The Tumor Microenvironment

A Critical Component of Tumor Progression and Metastasis And a Target for Therapeutic Intervention

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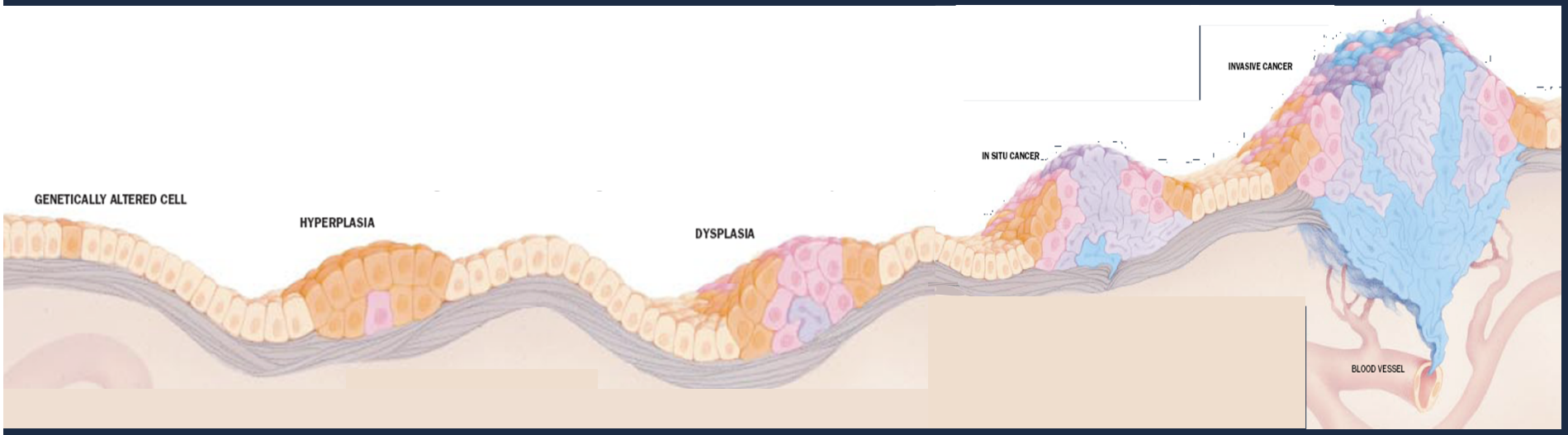
Harvard Medical School

Former View: Tumors are autonomous cell masses whose progression is driven by genetic alterations

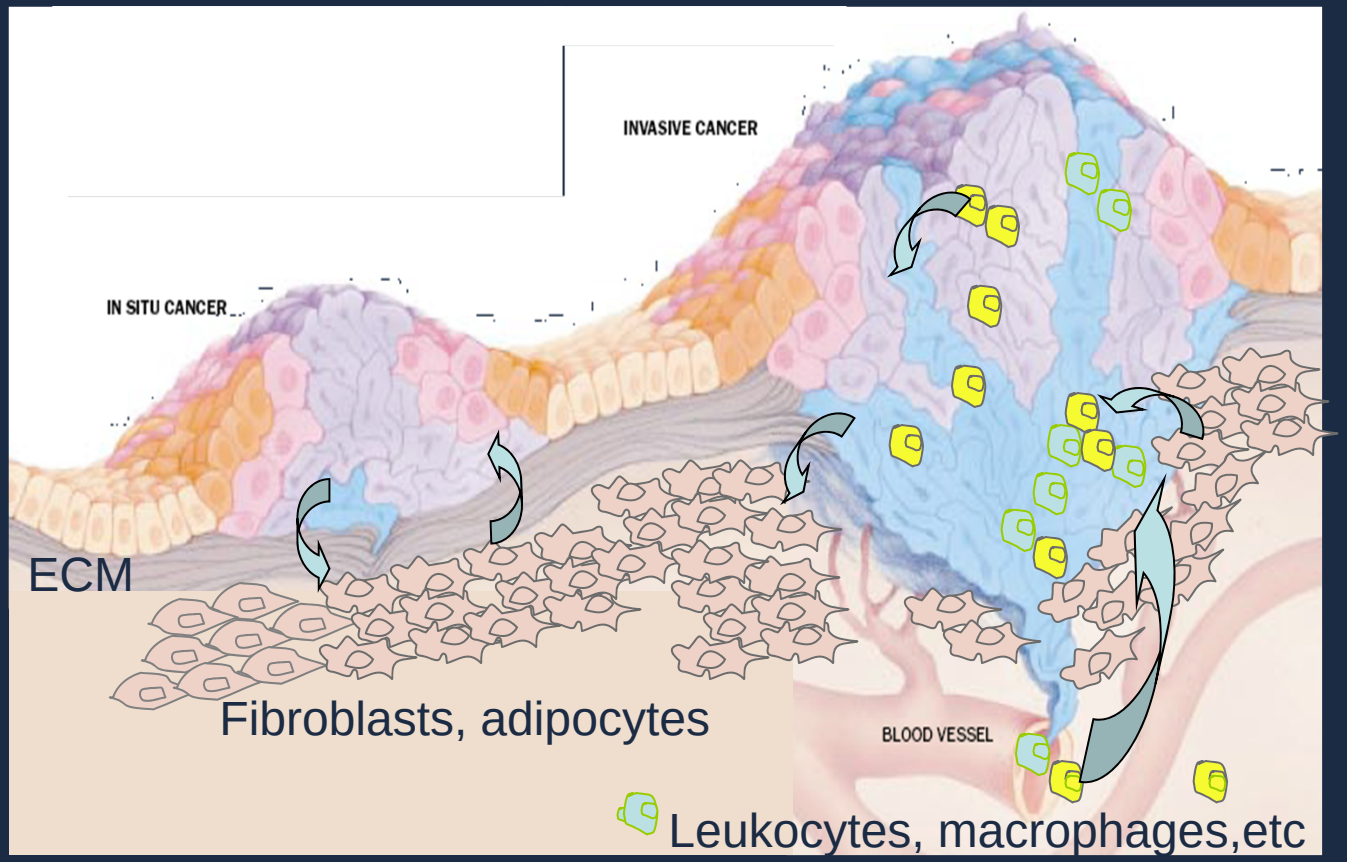


Normal colorectal Epithelium	Early Adenoma	Intermediate Adenoma	Advanced Adenoma	Colorectal Carcinoma	Invasive Carcinoma	Metastatic Carcinoma

→ APC → K-ras → Unknown Locus on 18Q → P53 → ? → ? →

From: RA weinberg How Cancer Arises Sci Am 1996

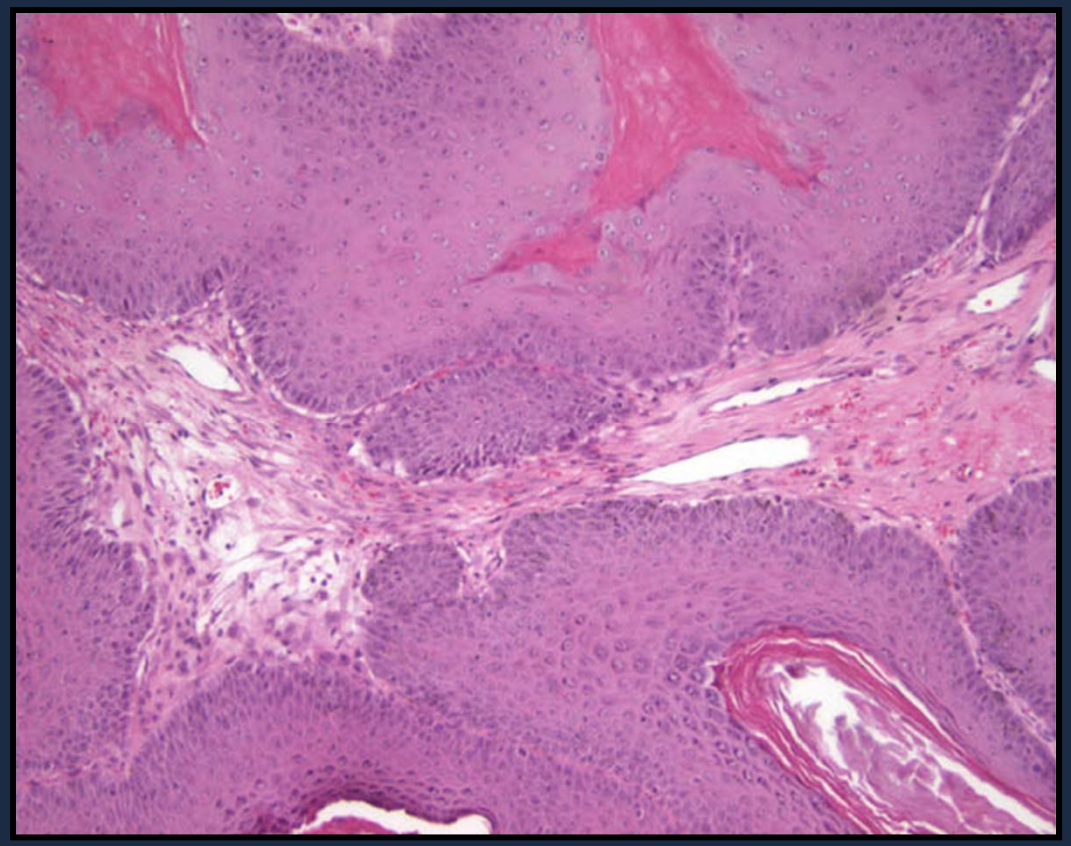
Current View: Tumors are “organs” composed of many interdependent cell types that contribute to tumor development and metastasis



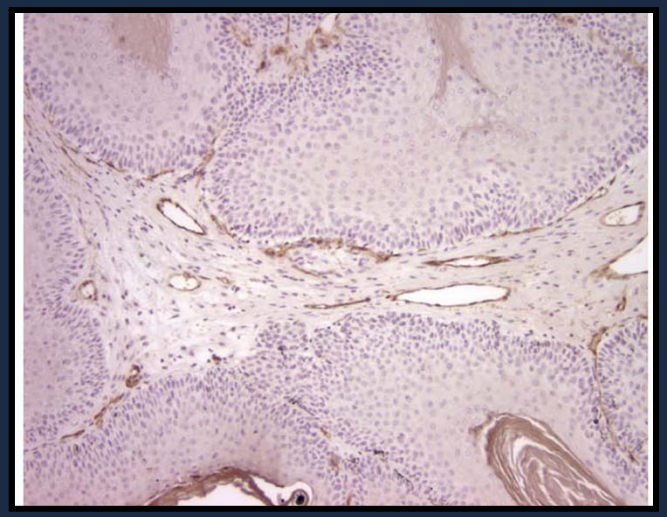
Modified from: RA Weinberg Sci Am How Cancer Arises 1996

