Cancer Biol: Metastasis

Lecture : 08-04-2011

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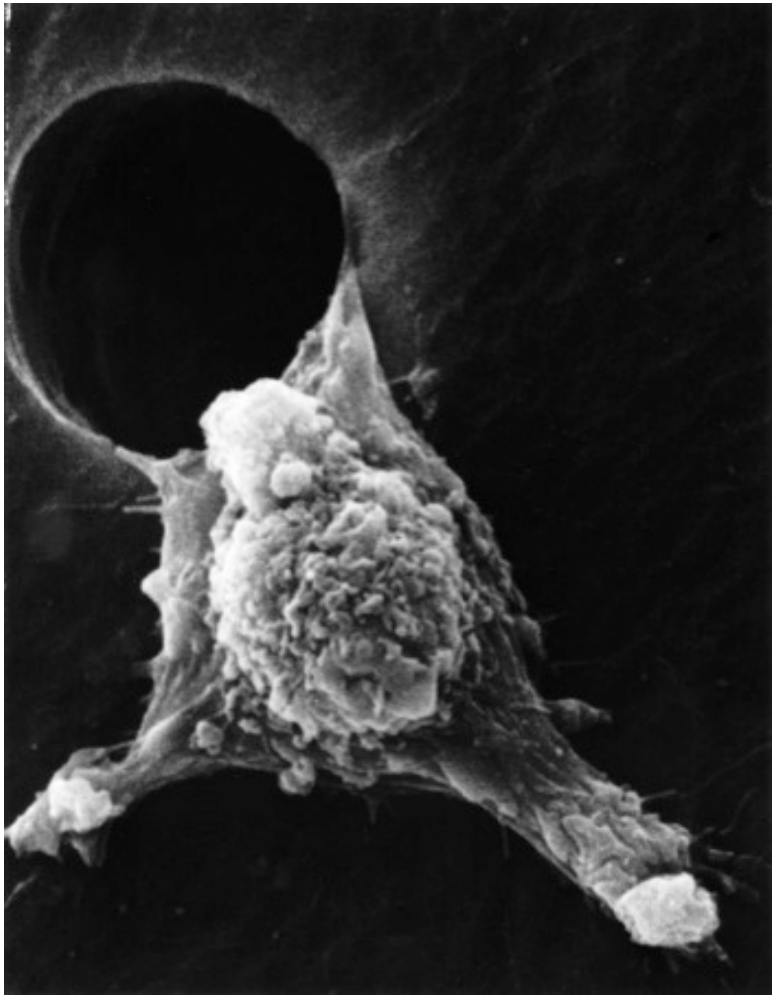
Table 9.5 Examples of anti-apoptotic alterations found in human tumor cells

Alteration	Mechanism of anti-apoptotic action	Types of tumors
CASP8 promoter methylation	inactivation of extrinsic cascade	SCLC, pediatric tumors
CASP3 repression	inactivation of executioner caspase	breast carcinomas
Survivin overexpression[a]	caspase inhibitor	mesotheliomas, melanomas, many carcinomas
ERK activation	repression of caspase-8 expression	many types
ERK activation	protection of Bcl-2 from degradation	many types
Raf activation	sequestration of Bad by 14-3-3 proteins	many types
PI3K mutation/activation	activation of Akt/PKB	gastrointestinal
NF-κB constitutive activation[b]	induction of anti-apoptotic genes	many types
p53 mutation	loss of ability to induce pro-apoptotic genes	many types
$p14^{ARF}$ gene inactivation	suppression of p53 levels	many types
Mdm2 overexpression	suppression of p53 levels	sarcomas
IAP-1 gene amplification	antagonist of caspases-3 and 7	esophageal, cervical
APAF1 methylation	loss of caspase-9 activation by cytochrome *c*	melanomas
BAX mutation	loss of pro-apoptotic protein	colon carcinomas
Bcl-2 overexpression	closes mitochondrial channel	~ of human tumors
PTEN inactivation	hyperactivity of Akt/PKB kinase	glioblastoma, prostate carcinoma, endometrial carcinoma
IGF-1/2 overexpression	activates PI3K	many types
IGFBP repression	loss of anti-apoptotic IGF-1/2 antagonist	many types
Casein kinase II	activation of NF-κB	many types
TNFR1 methylation	repressed expression of death receptor	Wilms tumor
FLIP overexpression	inhibition of caspase-8 activation by death receptors	melanomas, many others
Akt/PKB activation	phosphorylation and inactivation of pro-apoptotic Bcl-2-like proteins	many types
Stat3 activation	induces expression of Bcl-X_L	several types
TRAIL-R1 repression	loss of responsiveness to death ligand	small-cell lung carcinoma
FAP-1 overexpression	inhibition of Fas receptor signaling	pancreatic carcinoma
XAF1 methylation[c]	loss of inhibition of anti-apoptotic XIAP	gastric carcinoma
Wip1 overexpression[d]	suppression of p53 activation	breast and ovarian carcinomas, neuroblastoma

[a]Survivin is an inhibitor of apoptosis (IAP) in gastric, lung, and bladder cancer and melanoma in addition to the mesotheliomas indicated here. The expression of a number of IAP genes is directly induced by the NF-κB TFs.
[b]Induces synthesis of c-IAPs, XIAP, Bcl-X_L, and other anti-apoptotic proteins.
[c]XAF1 (XIAP-associated factor 1) normally binds and blocks the anti-apoptotic actions of XIAP, the most potent of the IAPs.
[d]Wip1 is a phosphatase that inactivates p38 MAPK, which otherwise would phosphorylate and stimulate the pro-apoptotic actions of p53.

Table 9.5 *The Biology of Cancer* (© Garland Science 2007)

A Metastatic Tumor cell exiting a blood vessel



- **Metastasis is the cause of 90% of cancer deaths.**
- **This process is poorly understood!**

Basics on Metastasis

- **Metastases is the spread of a tumor from its primary site to a distant site—usually in another organ. Metastases are often called secondary tumors.**

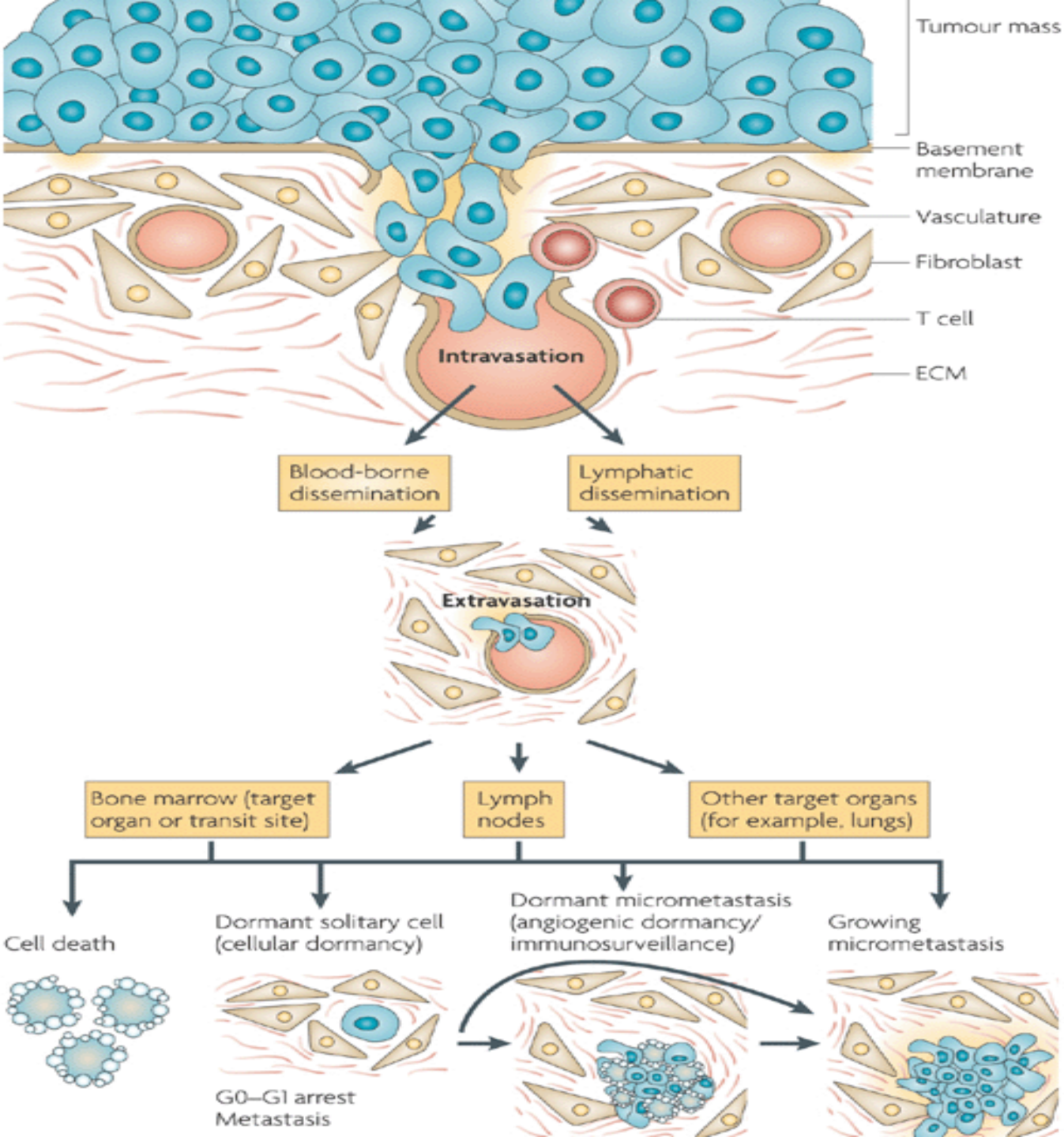
- **Metastasis is the cause of 90% of cancer deaths.**

