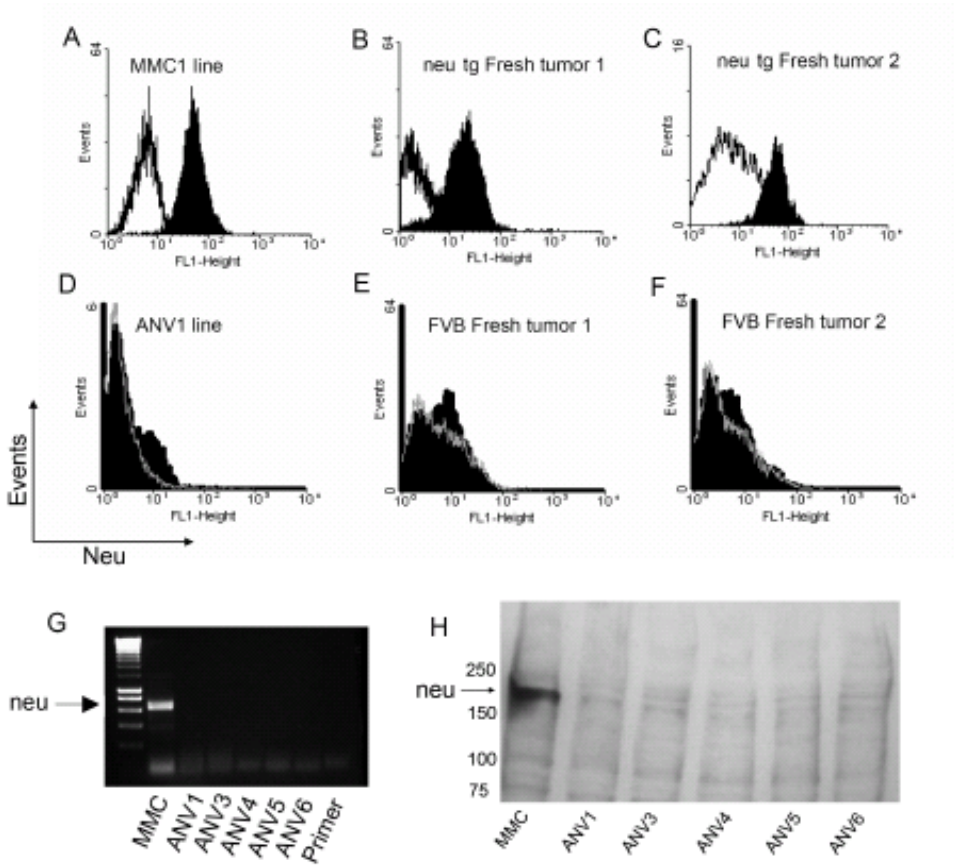
Knutson, et al.: Immunoediting

- Immunoselection of tumor variants
- Tumor responds by altering gene expression
- Epithelial neu-expressing breast cancer cell line

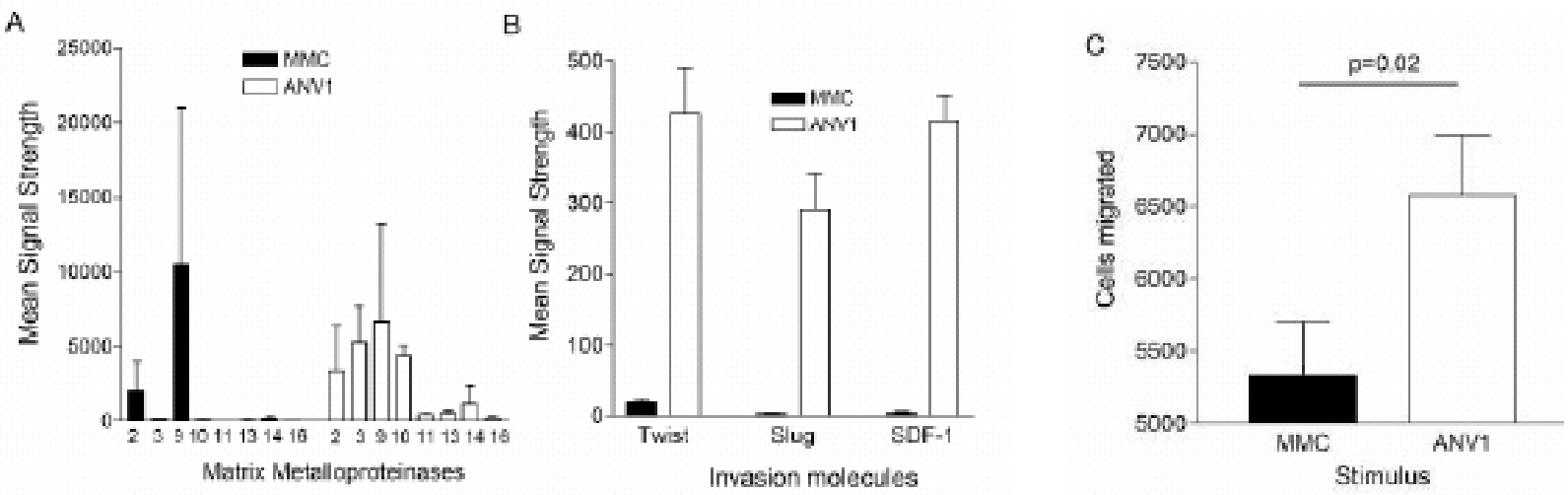
MMC Cell Line Immunogenic and Elicits Protective Response