Heterogeneity of Cells Within Tumors

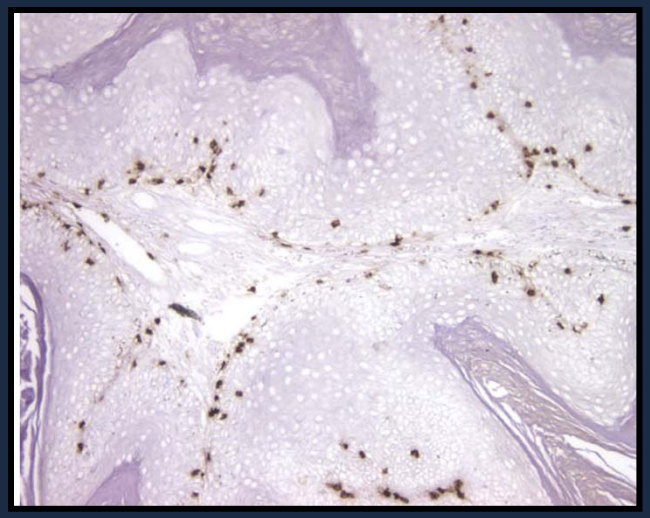
Squamous cell carcinoma



H&E



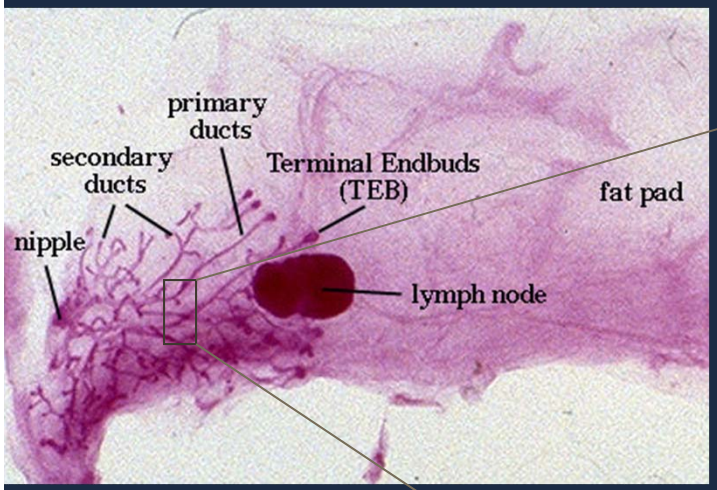
α-Pecam/CD31



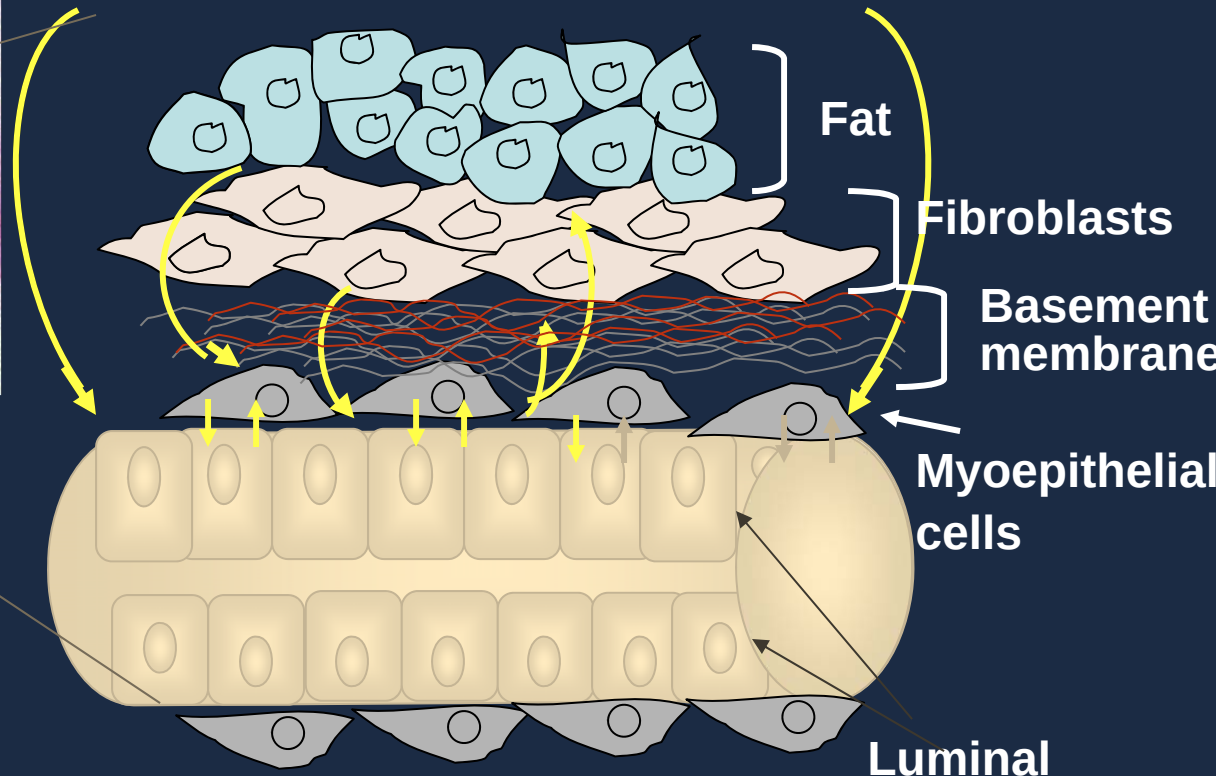
α-neutrophil

Lisa McCawley/ Lynn Matrisian

The Microenvironment Controls Normal Development and Homeostasis



Developing mammary gland

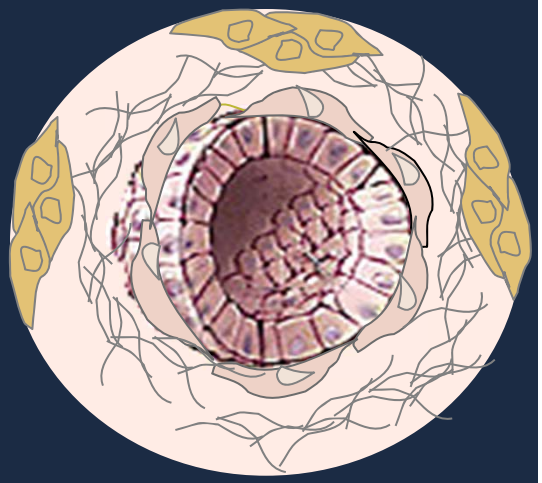


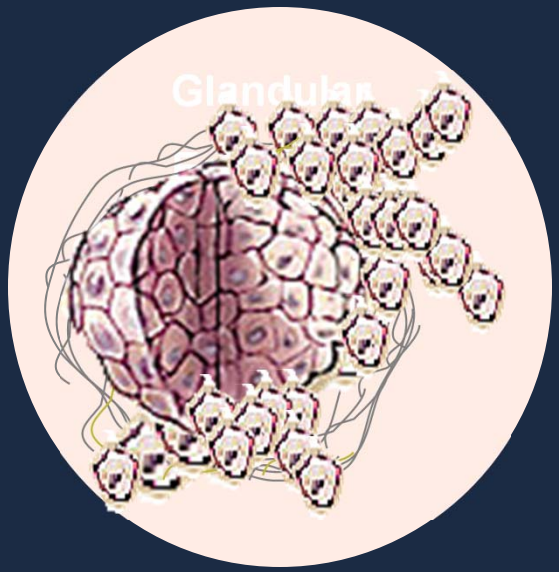
The Microenvironment (μE) Can Exert Positive and Negative Influences on Tumors

Tumor Suppressive Influences of the Microenvironment

- Cell-cell and cell-matrix interactions as well as hormones and growth inhibitory factors present in the μE can suppress aberrant behavior of genetically altered cells

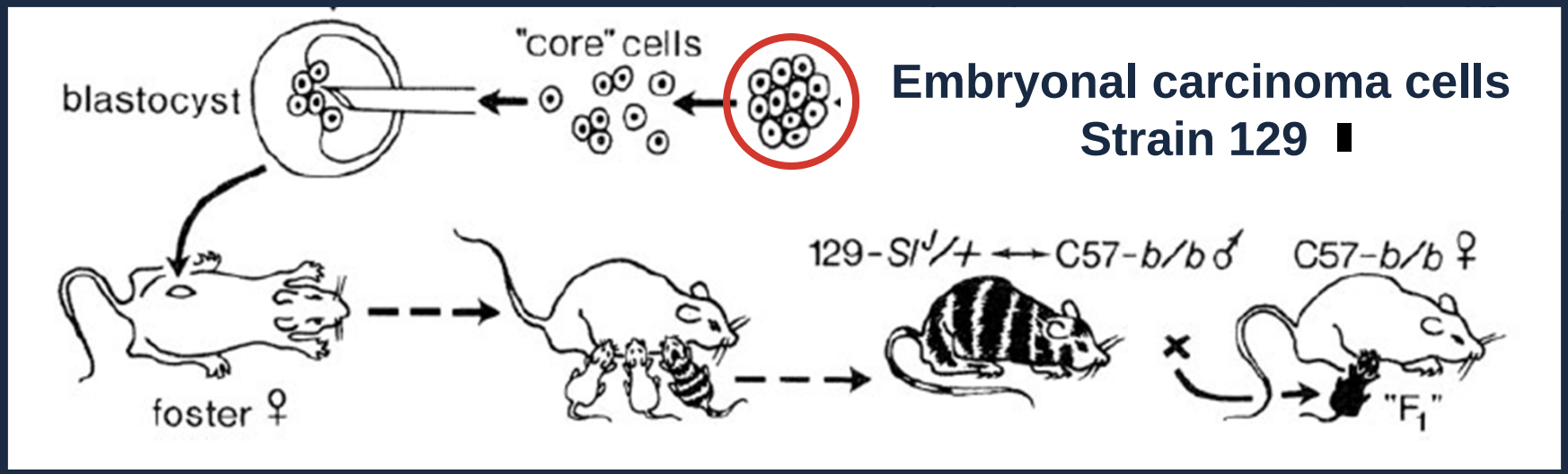
- These play an important role in early lesions where suppressive interactions limit the proliferation and invasion of dysplasias, hyperplasias and in-situ tumors