- **Metastatic process requires anchorage independent tumor cells which have degraded the surrounding ECM and entered circulation (blood or lymph).**

- **So, the tumor goes from being local to systemic.**
- **Circulating tumor cells are rare-but have been detected !**

- **To become a successful secondary tumor, the Metastatic cells must first get established (colonized) at their new site.**

- **Then, in order to re-grow, the secondary tumor must activate Angiogenesis**


Tumour mass
Basement membrane
Vasculature
Fibroblast
T cell
ECM
Intravasation
Blood-borne dissemination
Lymphatic dissemination
Extravasation
Bone marrow (target organ or transit site)
Lymph nodes
Other target organs (for example, lungs)
Cell death
Dormant solitary cell (cellular dormancy)
Dormant micrometastasis (angiogenic dormancy/ immunosurveillance)
Growing micrometastasis
G0–G1 arrest
Metastasis

Previous Figure ; Succession of cellular changes for Metastasis

Begin with local invasion of the tumor, and breakage of the Basement membrane

Intravasation: Movement of cancer cells into nearby blood and lymphatic vessels,

Extravasation : Escape of cancer cells from lymph/blood vessels into the parenchyma of distant tissues

EXTRA-VASATED TUMOUR CELLS HAVE FOUR POSSIBLE FATES:

- **1. Vast majority of tumor cells have lost anchorage and undergo apoptosis .**
- **2. Cells enter a state of dormancy, either as a single cell or as a micro-metastatic lesion that proliferated but could not recruit a vascular bed**
- **3. Dormant Cells can resume proliferation (growing micro-metastasis).**
- **4. Micrometastasis can enlarge if angiogenesis begins. If micro-metastatic lesions enlarge successfully, we get macroscopic tumors!**

Colonization: Rate Limiting step in Metastasis

Macroscopic tumors must adapt to local conditions of the secondary site.

Then, these macroscopic tumors must start re-growing…only then can we say that the primary tumor has successfully colonized a secondary site and become metastatic! …..that is why Colonization is rare!!

In fact, Colonization is a Rate Limiting step in Metastasis

Early view of Colonization: Paget's Seed and Soil Hypothesis:

- Primary Tumor cells spread randomly to all parts of body via circulation and lymph---but only few survive, adapt and proliferate in a new organ.

This is just like seeds which land everywhere, but only few will germinate!

Modern view of Colonization : Clinical data suggest that Metastasis is not random. It is usually found in certain target organs.

Organ Specific Metastasis

There is Good clinical evidence for Organ Specific Metastasis

Colon cancer cells metastases is mainly in liver due to portal vein circulation.

Lung metastases of breast cancers may also be due to pattern of blood flow.

Bone metastasis : This process is well understood for Breast cancer

- **How to explain Organ Specific Metastasis??**

- **1. Circulating tumor cells get preferentially trapped in certain organs due to body's vascular system. This may explain about 70% of organs which are secondary sites for metastasis.**

- **2. Ability to colonize is a rare event. Some primary tumors are more successful in targeting certain organs for metastases. This may explain about 20% of secondary sites for metastasis..**

The Metastatic Process is Usually Very Inefficient !!

- **Based on animal models, it has been estimated that only about 0.01% of disseminated tumor cells will actually progress to macro-metastasis.**

  - **WHY??**

- **Extra-Vasated tumour cells have lost Anchorage Dependence. So, they must survive as suspended cells (by avoiding apoptosis). This is very rare!**

- **One possibility ….Suspended tumour cells expressing Integrin αvβ3 resist apoptosis. Integrin αvβ3 signals via Src to increase cell survival (next slide)**

- **Early stages of forming a viable micro-metastases is a rate limiting step ….so macroscopic tumors are also rare!**

- **The few macroscopic tumors which form , can only survive and re-grow if they successfully colonize a secondary site . This is a late step-and is also rate limiting!**

Metastatic Process Can be Greatly Delayed !!

- Q. ***How can Micrometastases remain dormant for long periods and then suddenly develop into macroscopic secondary tumors ??***

- ***Understanding this process may help us figure out why certain cancer patients relapse after many healthy years of tumor-free life !***

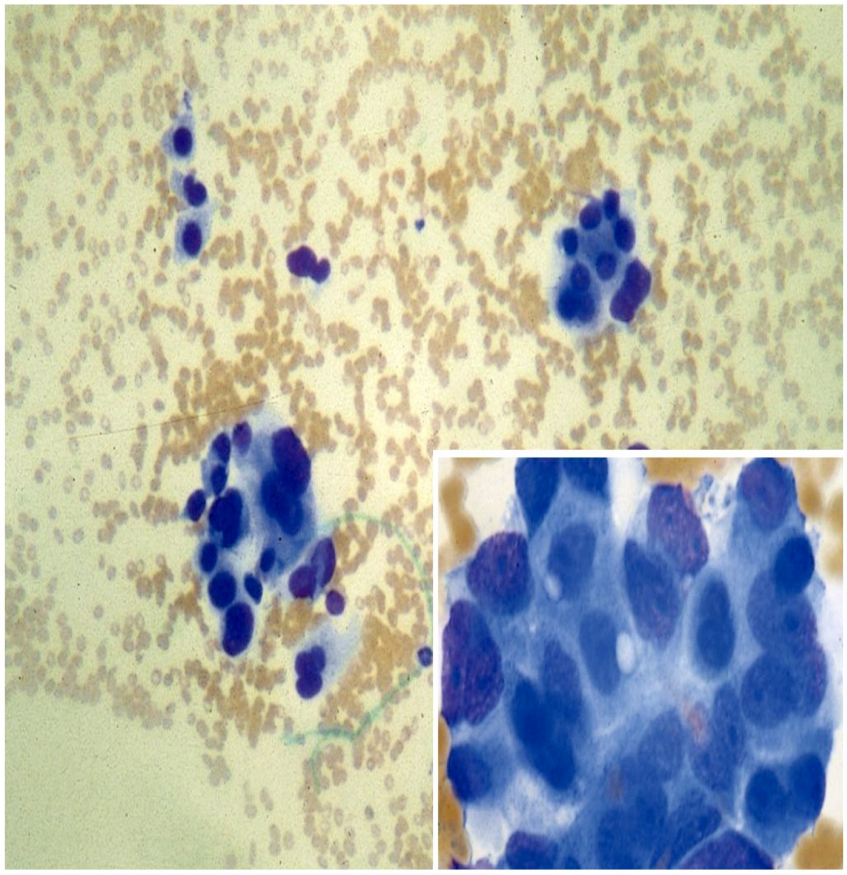
- **Recent experiments show that nutrient starvation can induce Autophagy that causes cancers to shrink and adopt a state of reversible dormancy…. …such cells may even resume proliferation when nutrients return!!**

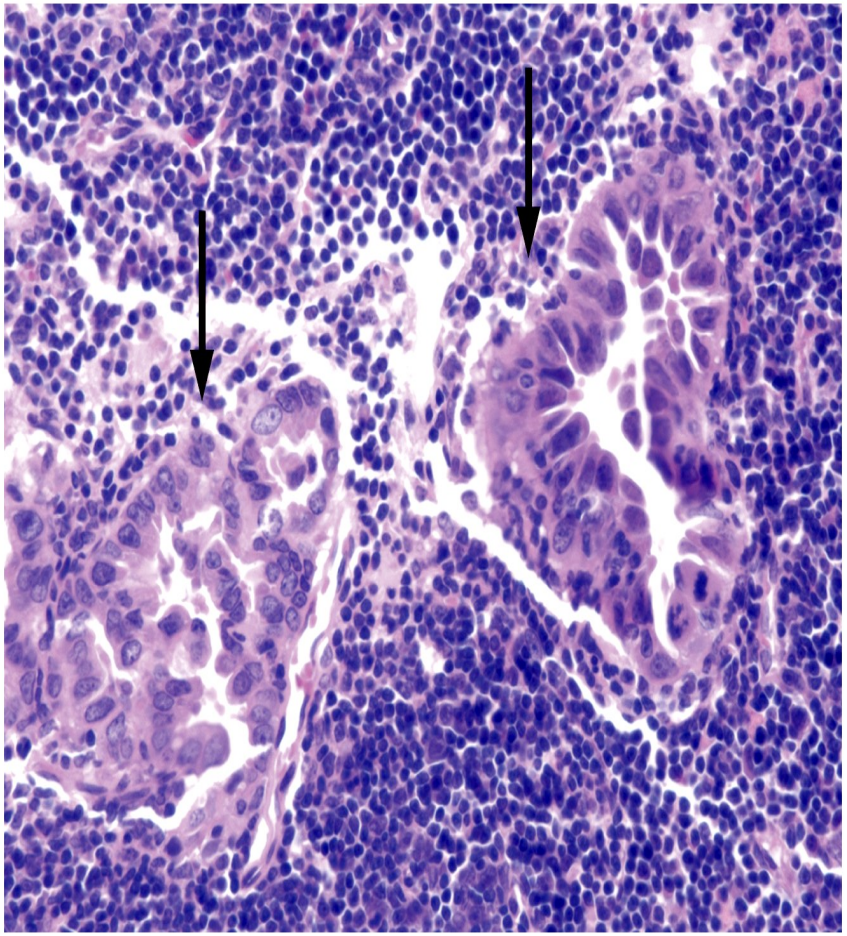
- **Micro-metastatic dormancy may involve anti-growth signals embedded in normal tissue extracellular matrix.**

- **Micro-metastatic dormancy may also happen because of tumor-suppressing actions of the immune system**

Left: Epithelial markers stain a Cancer which Metastasized into Bone marrow

Right: Two Micro-metastatic regions of Lung Cancer are seen in a Lymph Node.