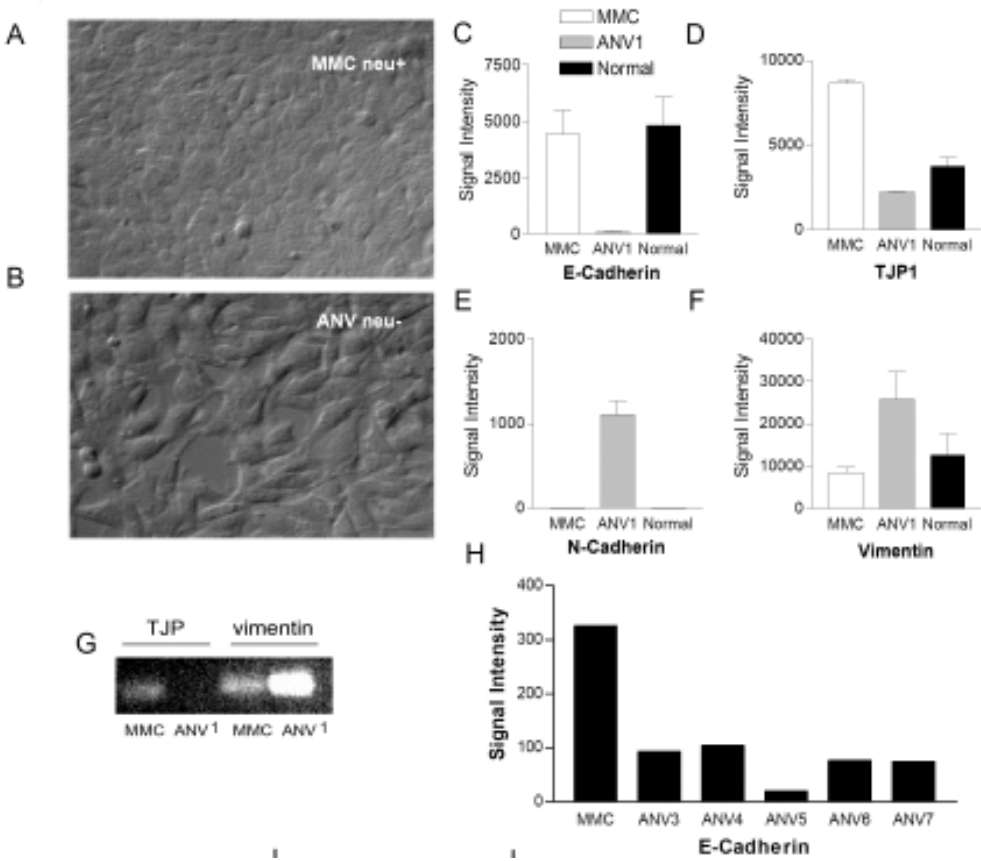
Tumors that Emerge Following MMC Rejection Have Neu Loss