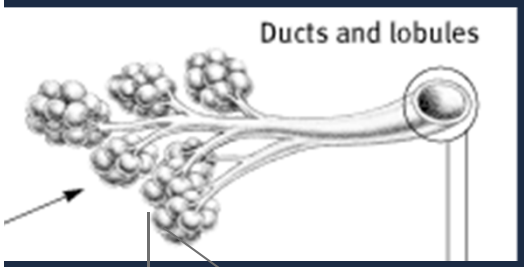
Alterations in cells/matrix in the tumor μE can release these negative influences and allow uncontrolled proliferation and invasion

The Malignant Potential of Teratocarcinoma Cells Can Be Restrained During Embryonic Development

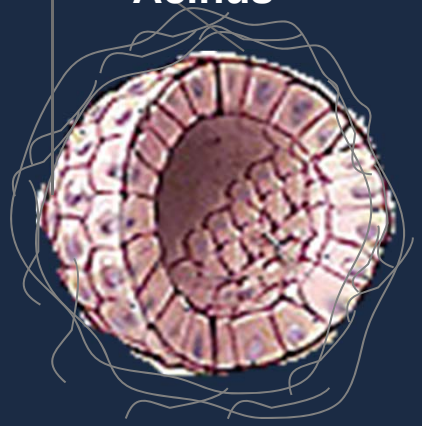


Mintz and Illmensee PNAS 1975; cartoon modified from Kenny and Bissell Int. J. Cancer 2003

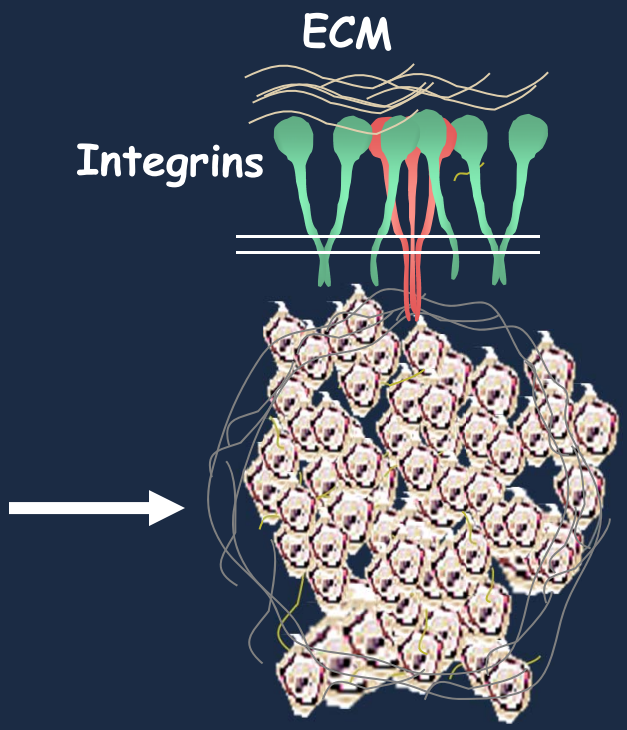
Reversion of Tumorigenicity Through ECM Receptor



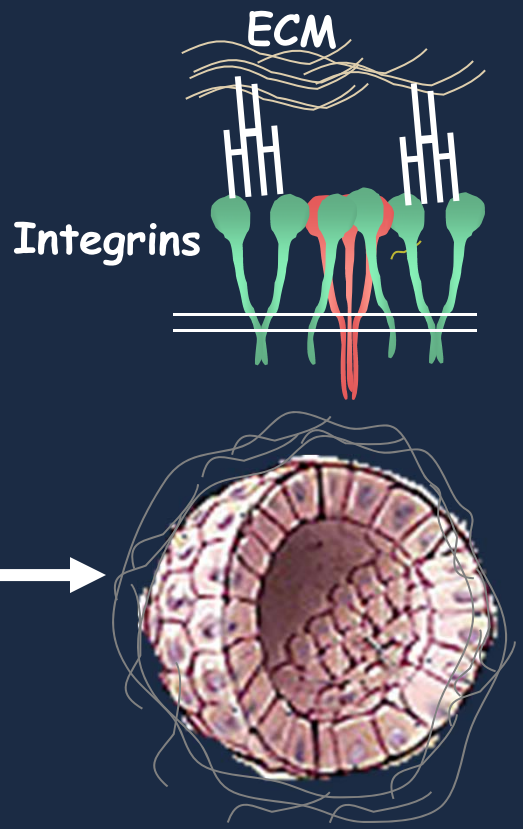
Acinus



Immortalized mammary epithelial cells cultured in basement membrane proteins



Spontaneously transformed derivative

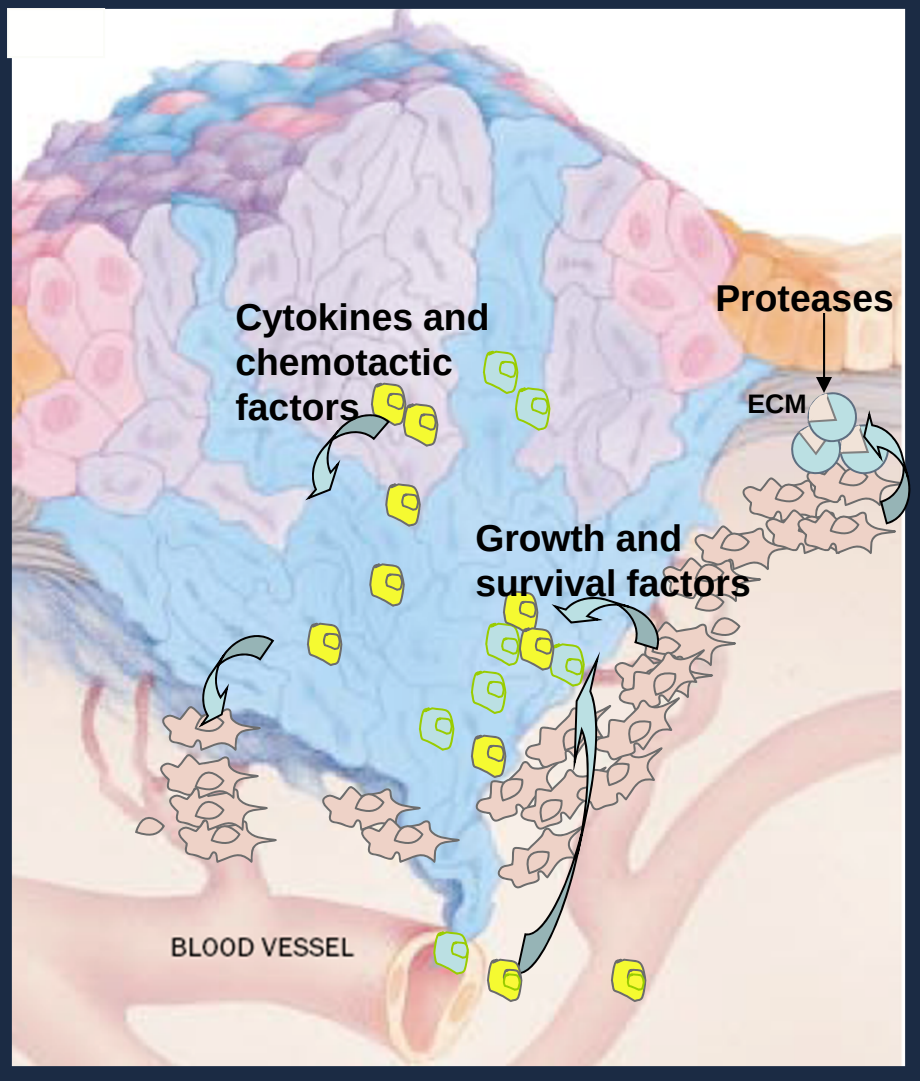


Weaver…. Bissel JCB 1997

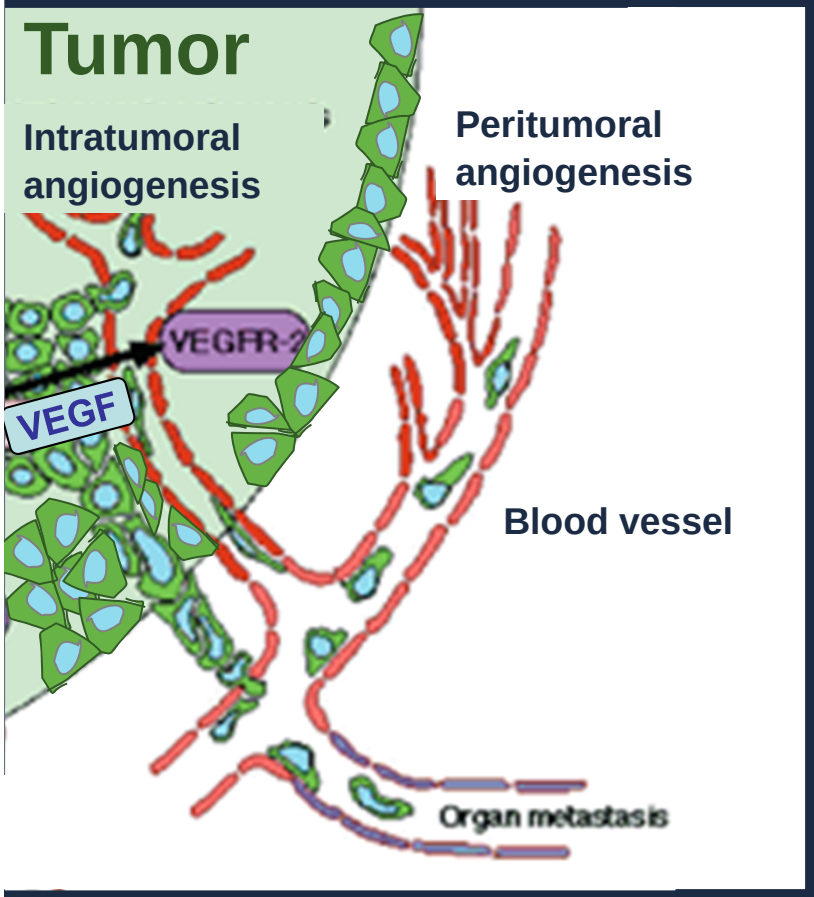
Tumor Promoting Influence of the Microenvironment

Factors produced in cells within the tumor μE can alter many different aspects of tumor cell behavior e.g.