Cancer Patients with affected Lymph Nodes are at high risk for Distant Metastasis:

Clinical Observation: **Patients who relapse after cancer therapy usually have several Lymph Nodes which test Positive for tumor cells.**

Patients who survive 5years after cancer therapy show few Positive Lymph Nodes

Early view:

Lymph Node Positivity is an intermediate step in Metastases.

So, patients with affected Lymph Node will always get distant Metastases.

This Early view is false ….Because…..

Removal of affected Lymph Nodes does not improve patient survival !!

New View:

Cancer Patients with > 4 Lymph Nodes which test Positive for tumor cells (Lymph Node Positive patients) are at very high risk for distant metastases .

Is there a gene signature for Lymph Node metastasis ?

J Clin Oncol 28:15s, 2010 (suppl; abstr 1075) ***ASCO ABSTRACT:*** *R. E. Ellsworth et al.*

- **Problem:** Lymph node status is one of the most useful prognostic indicators in breast cancer. **But, current methods to assess lymph node status are poor. Moreover, 10-33% of women diagnosed with negative lymph nodes develop metastases !!**

- **Sample: Frozen breast specimens were collected from women with negative (n=39) and positive (n=35) lymph node status.** Patients with positive BRCA1/BRCA2 status, neoadjuvant therapy or negative lymph node status who later developed distant metastasis were not included in this study

- **Methods:** RNA was isolated from pure tumor cell populations after laser micro-dissection and gene expression data generated using Microarrays (Affymetrix).

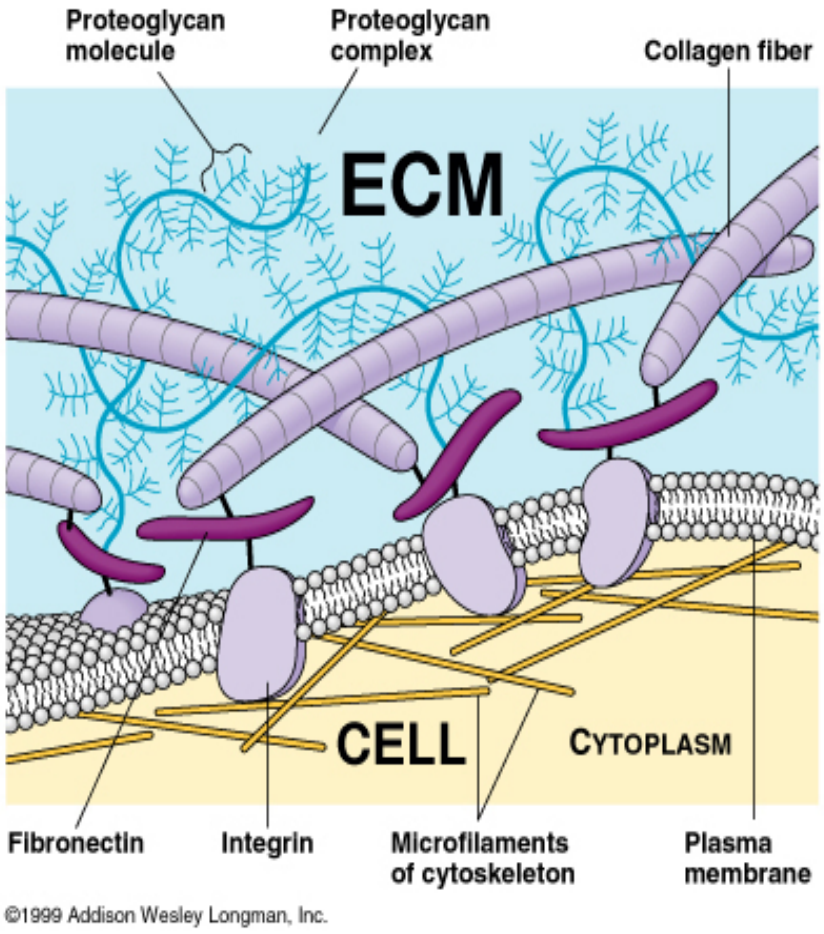
- **Results: Data suggested that a molecular signature to discriminate between primary breast tumors with and without lymph node metastases does not exist.**

-

Properties of Metastatic cells

- **Anchorage Independence**
- **Motility**
- **Invasiveness (can degrade proteins of BM and ECM)**
- **Most Metastases originate from Epithelial tumors…and the Epithelial form of tumor cell can temporarily show a fibroblastic form!**

Extracellular Matrix (ECM) of Normal Cells



ECM is composed of 3 major classes of molecules:

Structural proteins - Collagen and Elastin,

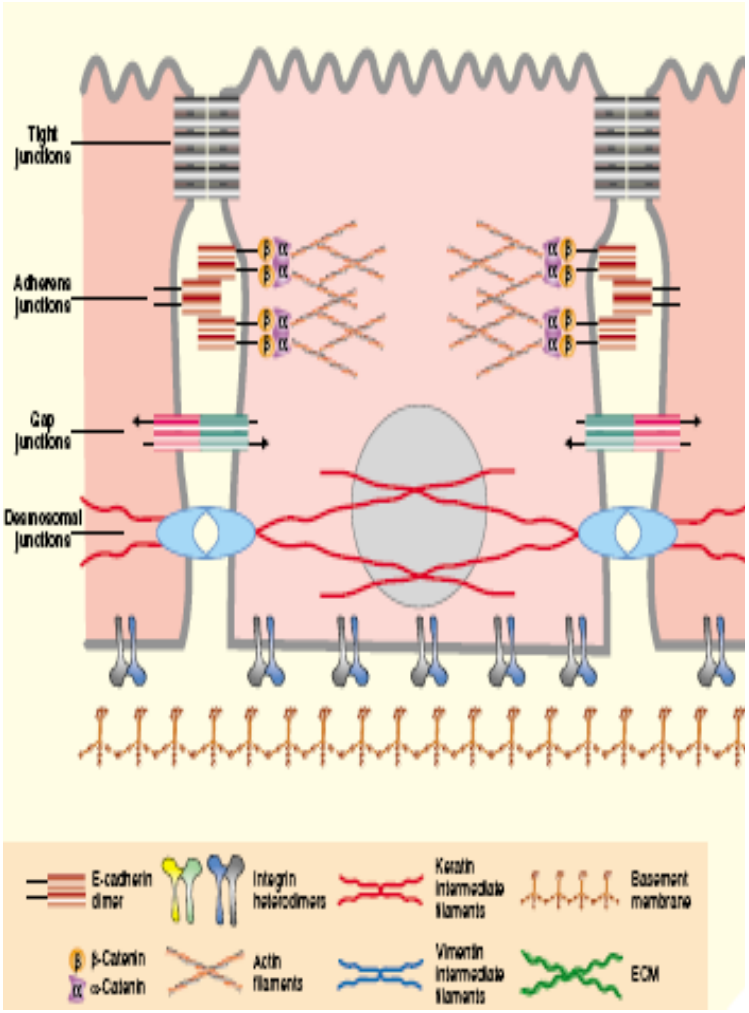
Specialized proteins – for adhesion, and conveying signals from growth factors and cytokines (e.g. fibronectin, and laminin)

Proteoglycans - complex proteins linked to long chains of repeating disaccharides (glycosaminoglycans) attached to backbone of Hylauronic acid

Integrins connect ECM to cytoskeleton.

Integrins play big role in ANCHORAGE DEPENDENCE!

Cell Adhesion in Normal Epithelial cells



E-cadherins have major role in connecting epithelial cells together

The C-terminal region of E-cadherin interacts with Catenins.

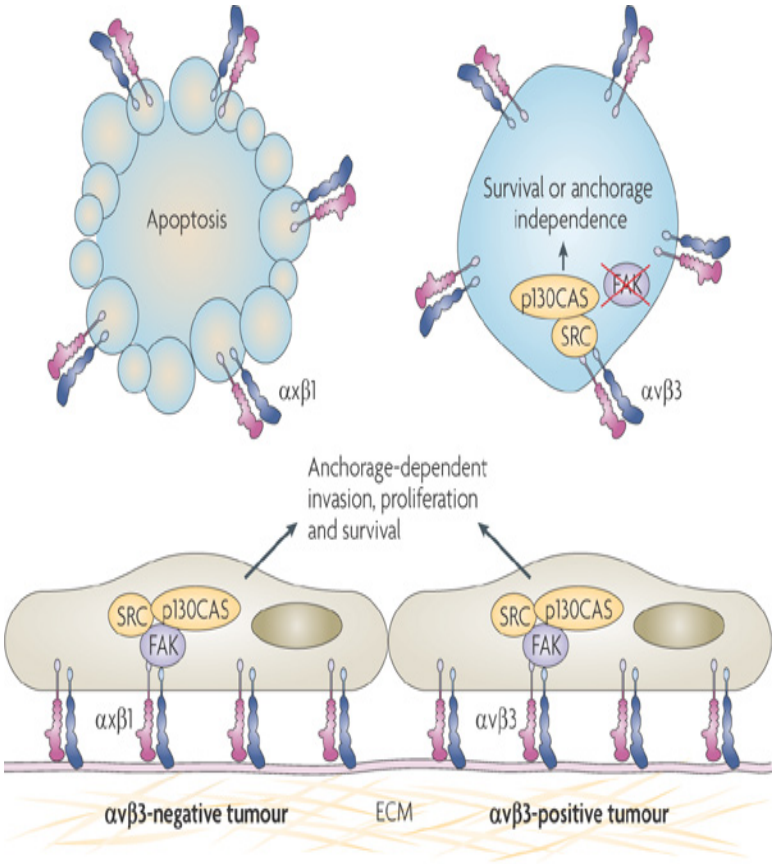
Catenins connect the cadherins to the actin cytoskeleton.