ANV More Invasive than MMC Tumor Cells



MMC to ANV: Epithelial-Mesenchymal Transition

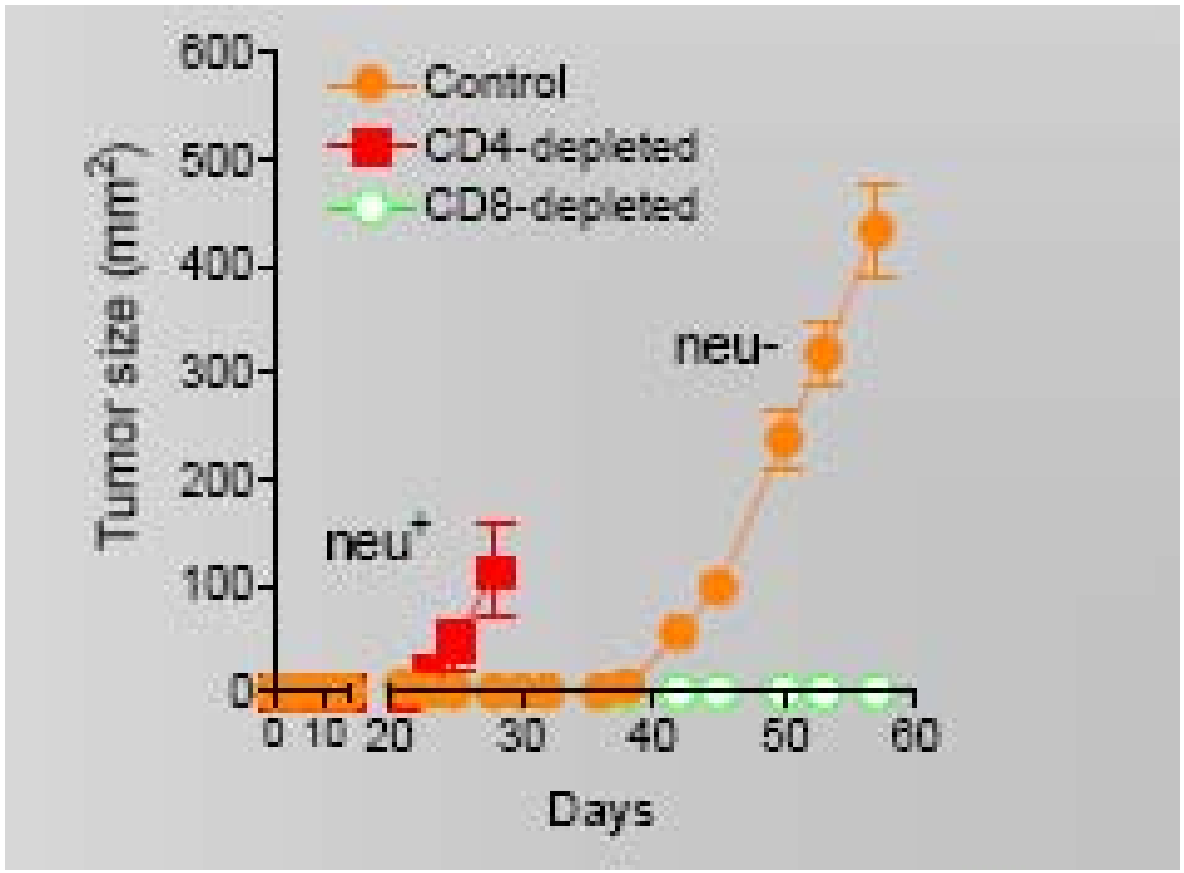


Knutson et al.: Role of CD8

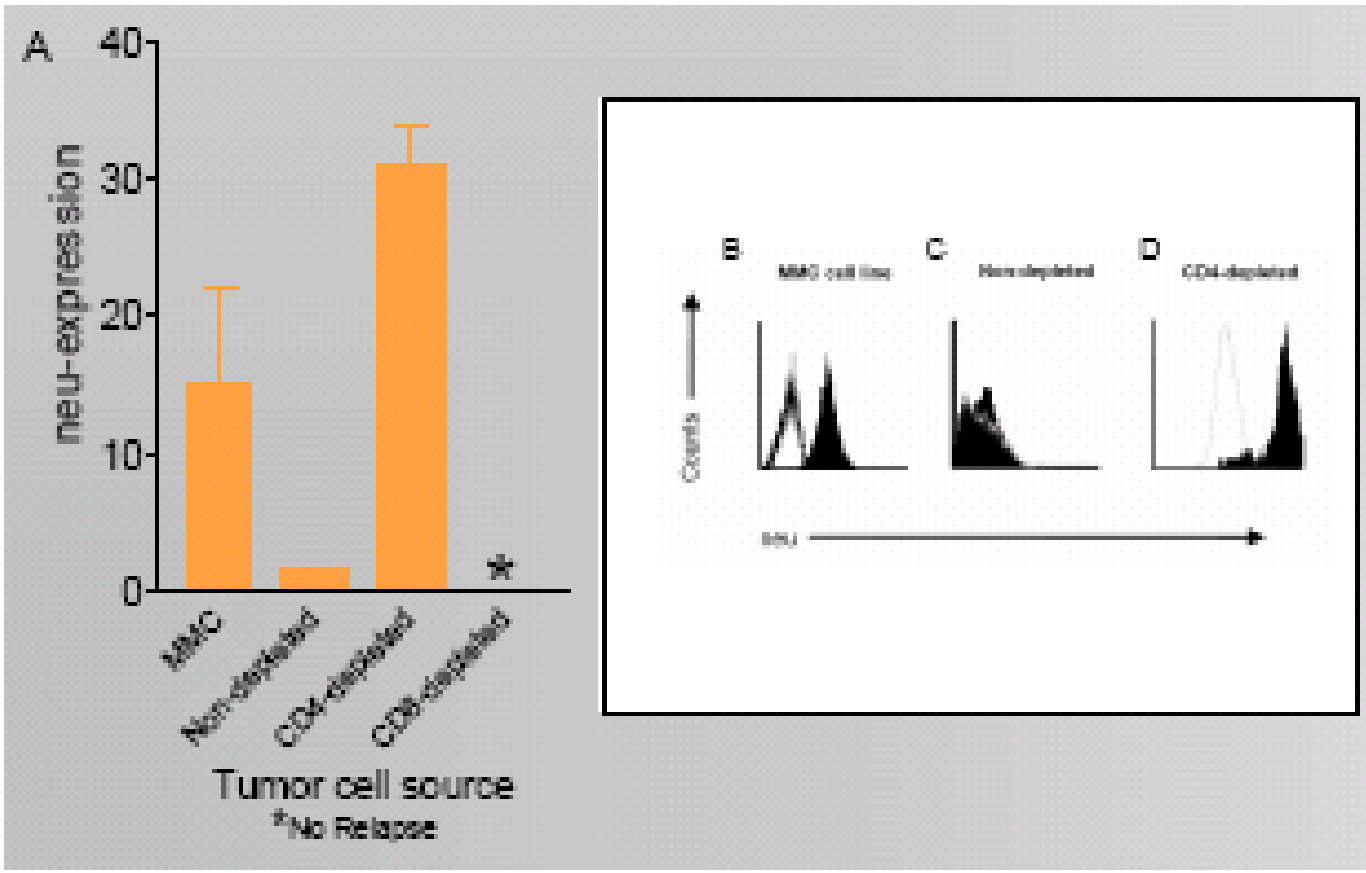
What subset of T cells responsible for tumor rejection and relapse?