- growth factors produced in adjacent cells promote cell proliferation and survival
- cytokines and chemotactic factors produced by inflammatory cells and other stromal promote cell migration and invasion
- proteases produced by the μE break down basement membrane, altering the architecture of tissue structures, and migration/invasion of tumor cells



Tumor Vasculature- Blood Vessels



Tumors produce factors that recruit endothelial cells

Tumor endothelial cells are architecturally distinct -- irregular,dilated, tortuous, and express unique sets of proteins

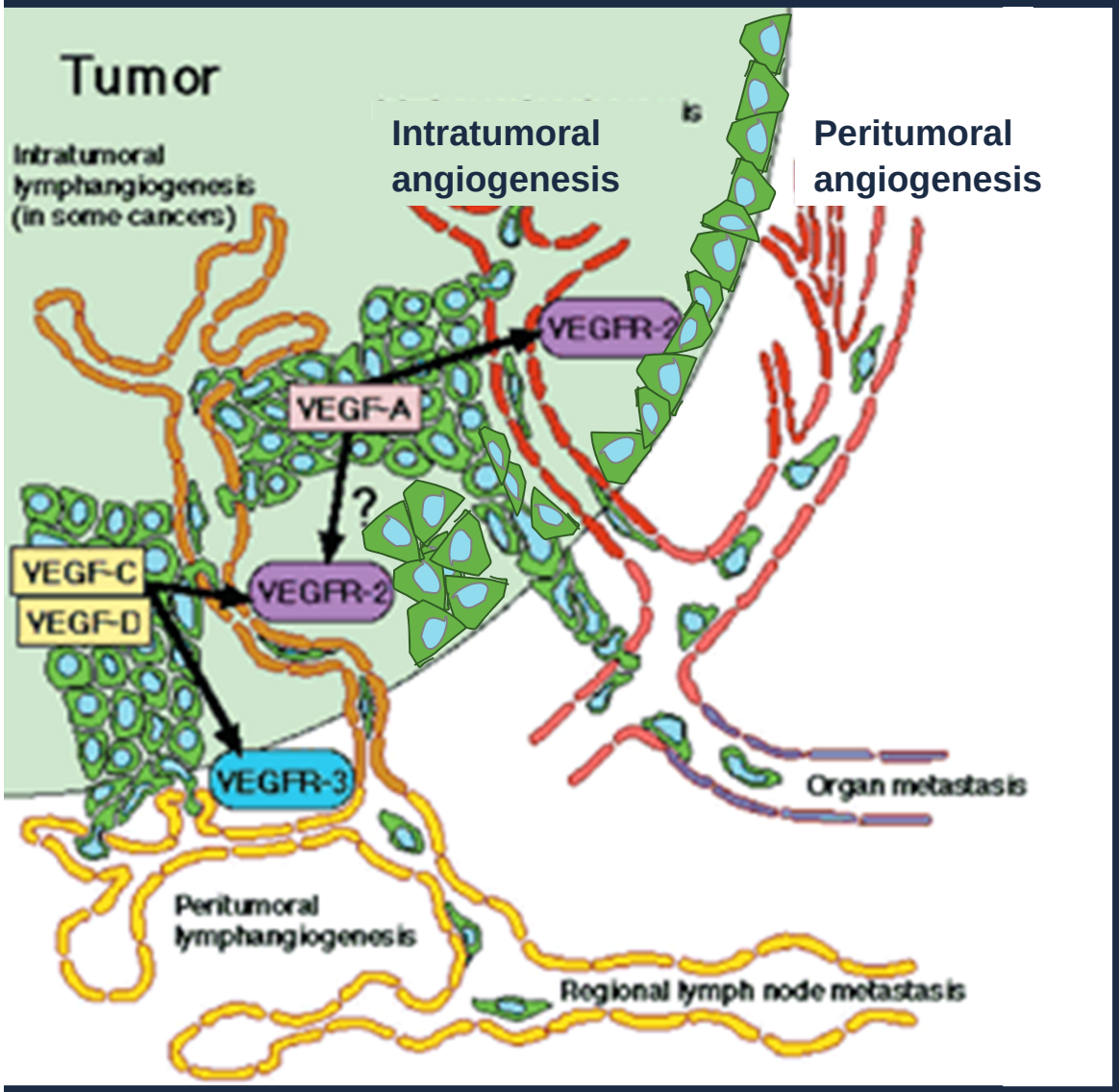
Angiogenesis orchestrated by a variety of activators and inhibitors

Critical factors that mediate blood vessel recruitment and development have been identified

An anti-angiogenesis drug that targets VEGF is showing clinical efficacy in initial trials (Avastin- Genentech)

Modified from http://www.the-scientist.com/images/yr2003/sep08/tumor.gif

Tumor Vasculature- Lymph Vessels



Lymphatic endothelial cells are also recruited by tumor cells and the lymph vessels provide a gateway for metastasis.

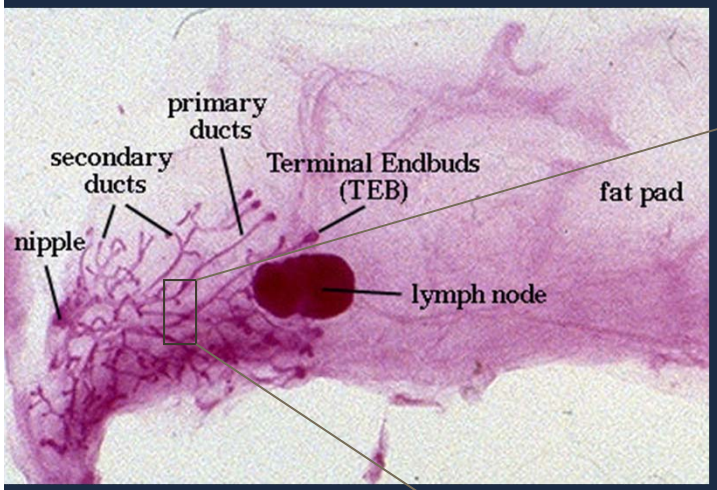
Critical molecules the mediate lymphangiogenesis have now been identified.

The importance of VEGF-C/D in metastasis has been shown in mouse models of cancer.

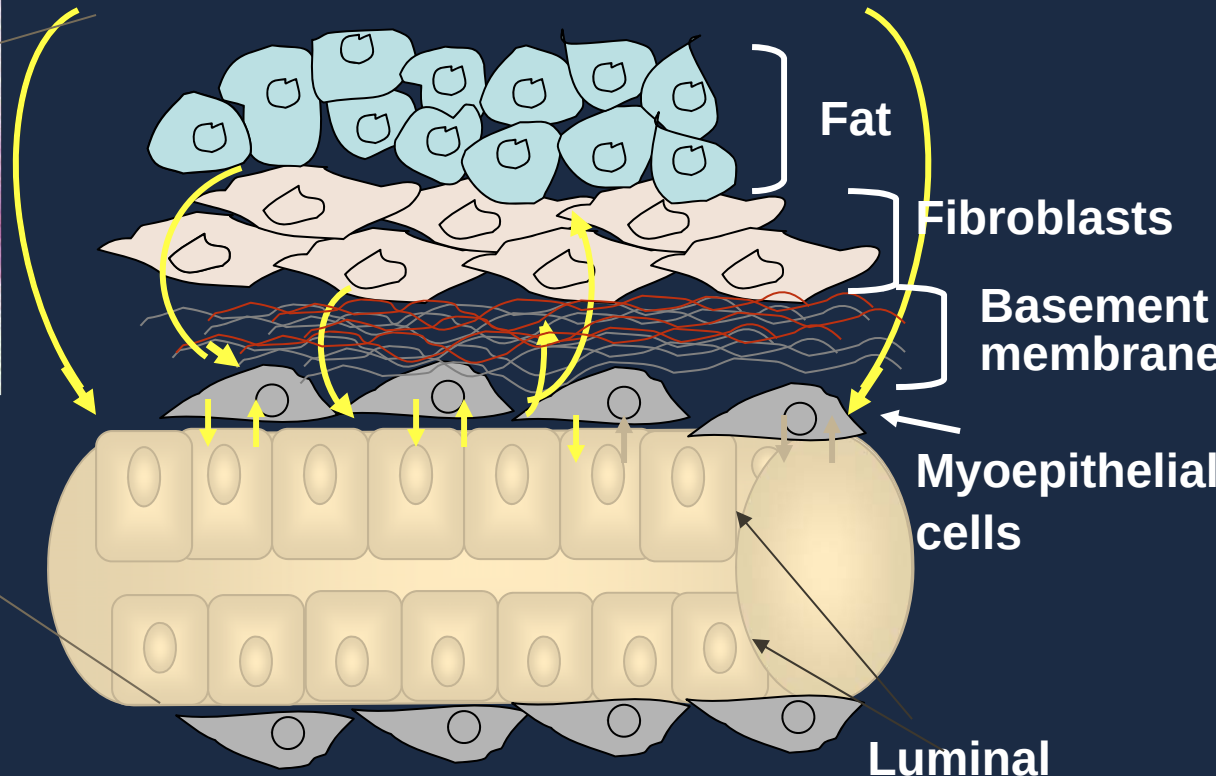
Modified from http://www.the-scientist.com/images/yr2003/sep08/tumor.gif

Inflammatory Cells Influence Tumor Progression

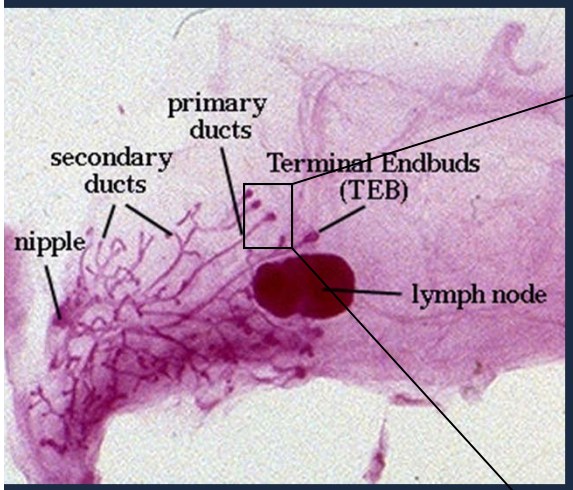
The Microenvironment Controls Normal Development and Homeostasis