Integrins play a role in

1. **Transmitting mechanical stimuli from ECM to the cytoskeleton.**
2. **Anchorage of cell**
3. **Regulating cell Motility**
4. **Activation of Ras-MAPK pathways**

Certain Integrins help Anchorage Independent Tumor cells Survive

Nature Reviews Cancer 10, 9-22 (January 2010)



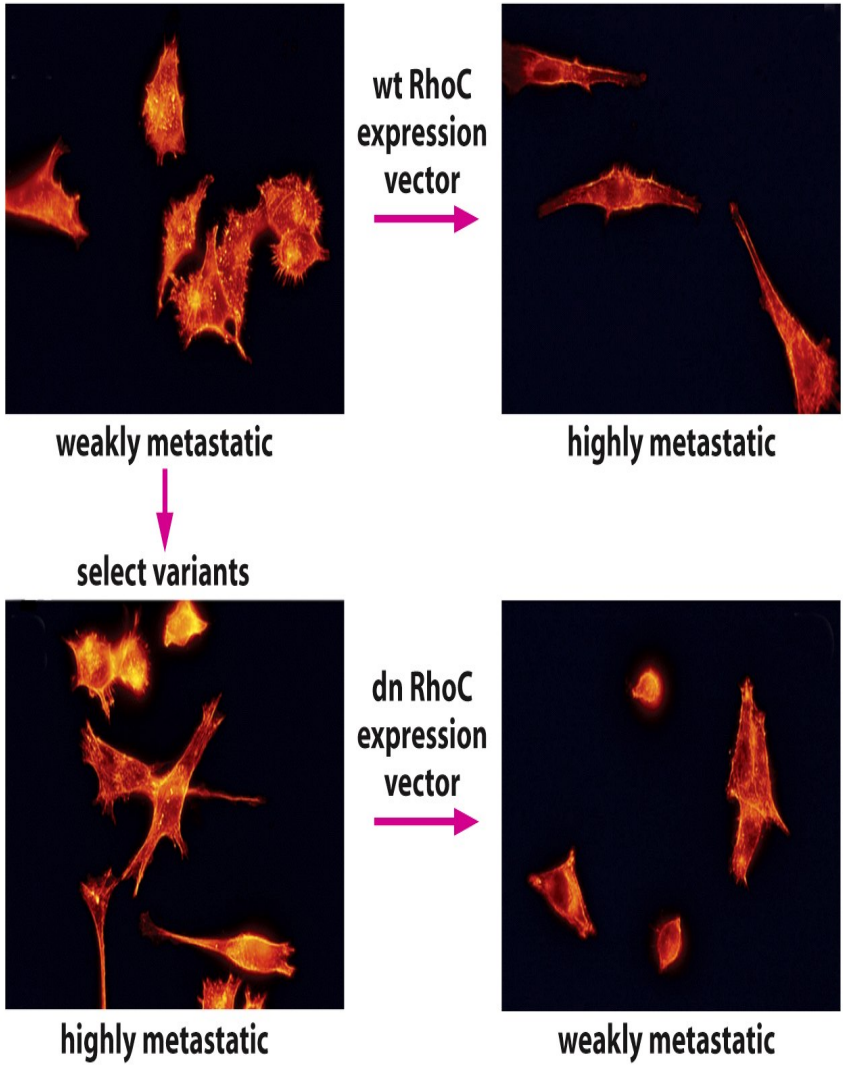
Left: When it looses anchorage, tumor cell lacking Integrin αvβ3 expression dies.

Right: When it looses anchorage, the tumor cells expressing Integrin αvβ3 can survive!

This happens because Integrin αvβ3 can signal via Src-CAS pathway…and turn on cell cycle.

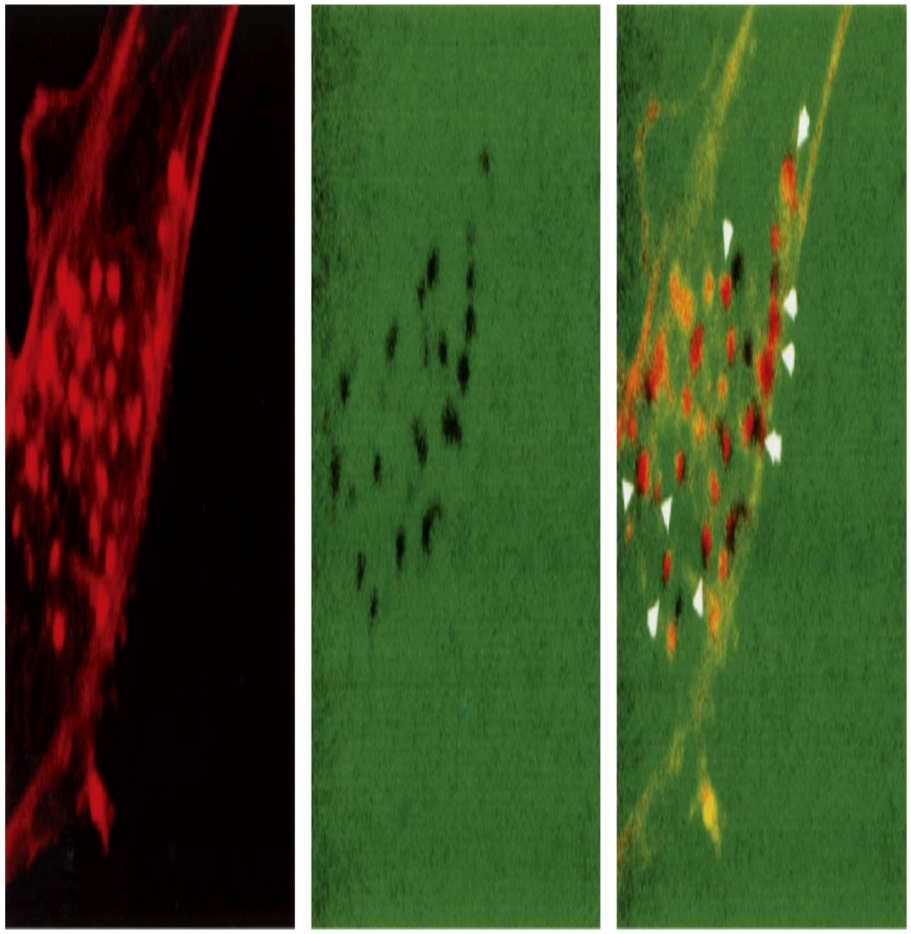
This happens even though FAK is inactive!

Rho C protein can make Epithelial cells more Fibroblastic and Metastatic



- **Top Left:** Weakly metastatic melanoma cells appear epithelial
- **Top Right:** Overexpression of RhoC in these cells triggers a fibroblastic form which is highly metastatic
- **Bottom left:** Highly metastatic melanoma cells appear fibroblastic
- **Bottom Right:** Overexpression of a Dominant Negative RhoC in highly metastatic cells has 2 effects….
- Reduces the fibroblastic form
- Suppresses metastatic ability!

Src Transformed Fibroblasts are Invasive (destroy ECM)



- **Left:** Clusters of Actin fibers stained RED
- **Middle:** Cells are growing on an ECM protein (fibronectin) which is tagged to GREEN dye.
- Holes indicate that cells degraded the fibronectin in the ECM by secreting proteases like MMPs
- **Right:** White arrows show merging of the ECM holes with the actin clusters….

Epithelial-mesenchymal transition (EMT)

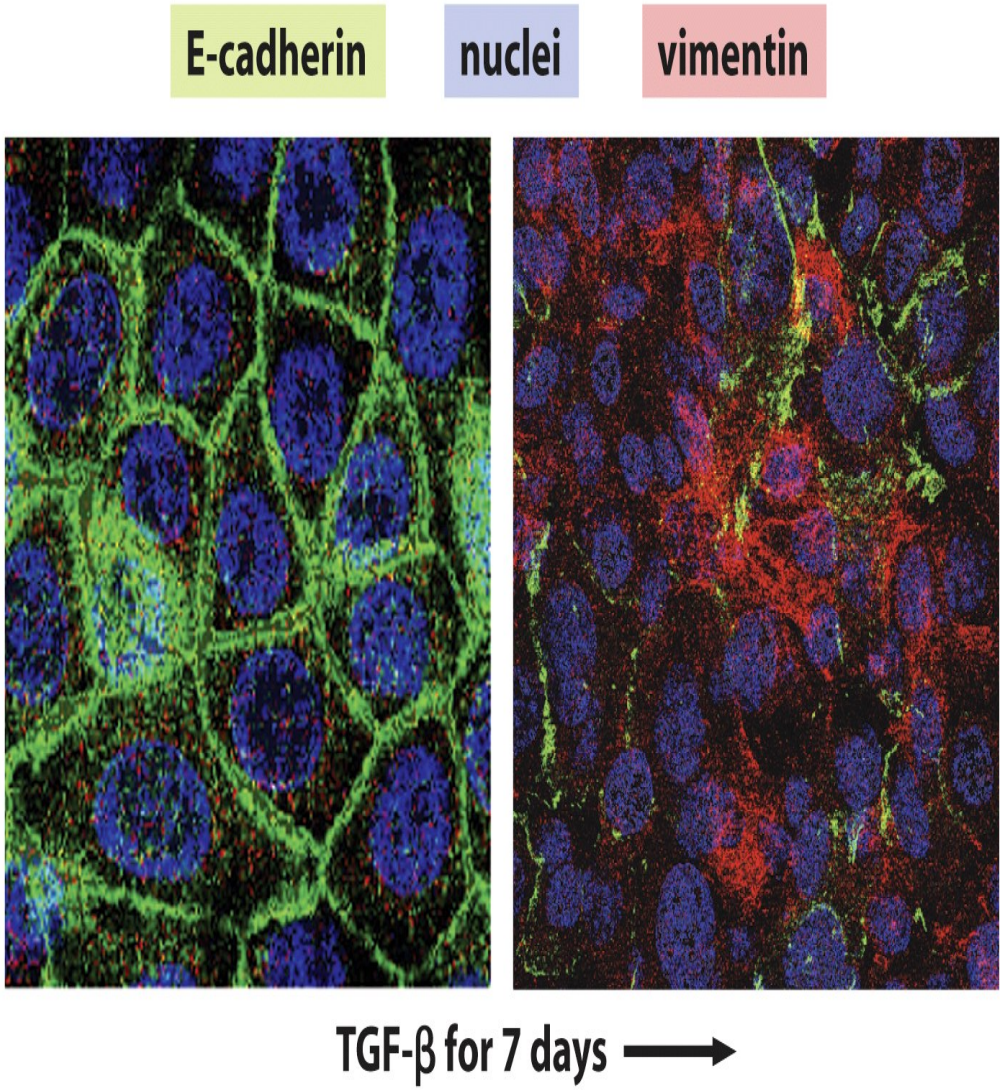
Most Metastases originate from Epithelial tumors !!!