- CD4 vs. CD8 depletion

CD8 Cells Promote Breast Cancer Relapse



CD8 Depletion Prevents Tumor Relapse



Role of CD8 in EMT

- CD8 cells not involved in tumor rejection but promote EMT
- Gene expression and cell contact by CD8 required for EMT
- Immunoselection vs. Pressure for Tumor Cells to Alter Gene Expression

EMT and Cancer

- Activated Macrophages produce TNF-α that accelerates EMT
- Human breast cancer: TNF-α associated with increased metastatic potential
- EMT typically induced by TGF-β
- Strong immune response: induces TGF-β Treg that leads to EMT

TGF-β

- Roles in tumor suppression and progression
  - Fibroblasts, extracellular matrix, immune cells influence effects of TGF-β
  - SMAD Pathways
- Regulates:
  - Apoptosis
  - Senescence
  - Proliferation
  - Invasion/Migration

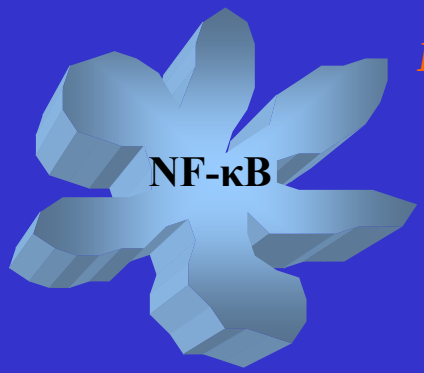
Targeting TGF-β

- Immunotherapy
- Antibodies (under development)
- Small Molecule Inhibitors (under development)
- Antisense
- Soluble Proteins

Other EMT Factors:
Complex Networks

- Ras Signaling Pathway
- Wnt, Notch, Hedgehog
- Snail/Twist: suppress E-cadherin
- PDGF-R Pathway (STAT1/STAT3b)
- Gene Profiling for EMT Regulation
- Immune Dysregulation
- Link Chronic Inflammation and Cancer

NF-κB:
A Link Between Inflammation and Cancer



Inflammatory Cell

Bacteria, Viruses,
Cell Necrosis

Secreted Factors that
Promote Tumor Growth,
Vascularization and
Survival

Malignant Cell

NF-κB

Elevated Cyclin D1,
IAPs, MMPs

Back to Basics: Immunology Lessons Learned from Primary Wound Healing

- Phase I:
  - Inflammation
  - "Clean up wound": effective antigen presentation
- Phase II:
  - Collagen/Scar Formation: EMT
- Dysregulation of Phases of Immune Reponses
  - Non-healing wounds
  - Autoimmune Diseases: Lupus, Scleroderma
  - Immune Dysregulation of Cancer: TGFβ, PDGFR
- Therapeutic Implications: Targeting Pathways

Tumor Microenvironment: Immune Responses and Tumor Progression

- Immune regulation of cancer is complicated
- Better defining regulatory cell subsets
- Cytokine profiles of tumor microenvironment leading to immune regulatory cells
- Cytokine profiles contributing to the progression of cancer
- Link of Chronic Inflammation and Cancer
  - Immune Dysregulation
- Therapeutic Interventions
  - Reprogramming