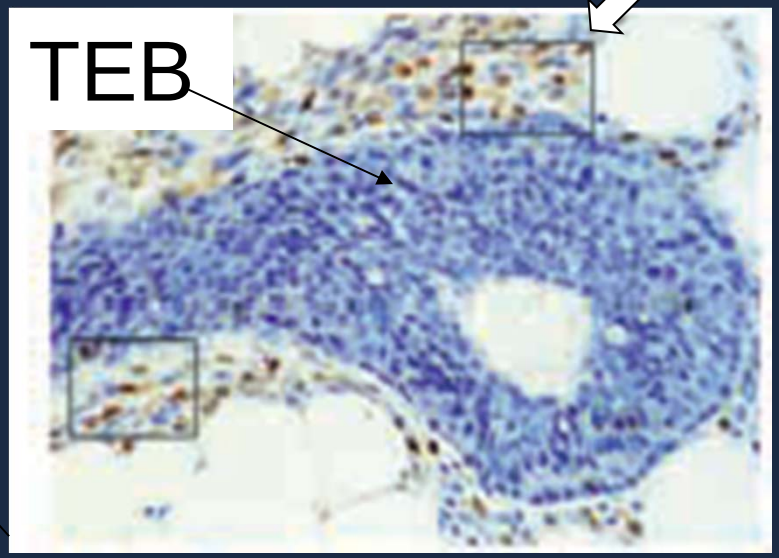


Developing mammary gland

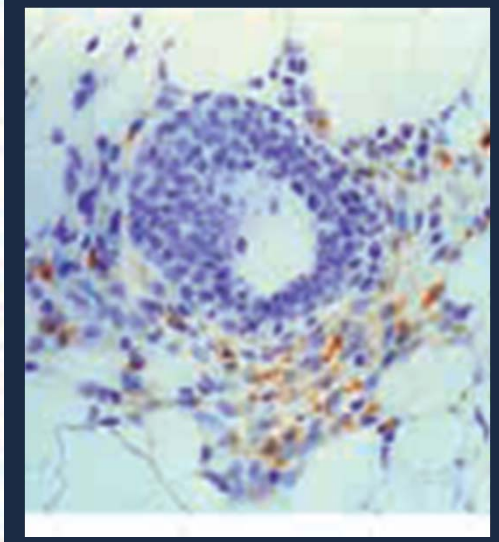


Macrophage and Eosinophil Association with Developing Mammary Gland

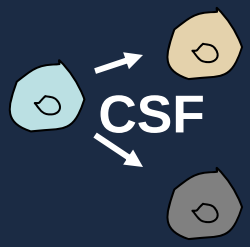




Macrophage (brown stain) associated with TEBs in developing mammary gland



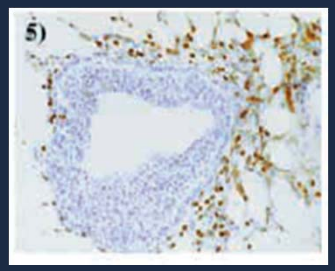
Cross section through TEB



Similar staining observed with anti- eosinophil antibodies.

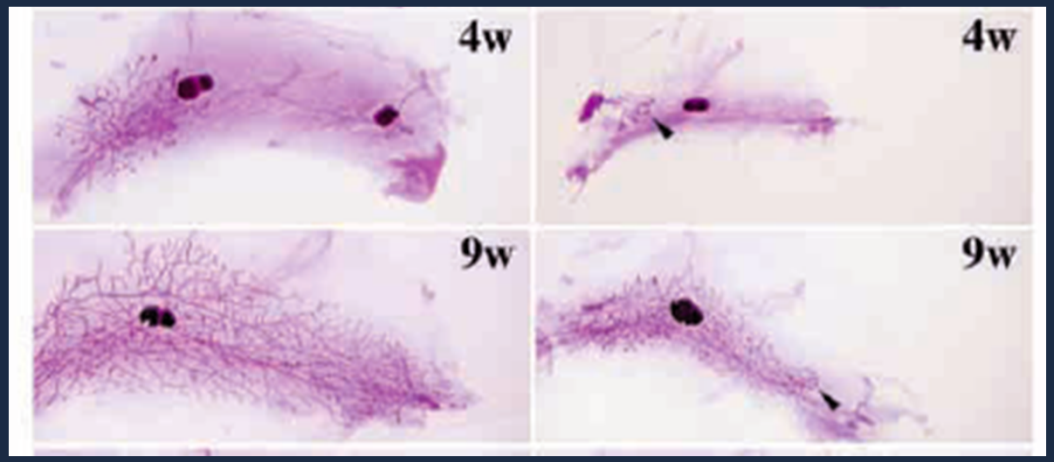
Gouon-Evans, Rothenberg, Pollard Development 2000

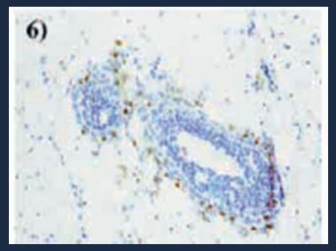
Knockout of CSF-1 or its Receptor Results in Defective Mammary Development



Macrophage staining

+/- mice (with CSF) **-/- (no CSF)**





Macrophage staining

- Impaired ductal branching and failure to lactate
- Loss of macrophage recruitment around endbuds
- Defect in mammary development with irradiation, rescued with bone marrow (eotaxin/ eosinophils also required)

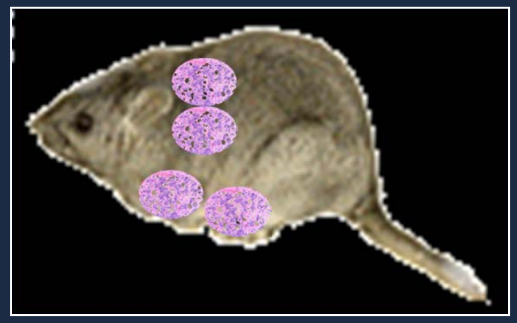
Gouon-Evans, Rothenberg, Pollard Development 2000

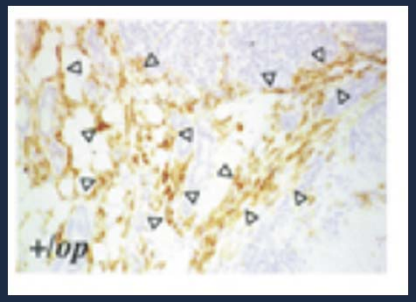
Loss of CSF-1 Prevents Metastasis in Mouse Tumor Model

Mammary tumor with lung mets

MMTV-PyMT

Breast tumor



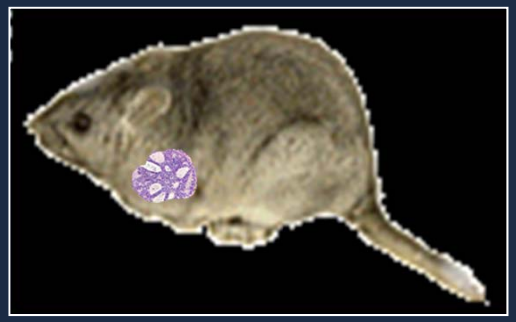


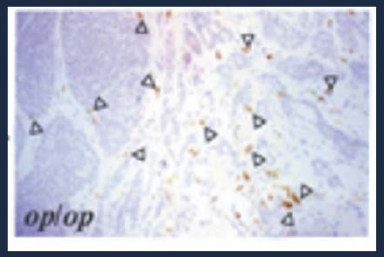
Tumor ass'd macrophages

→ Lung mets

Cross with CSF -/- mouse ↓

Mammary papilloma with no mets and dramatic reduction in invasion





Tumor ass'd macrophages

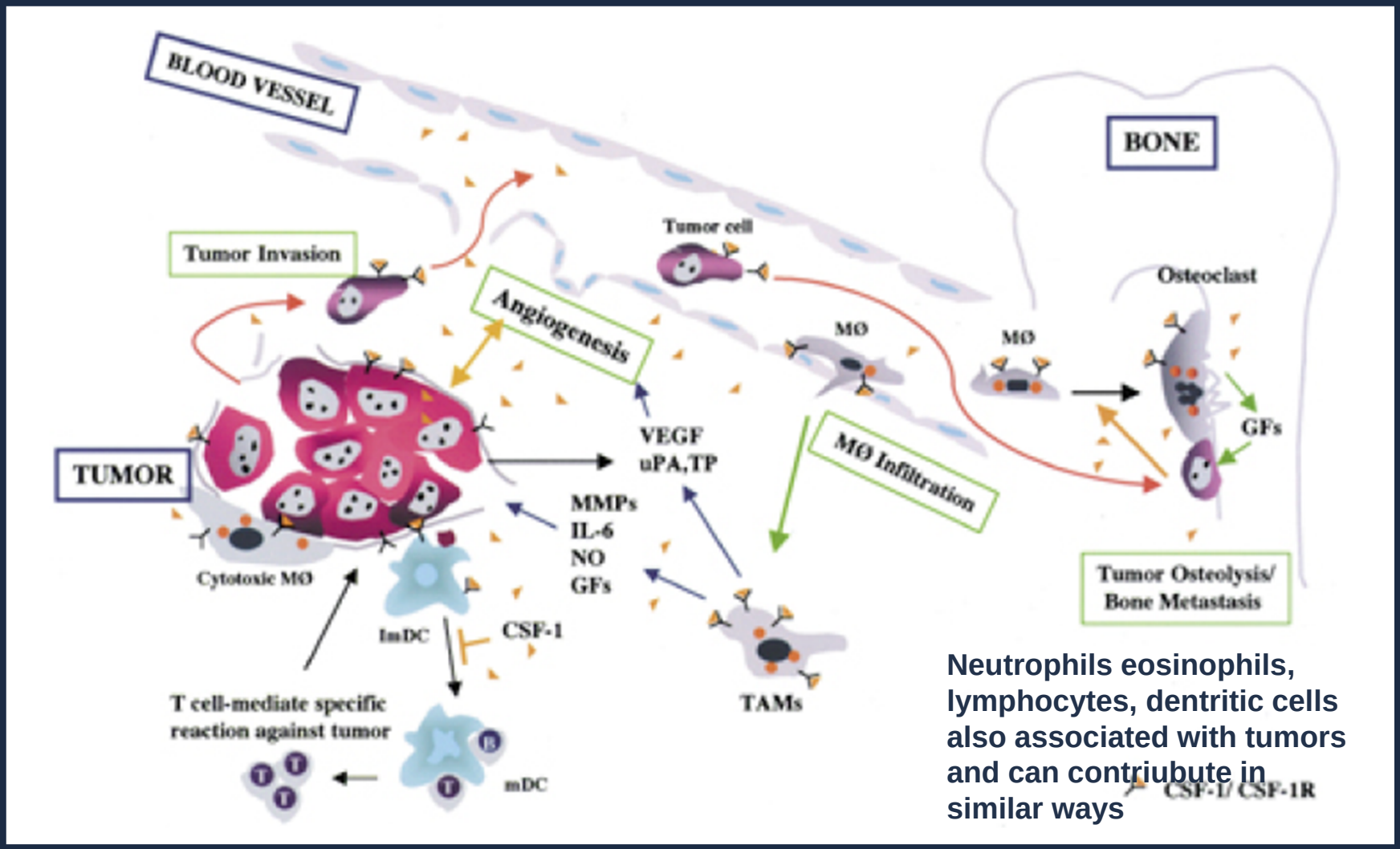
→ No lung mets

Lin EY, Nguyen AV, Russell RG, Pollard JW. J. Esp Med 2001

CSF-1 in Human Tumors

- **CSF-1 and its receptor have been shown to be elevated in a high percentage of several types of carcinomas**
- **Coexpression of ligand and receptor common in invasive tumors**
- **Expression correlates with marked inflammatory cell**
- **infiltration and poor prognosis**
- **High level expression in sera (100ng/ml) of patients with neoplastic disease (work on ovarian particularly compelling - CSF-1 + CA-125 -95% pred.)**