- ***So-we need to better understand Epithelial cells!***

- ***Epithelial-mesenchymal transition (EMT)*** *is a genetically regulated process by which* ***epithelial cells convert into*** *a more fibroblastic or* ***mesenchymal form***

- ***When the Epithelial tumor cell becomes 'mesenchymal' it moves and invades the stroma…this property is essential for Metastases***

TGF-beta is a strong inducer of EMT

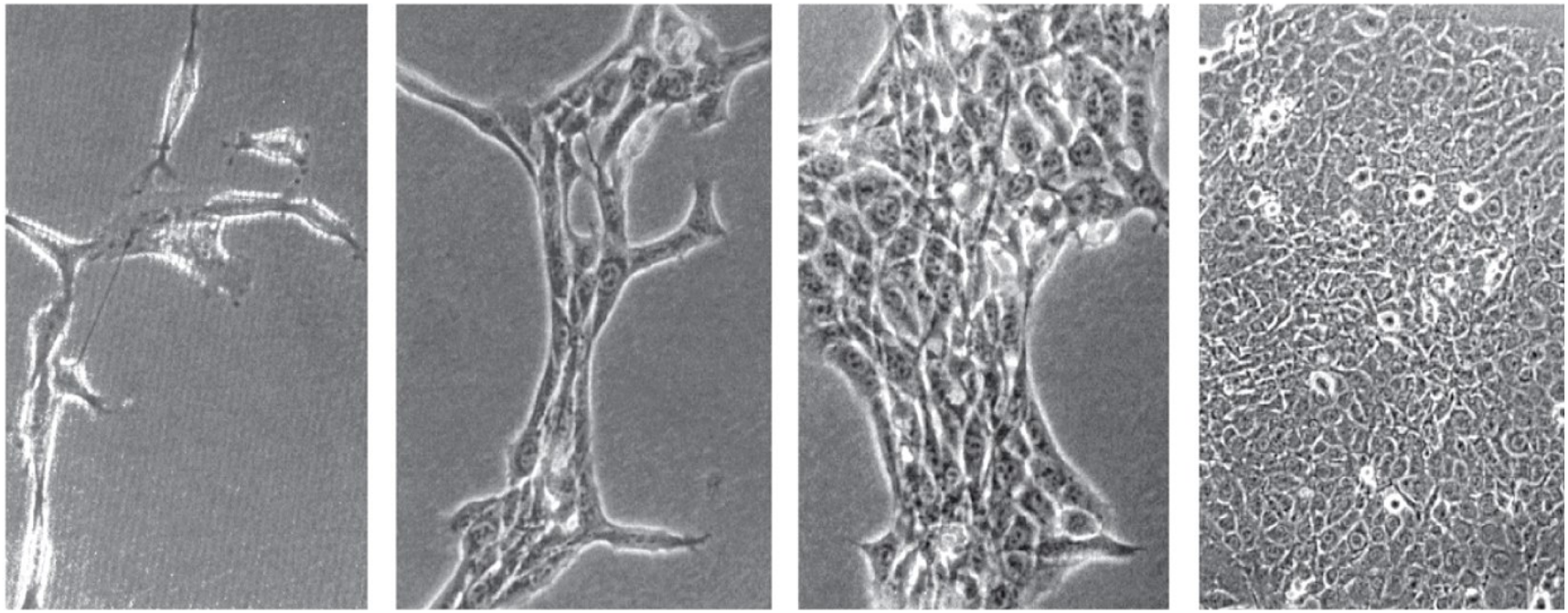


Mammary epithelial tumor cells (Ras transformed) show high expression of E-cadherin (GREEN)

TGF-beta treatment is sufficient to induce EMT….!

Vimentin (RED) is a cytoskeletal marker for mesenchymal cells !

EMT Induced by TGF-beta is Reversible!



remove TGF-β + 3 days + 5 days + 10 days

Reversibility of EMT explains a few mysteries!

Most metastatic cells isolated from tumors have epithelial morphology and behaviour…If EMT occurred…. Why don't they appear fibroblastic?

Histo-pathologists never see EMT in sections of cancer tissue!

WHY?????????

Answer to above queries:

EMT is not detectable in cancer tissues because it is transient..It mainly happens just before tumor cells move from primary to secondary site!

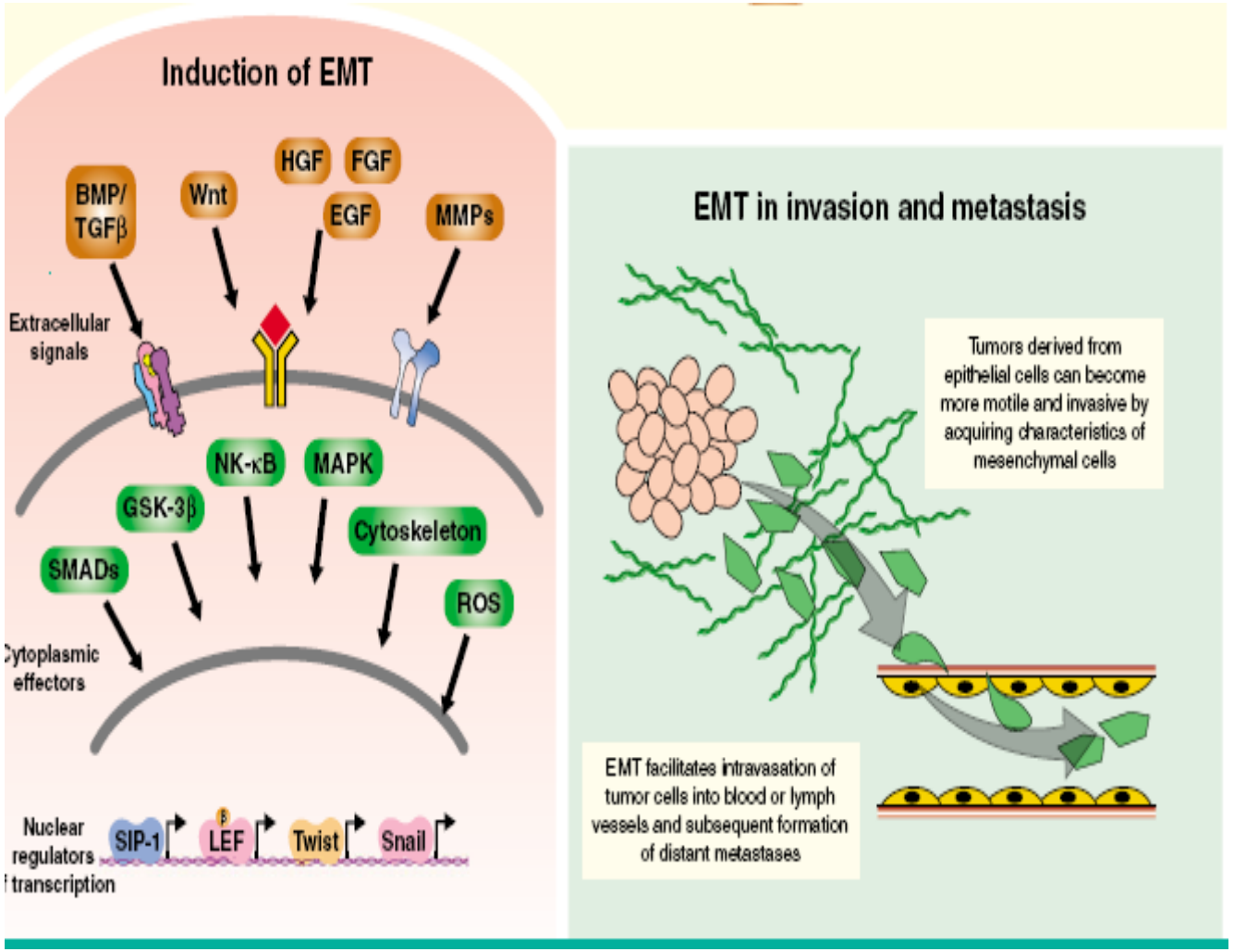
EMT may be reversed after tumor cells metastasize (colonize and adapt to new site).