Contributions of CSF-1 and Macrophage to Tumor Progression



Neutrophils eosinophils, lymphocytes, dentritic cells also associated with tumors and can contriubute in similar ways

From Lin EY, Gouon-Evans V, Nguyen AV, Pollard JW J. Mam. Gland. Neoplasia 2002

Summary: Role of Inflammatory Cells in Tumor Development

Early

Late

Function as tumor promoters

- Produce cytokines that promote proliferation
- Facilitate genomic instability
- Promote angiogenesis

Promotes spreading and metastasis

-Proteases

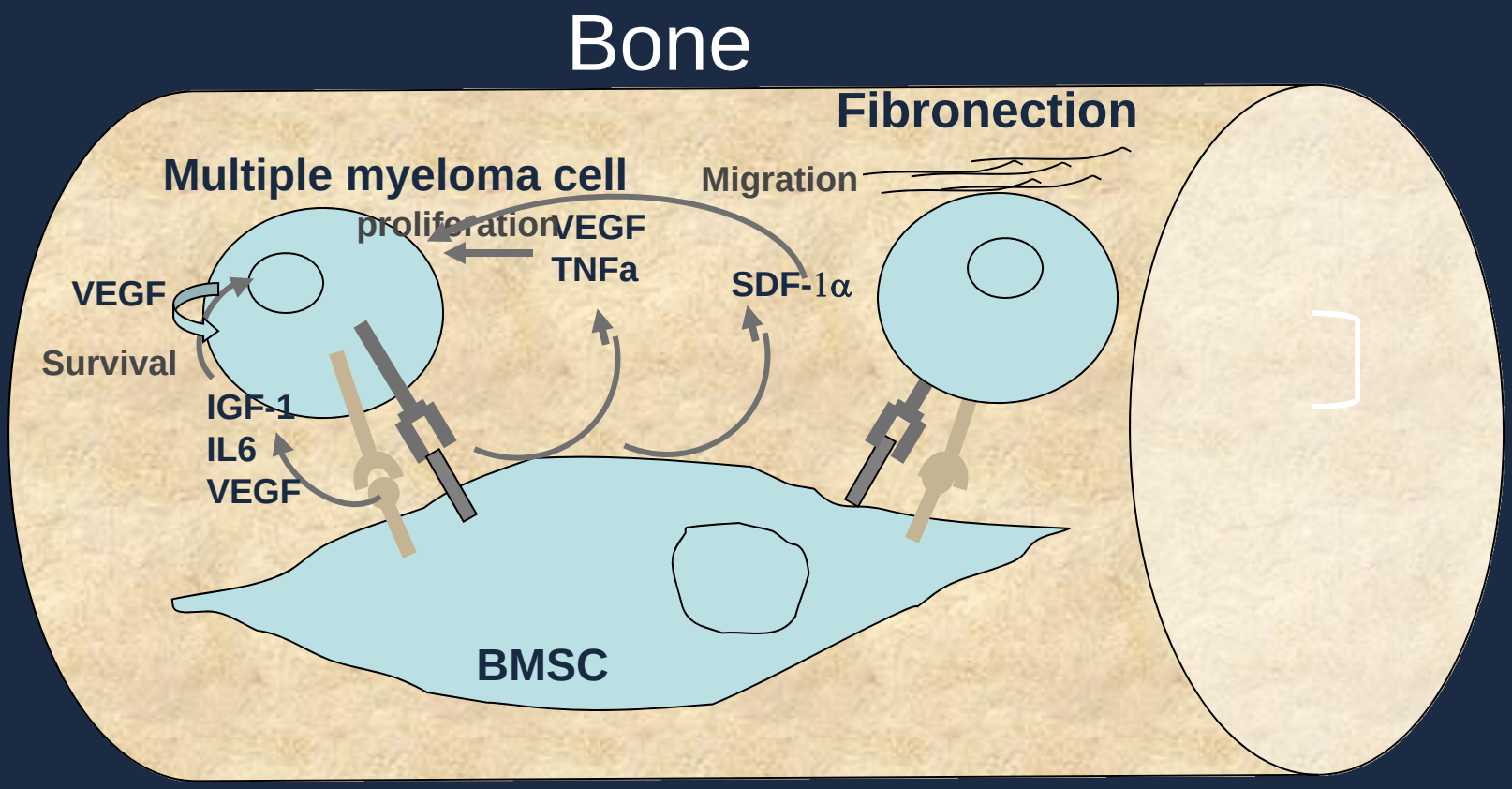
- remodel ECM and disrupt tissue architecture
- break down basement membrane

-Chemokines

- promote cell migration and invasion

Stromal Cells
Influence Tumor Progression

Multiple Myeloma Dependence on the Microenvironment



Derived from Hideshima and Anderson Nature Rev Cancer 2002

Effects of a Thalidamide Analog (Revamid) and Velcade on MM cell-Stroma Interactions

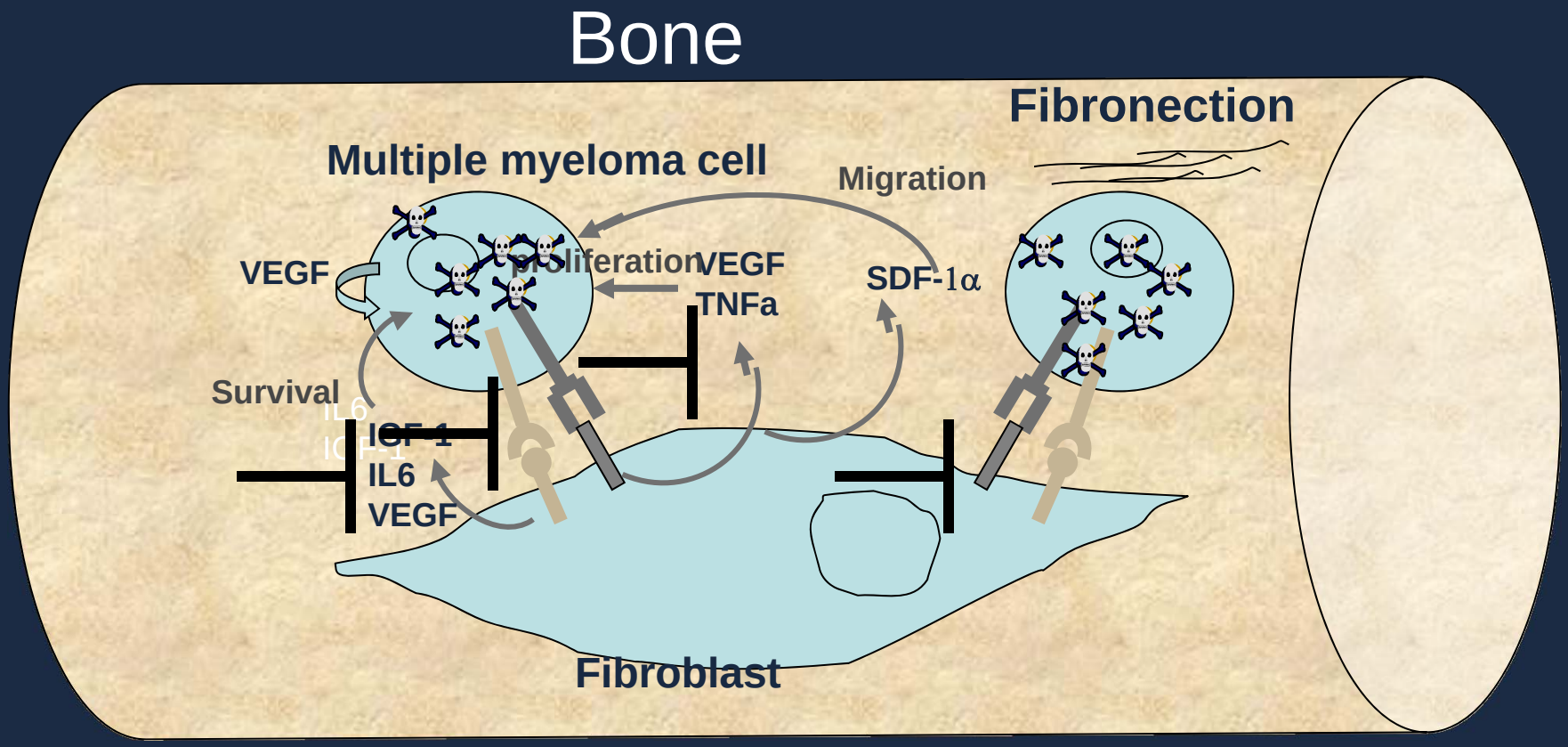


Table 2 | **Multiple myeloma paraprotein decline after IMiD3 treatment**

	Maximal decrease				Maximal increase
	<25%	*25–50%*	*50–75%*	*75–99%*	*≥25%*
% of patients (n=24)*	8%	42%	17%	13%	20%

* All patients had progressive diseases on salvage therapies[84].