Still…..controversy exists over whether or not EMT actually takes place in vivo, and whether EMT is really required for metastases of solid tumors (of epithelial origin) !!

Table 14.2 Cellular changes associated with the epithelial–mesenchymal transition

Loss of
Cytokeratin (intermediate filament) expression
Epithelial adherens junction protein (E-cadherin)
Epithelial cell polarity
Acquisition of
Fibroblast-like shape
Motility
Invasiveness
Mesenchymal gene expression program
Mesenchymal adherens junction protein (N-cadherin)
Protease secretion (MMP-2, MMP-9)
Vimentin (intermediate filament) expression
Fibronectin secretion
PDGF receptor expression
$\alpha v\beta 6$ integrin expression

EMT by D.C. Radisky J. Cell Sci. p.4325-4326, 2005



Previous slide: Regulation of EMT

- Several oncogenic pathways (peptide growth factors, Src, Ras, Ets, integrin, Wnt/beta-catenin and Notch) may induce EMT.

- In particular, Ras-MAPK has been shown to activate two related transcription factors known as Snail and Slug.

- Snail and Slug are transcriptional repressors of E-cadherin.
- Expression of Snail and Slug can induce EMT !

- Twist, another transcription factor, may induce EMT, and is also implicated in the regulation of metastasis.

- EMT MAY BE INDUCED BY CERTAIN PROTEINS IN STROMA (type I collagen, mediated by integrin α1β2.)

Metastasis requires active Stroma

Tumour stroma (connective tissue surrounding the tumor) **is characterized by the presence of specific cells and proteins which get 'Activated'.**

- **The Activated tumour Stroma can promote Metastasis of the tumor**

  - ***What does the Activated Tumour Stroma contain??***

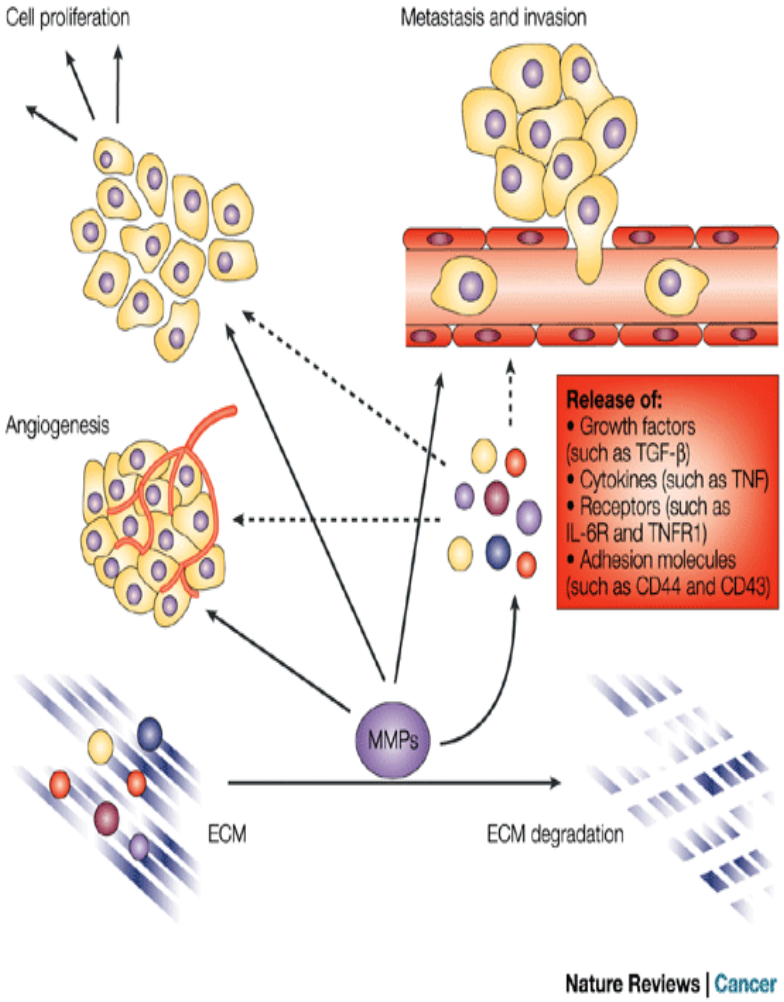
- **Proliferating endothelial cells expressing the receptor for vascular endothelial growth factor (VEGF)**

- **Fragments of extracellular-matrix (ECM) molecules**
- **Broken parts of BM (basement membrane).**
- **Active proteases (Matrix Metalloproteases-MMPs)**
- **Tumor Associated Macrophages (TAMs)**
- **Carcinoma Activated Fibroblasts (CAFs)**

TUMOR INVASION

Molecular mechanisms of glioma invasiveness: the role of Matrixmetalloproteases (MMPs)

Jasti S. Rao *Nature Reviews Cancer* **3**, 489-501 (July 2003)



MMPs can alter availability of Growth Factors:

- **MMPs can cleave insulin-like growth factor**

MMPs can activate the trans-membrane precursors of growth factors, (TGF-alpha)

MMPs can Promote Release of TGF-Beta

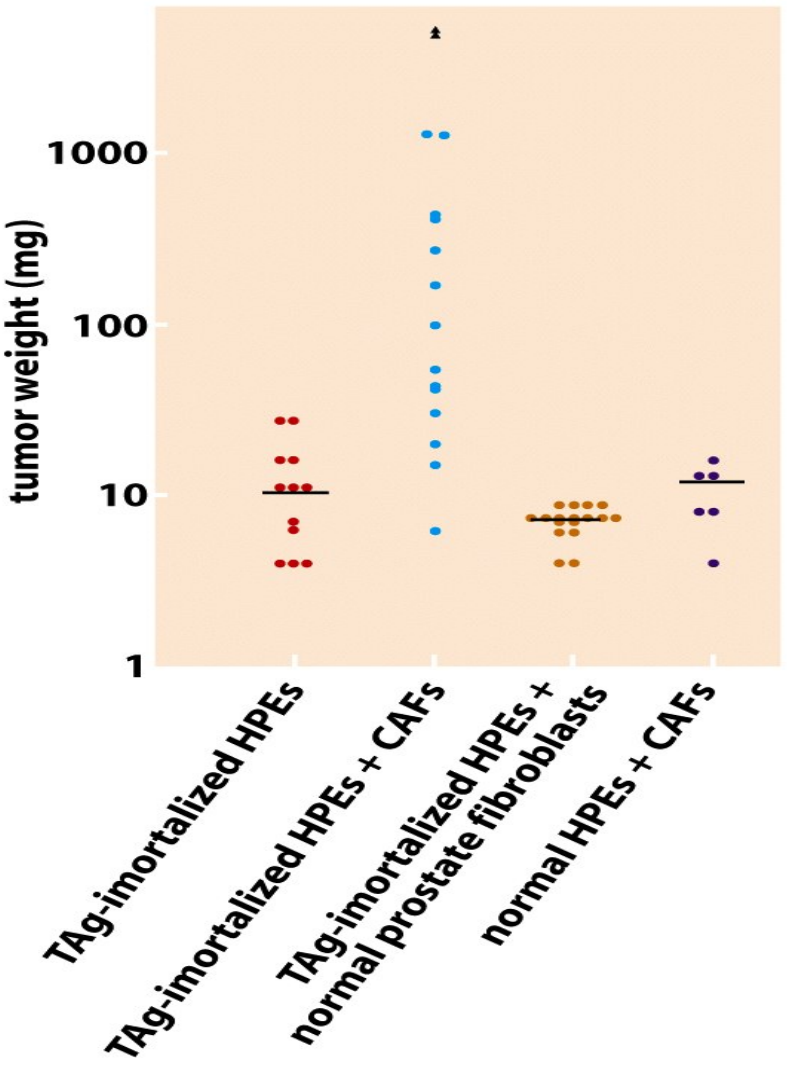
- **and Tumour necrosis factor alpha.**

MMPs Promote Angiogenesis by increasing the bioavailability of pro-angiogenic growth factors.

MMPs also regulate invasion and migration by degrading structural ECM components –

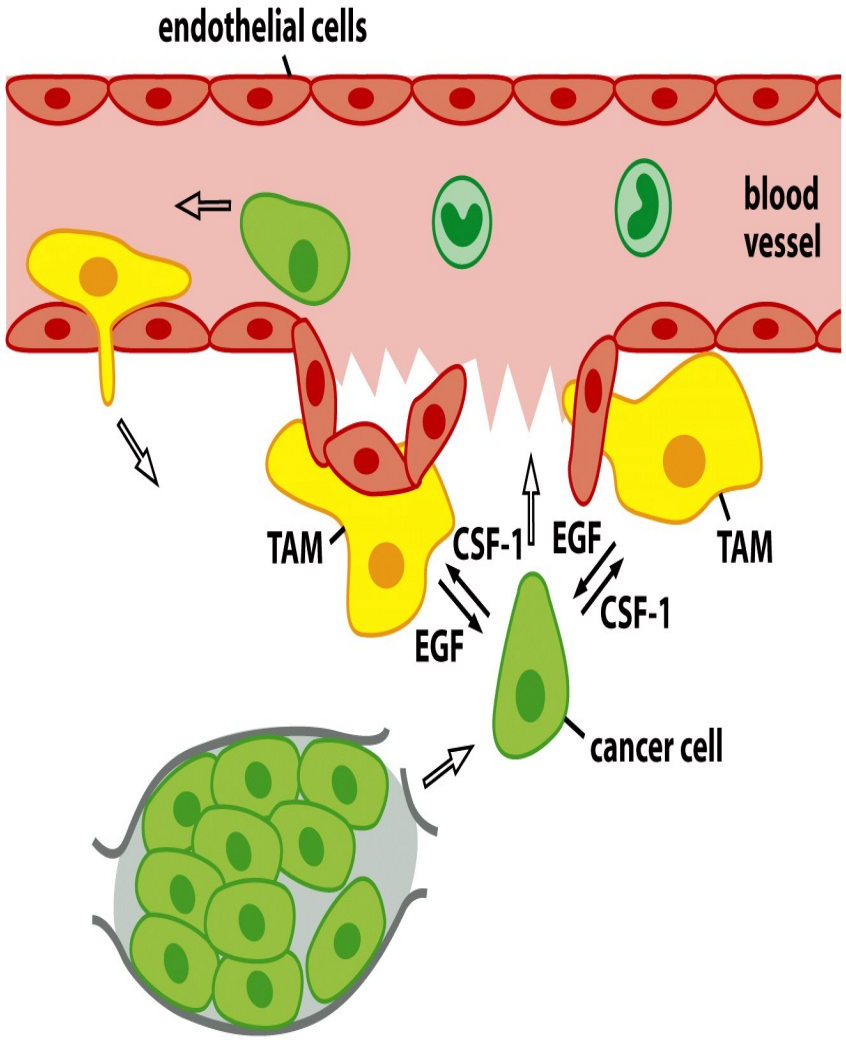
- **Laminin-5, Fibronectin, Aggrecan**

Fibroblasts from carcinoma stroma (CAFs) promote tumors!



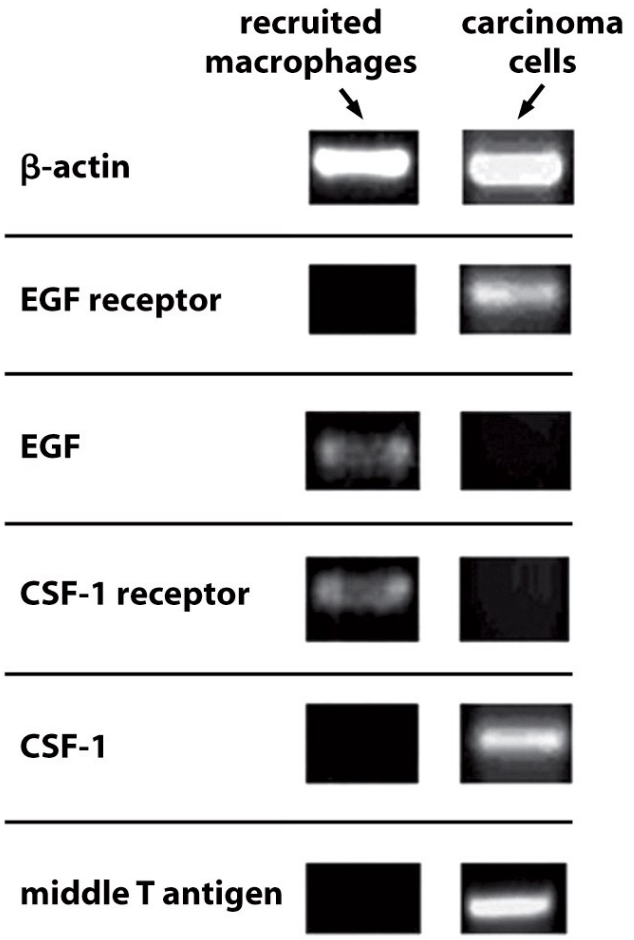
- Tag-HPE = normal human prostate epitheium immortalized with T-Antigen . These cells form small tumors in mice.
- Tag-HPE + carcinoma associated fibroblasts (CAF) promote formation of very large tumors!
- Tag-HPE + normal prostate fibroblasts have little/no effect on tumor size
- Normal prostate epitheium +CAF do not form tumors.

Reciprocal interactions between TAMs and tumor cells



- In repsonse to EGF, Cancer cells secrete CSF-1 which acts on TAMs (which have CSF-1R)
- TAMs secrete EGF which stimulate tumor cells to proliferate and invade ECM !
- So..its a perfect Reciprocal interaction …..!!
- Cancer cell provides CSF for TAM, and
- TAM provides EGF for cancer cell!

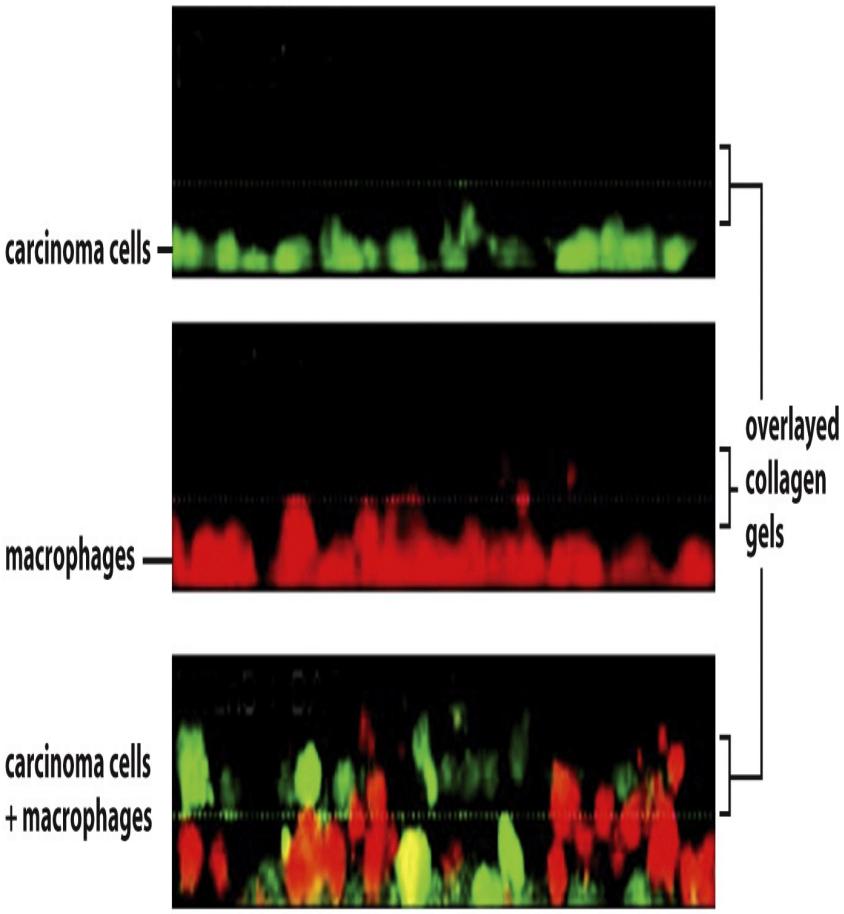
RT-PCR data proving paracrine loop between TAM and carcinoma cells



- CSF-1 production by Carcinoma cells
- EGF production by TAM/recruited macrophages

- Note:
- EGF-R expression in carcinoma cells only
- CSF1-R expression in TAM/recruited macrophages only

Macrophages can stimulate motility and invasiveness of Breast Cancer cells



- **Top:** Breast cancer cells (GFP tagged) at bottom of dish. Overlay of collagen gel
- **Middle:** Macrophages at bottom of dish. Overlay of collagen gel
- **Bottom: Co-culture of both cell types at bottom of dish……**
- **….Now, the Breast cancer cells start invading the Overlay of collagen gel !**

Quote from Weinberg's 2011 Hallmarks of Cancer pdf

- "It is increasingly apparent that crosstalk between cancer cells and cells of the neoplastic stroma is involved in the acquired capability for invasive growth and metastasis .

- Their concerted interactions facilitate intra-vasation into the circulatory system and metastatic dissemination of the cancer cells "

Is there a 'Gene signature for Metastasis' ??

The data are varied…..

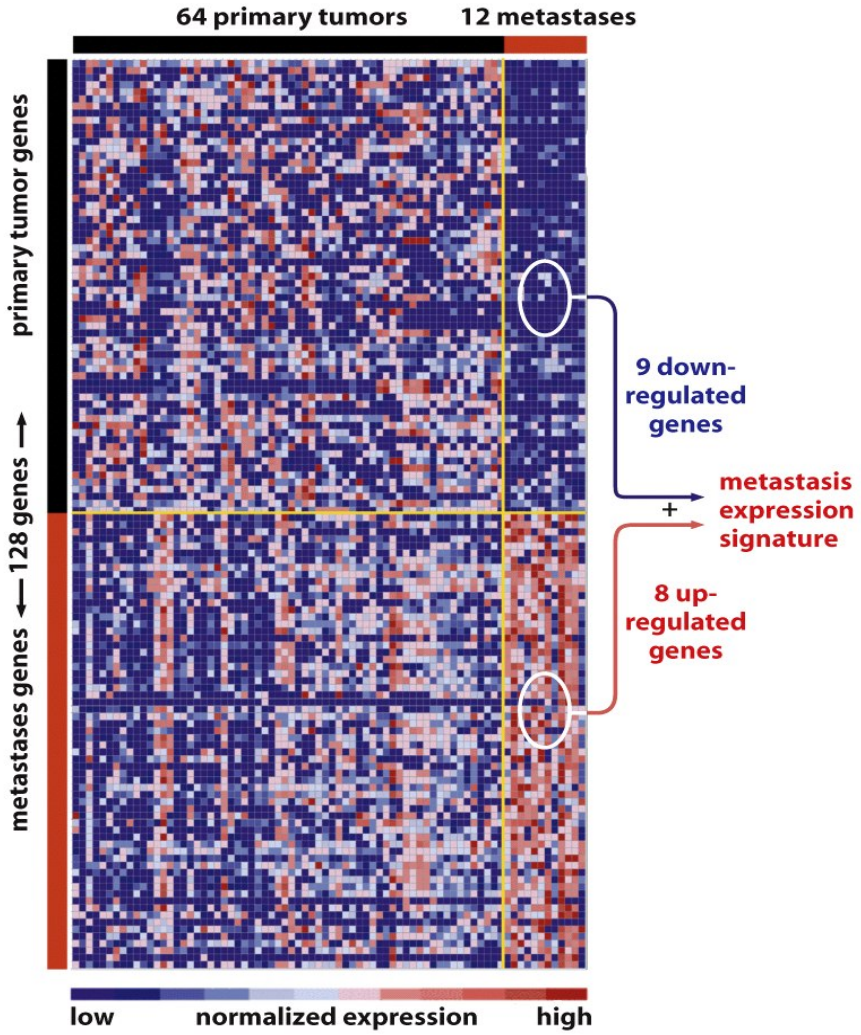
Some primary tumors may have a small subset of cells which express genes that specifically favour metastasis!