Derived from Hide

Velcade shows similar broad activities and promising clinical efficacy

The Seed and Soil Hypothesis

Nature Reviews | Cancer

Stephen Paget, 1889
The Lancet

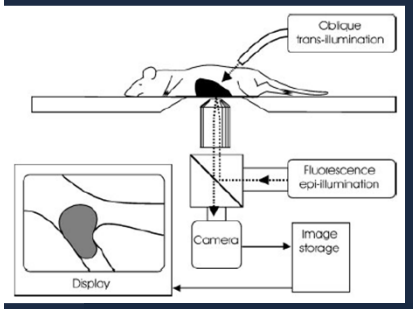
Growth in organ microenvironment requests compatible "seed" and "soil"

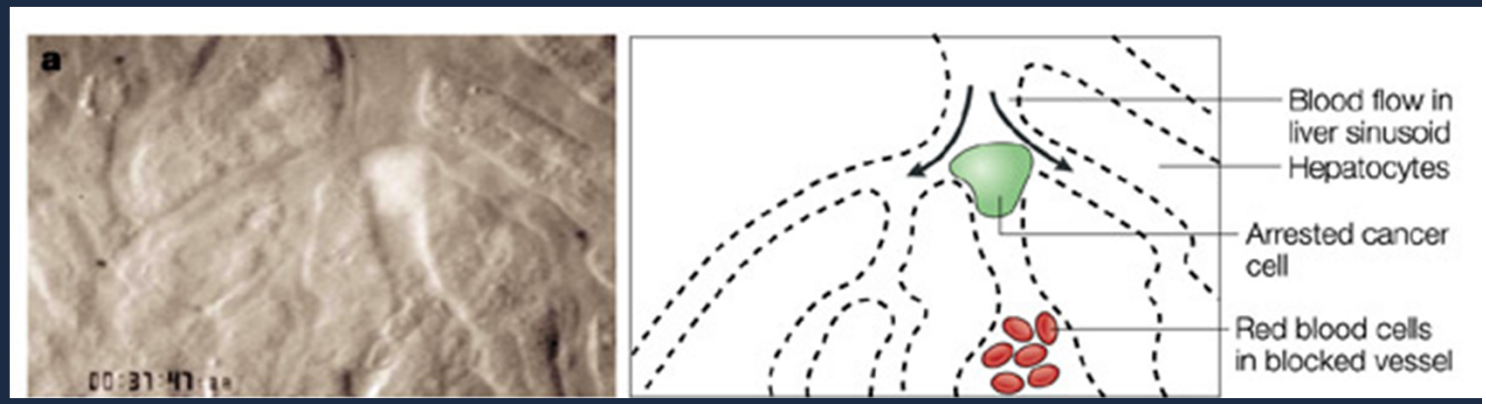
"When a plant goes to seed, its seeds are carried in all directions; but they can only live and grow if they fall on congenial soil."

"What is it that decides which organs shall suffer in a case of disseminated cancer?

What Steps in Metastasis are Inefficient?

In vivo video microscopy of metastasis



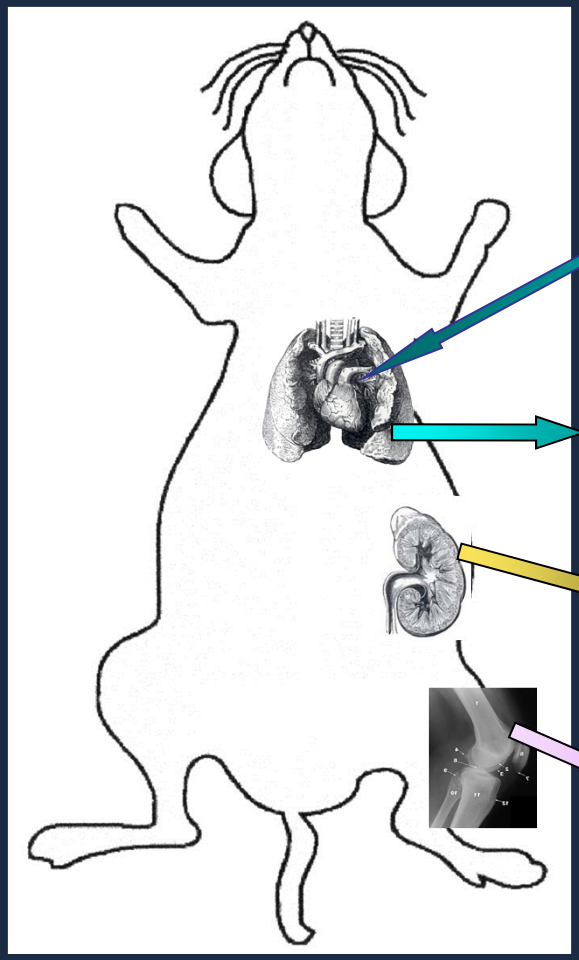


"Because growth of metastases is a primary determinant of metastatic outcome, the growth phase of the metastatic process is a promising therapeutic target. Treatments that target the specific 'seed–soil' compatibility that results in organ-specific metastatic growth would be especially useful."

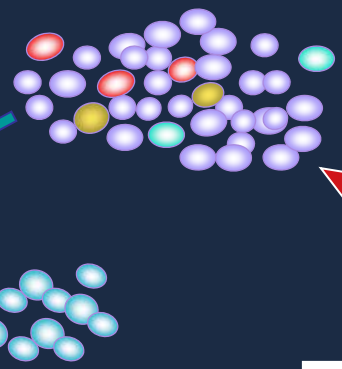
Chambers and MacDonald **Cameron et al Cancer Res. 2000**

What is the Molecular Basis of Site-Specific Metastasis?