So, these genes (which give signature for metastasis!) would be enriched in circulating tumor cells and early micro-metastasis.

Other studies suggest that all primary tumor cells carry such specific 'metastasis genes' ! There is no special subset of cells in primary tumor which expresses these genes!

Answer: Maybe…different tumor types have different ways of metastasizing!

Use of Microarrays to identify a set of metastasis associated genes---"gene signature for metastasis" ??



- After analyzing gene expression from
- 64 primary tumors and
- 12 metastases……

- A set of 17 genes strongly associated with metastases

- 9 Down-regulated genes
- 8 Up-regulated genes

- ***Genetics*** **33, 49 - 54 (2002)** Sridhar Ramaswamy et al.

Different views on Gene Signatures for Metastasis-1

A molecular signature of metastasis in primary solid tumors

***Genetics* 33, 49 - 54 (2002)** Sridhar Ramaswamy et al.

We found a gene-expression signature that distinguished primary from metastatic adenocarcinomas .

These results suggest that the metastatic potential of human tumors is encoded in the bulk of a primary tumor, thus challenging the notion that metastases arise from rare cells within a primary tumor that have the ability to metastasize !

Different Views on Gene signatures for Metastasis-2

A five-gene signature as a potential predictor of metastasis and survival in colorectal cancer (CRC). J Pathol. 2010 Mar;220(4):475-89. Hao JM, Chen JZ, et al.

Individual expression of LYN, MAP4K4, SDCBP, and MID1, as well as the combined five-gene signature, was significantly correlated with overall survival in CRC patients.

Thus, our five-gene signature may be able to predict metastasis and survival of CRC in the clinic, and opens new perspectives on the biology of CRC.

Genes associated with increased Metastasis

- TGF-beta (promotes EMT in vitro)
- Transcription factors: Snail, Slug, and Twist (favour EMT)
- MMPs and uPA (urokinase-type plasminogen activator)
- Angiogenic growth factors

Why bother about Hypoxia?

Hypoxia increases tumor glycolysis, angiogenesis, and other survival responses of the tumor.

Hypoxia works by activating transcription of specific target genes. This is done by hypoxia-inducible factors (HIF)

Hypoxia inhibits tumor cell proliferation and induces cell cycle arrest. This can result in resistance to chemo and radiation therapy…(remember: Anticancer drugs mainly target rapidly proliferating cells!)

HIFs are expressed in Circulating Tumor Cells, and studies suggest that each step of the metastasis maybe regulated by hypoxia

Hypoxia and Metastasis

- **Hypoxia and EMT:**

Hypoxia increased the expression of Slug and Snail, and decreased the expression of E-cadherin, (hallmark of EMT).

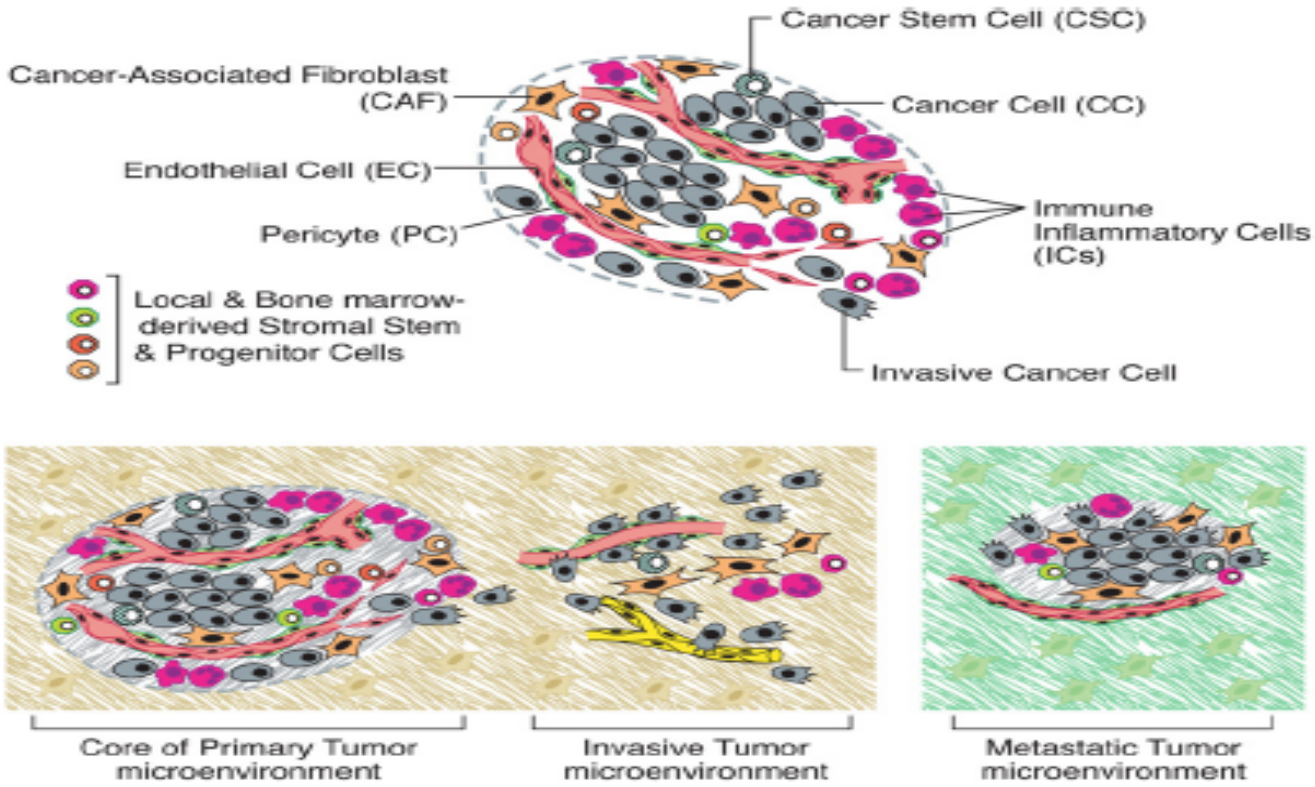
- **Hypoxia and Organ-specific metastasis:**

Chemokine SDF1alpha and its receptor CXCR4 have been implicated in organ-specific metastasis of many cancers (e.g. Breast cancer).

Up-regulation of CXCR4 (by hypoxia) is associated with increased migration and invasive potential of these cells. This effect can be abrogated by CXCR4 inhibition.

- **Hypoxia and CAF's:** **Hypoxia can modulate the differentiation and activity of Carcinoma activated Fibrobalsts (CAFs).**

Tumors contain distinct cell types that collectively enable tumor growth and progression.



Cancer Stem Cells (CSCs)

Hanahan and Weinberg: Hallmarks of Cancer -2011

- **CSCs may prove to be a common constituent of many if not most tumors**

- **CSCs are defined operationally through their ability to efficiently seed (produce) new tumors upon inoculation into recipient host mice**

  that means……

  CSCs have self-renewal capability that is crucial to their subsequent clonal expansion at sites of dissemination.

- **CSCs may represent a double-threat**, in that they are more resistant to therapeutic killing and, at the same time, endowed with the ability to regenerate a tumor once therapy has been halted.

- **Nevertheless, the importance of CSCs as a distinct phenotypic subclass of neoplastic cells remains a matter of debate!**

Summary -1

Metastasis : Basic cellular steps. Rate limiting steps

Organ specific Metastasis

Metastasis is inefficient and is often delayed!

Number of Affected Lymph Nodes indicate risk for Metastasis

Basics of epithelial cells and ECM : Role of cadherins, catenins, Integrins

Properties of Metastatic Cells: Figures showing importance of …….
Integrins, FAK, Src, Rho C in regulating these properties

BASIC CELLULAR STEPS OF Epithelial-Mesenchymal Transition (EMT)…WEINBERG'S TABLE

Summary -2

EMT …Continued:

- Role of TGF-β in the EMT process and Reversibility of EMT
- Regulation of EMT by Signalling pathways and Transcription factors

- Controversy : Is EMT really important as an in vivo mechanism for Metastasis ?

- ACTIVATED STROMA IN TUMORS HAS MANY COMPONENTS NEEDED FOR METASTASIS

- Role of MMPs, TAMs, CAFs, and CSCs in stroma… with Experimental proof

- Is there a 'gene signature for metastasis' ???

  Conflicting data! It depends on the type of tumor!

Summary -3

- **Why bother about Hypoxia?**
- **How Hypoxia affects several steps in the EMT process**