Select highly metastatic populations



Cell line

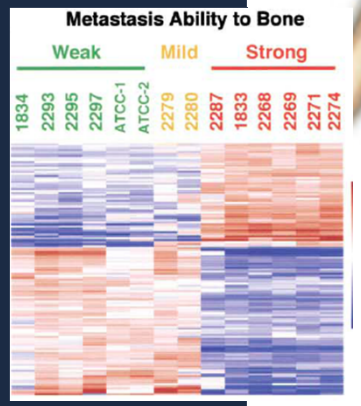


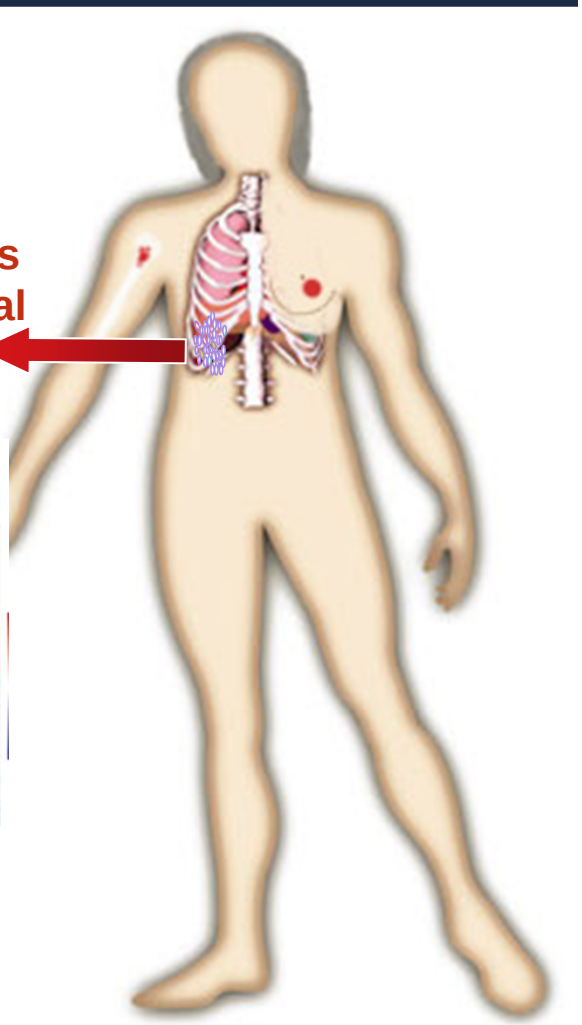
Tumor cells from Pleural Fluid

Lung Mets

Adrenal Mets

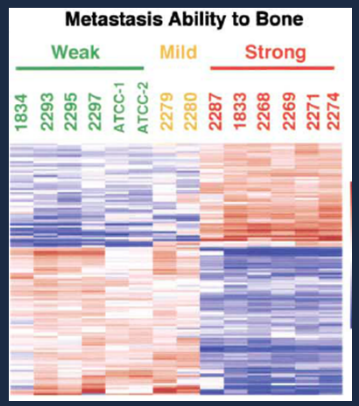
Bone Mets

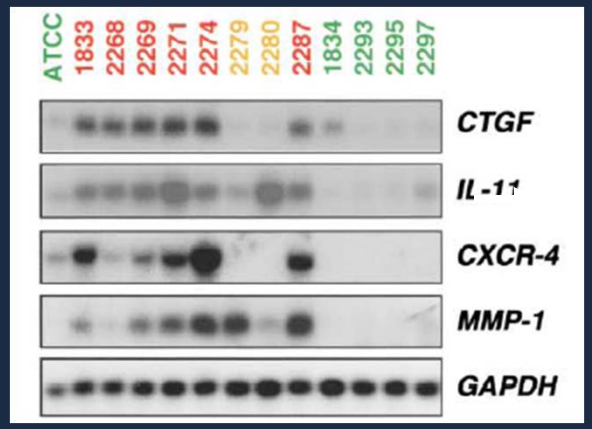


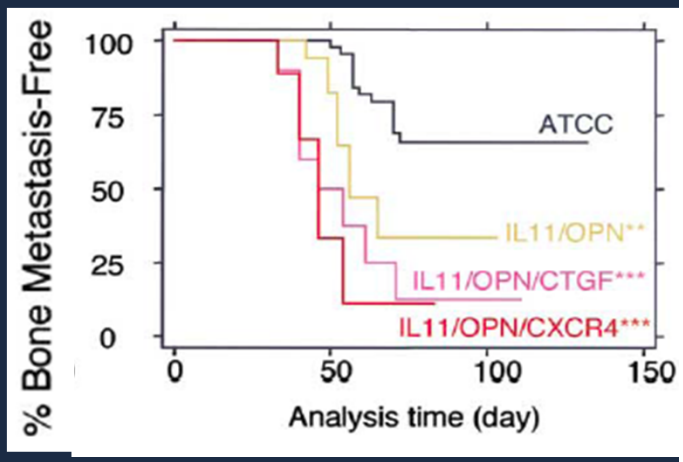


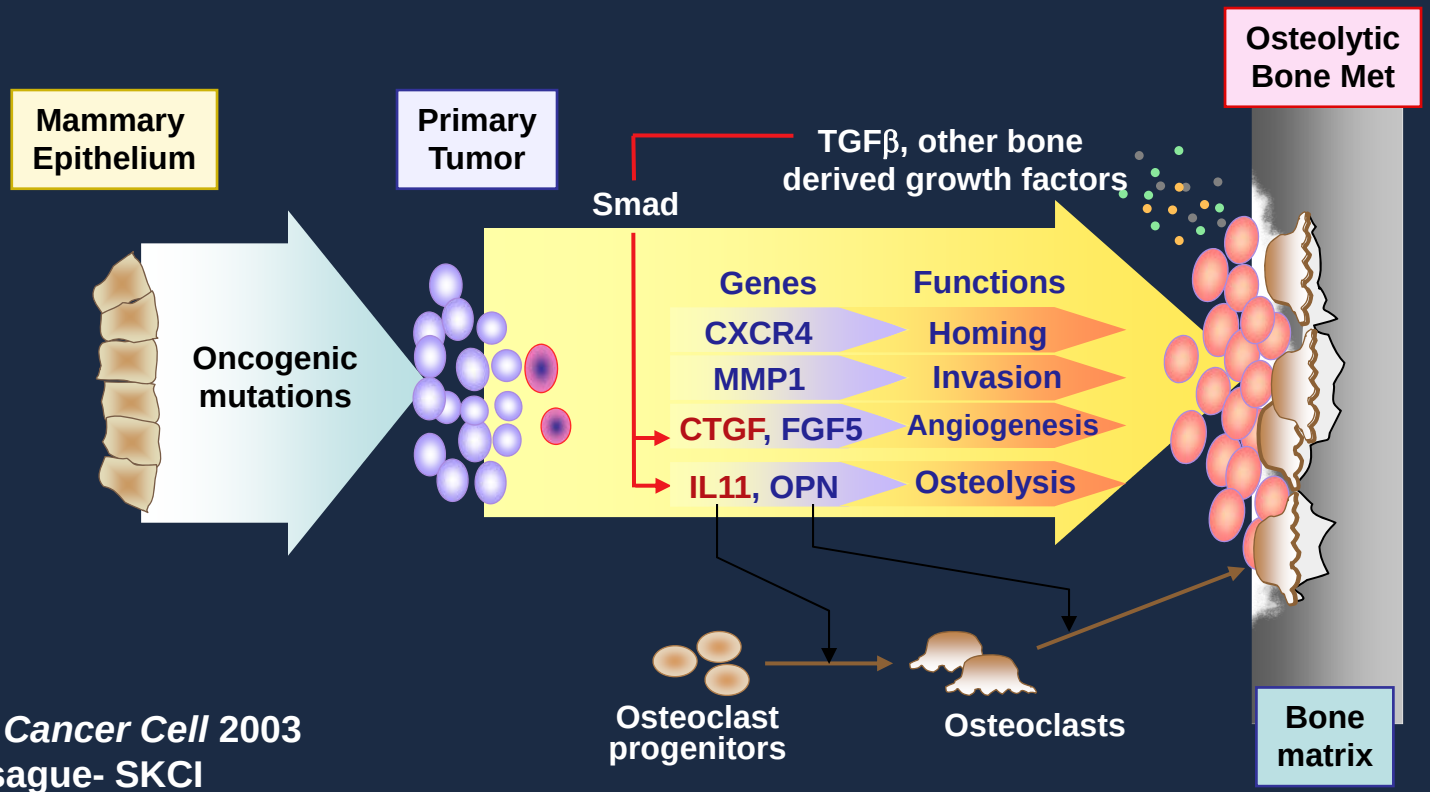
Kang et al *Cancer Cell* 2003
Joan Massague- SKCI

Molecular Basis of Site-Specific Metastasis









Kang et al *Cancer Cell* 2003
Joan Massague- SKCI

Summary

- **The cells that metastasize represent a small population of the cells in the initial population of the tumor cell line that express a specific SET of genes**
- **Metastatic lesions may be driven by a set of stochastcially accumulated functions**
- **Massague proposes that the functions contributed by CXCR4, CTGF, IL-11 and OPN may be fulfilled by other mediators in other tissues**

These types of studies provide the basis for developing therapeutic interventions.

“The Opportunity”

Understanding the tumor μE will:

- Lead to the development of therapeutics that significantly increase tumor cell killing or suppression of growth and invasion by
  - blocking critical factors in the microenvironment that are required for viability and invasive behavior of tumor cells
- Reduce the likelihood of development of drug resistance since the μE cells are not as genetically unstable and plastic as the tumor cells
- Lead to diagnostic tests that assess the state of the microenvironment for evidence of pre-disposure to tumor or for outcome of tumor

Feedback from NCI Funded Investigators

Workshops organized by Suresh Mohla:

- Matrisian LM, Cunha GR and Mohla S.: **Epithelial stromal interactions and tumor progression: meeting summary and future directions.** Cancer Res 61:3844-3846, 2001

- Reddi AH, Roodman D, Freeman C and Mohla S. **Mechanisms of tumor metastasis to the bone: challenges and opportunities**. J Bone Miner Res 18:190-194, 2003.

- Choi YS, Anderson K and Mufson RA. **Marrow microenvironment and hematological malignancies workshop.** Cancer Res., November 2003, in press.

Tumor Microenvironment Think Tank

Charge:

To make recommendations concerning:

- **Strategies to define the role of the tumor microenvironment in tumor initiation, progression and metastasis**
- **Strategies to use this information to develop applications that will impact the diagnosis, prevention and treatment of cancer**
- **Strategies whereby NCI can facilitate the acquisition of this information and its application**

Think Tank Participants

- Physician scientists
- Basic scientists
- Medical and surgical oncologists
- Industry representatives
- Genomics and molecular profiling experts
- Non-invasive imaging experts

The group contained a mixture of scientists with experience in the tumor mE as well as those from outside this area to give a good mix of perspectives.

Key Questions

What information about the microenvironment would facilitate the diagnosis, prevention, or treatment of cancer?

How can this information be translated into useful clinical applications?

Initial Goal of Preclinical Studies

To further our understanding of how the tumor microenvironment contributes to tumor cell progression and metastasis

Stage I

Identify the key components of the μE and define how these are altered during tumor development

- **cellular composition**
- **Genetic and epigenetic changes**
- **Changes in gene expression**
- **Changes in protein expression and modification or proteins**
- **Proteins secreted into the tumor microenvironment**

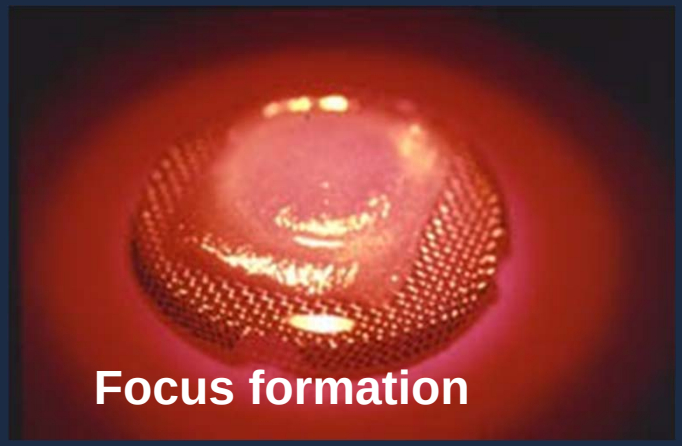
Stage II

Determine which alterations in the tumor microenvironment are critically involved in development, progression and metastasis of tumors

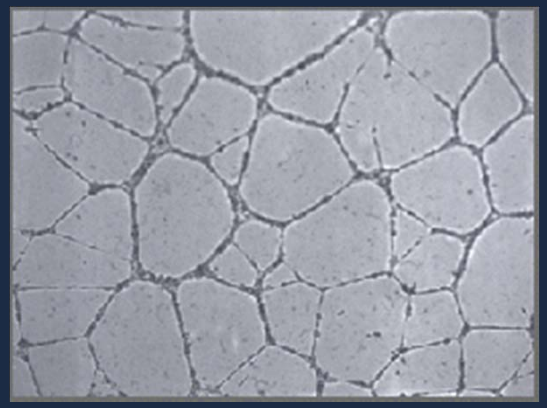
Elucidate the mechanisms responsible for induction of these changes

How can we determine which of the hundreds of alterations are ESSENTIAL for tumor development?

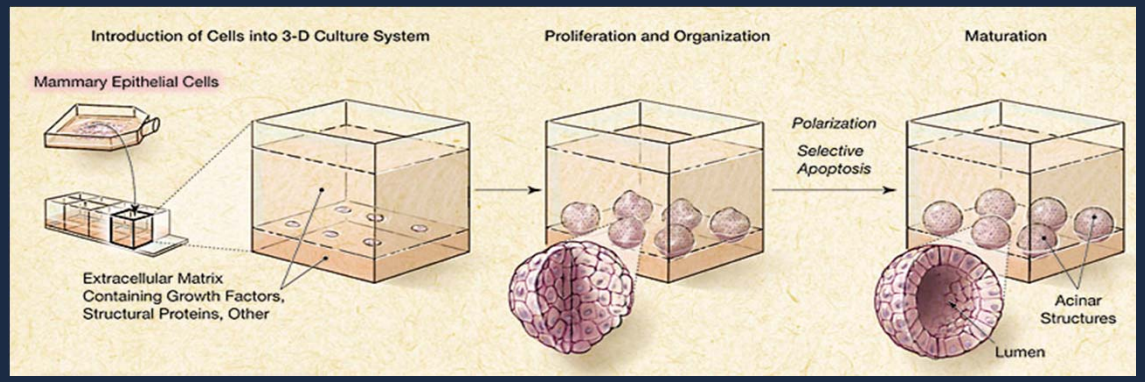
In vitro and Animal Models Can Help Facilitate Identification of the Critical Alterations Associated with the Tumor μE



Human keratinocyte and fibroblast raft co-cultures



Human endothelial tubes



Mammary epithelial cell acini

Illustration courtesy of Cassie Lynn, JAMA

Stage III: Translation

Develop therapeutic strategies to target the μE

Examples:

- Develop drugs that induce death or inhibit the function of the cells in the tumor μE that are required for tumor progression
- Develop drugs that specifically target the factors produced by cells in the μE that are responsible for tumor progression
- Block the induction of the μE factors responsible for tumor production

Stage III: Translation

Develop diagnostics tests to predict outcome and/or design treatment

Perform clinical trials to establish whether any of the alterations in the microenvironment correlate with outcome or specific treatment responsiveness

Stage III: Translation

Develop strategies to PREVENT development of tumors based on understanding of the μE changes required for tumor development

Recommendations for funding initiatives

- Initial efforts to define components of the μE and to identify differences relative to normal tissue would most likely involve small to midsize groups of investigators at each participating institution -- and would require systematic, well orchestrated approaches

- Effective communication and collaboration between groups studying different aspects of the environment and different types of tumors will be essential

Therefore, the funding format should facilitate effective cross talk, dissemination of information and reagents, and coordination of different efforts - will require considerable NCI staff support

Think Tank Recommendations (con't)

- Need to develop a format for reagent generation and dissemination of research tools and biologics

- Need core laboratories to carry out high technology sophisticated genomic and proteomic analyses

Think Tank Recommendations (con't)

- Need to develop new paradigms to establish mechanisms whereby the tumor microenvironment influences tumor development and metastasis

- Need additional in vitro organotypic models to facilitate mechanistic studies and continued improvement of animal models

Think Tank Recommendations (con't)

- Need to bring more scientists into this area of research through:

  - transdisciplinary training grants
  - training workshops to transfer expertise with models and new technologies
  - dissemination of reagents and cell lines