Positive and Negative Regulators of Metastasis

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TIME SCALE OF TUMOR PROGRESSION

CHEMOPREVENTION PERIOD

Rx

PRIMARY TUMOR

METASTASES

DIAGNOSIS

TUMOR SIZE

ANGIOGENESIS

INHERITED RISK

ACQUIRED RISK

DYSPLASIA

CA-IN-SITU

INVASION
The Metastatic Cascade
When does metastasis begin?

Commitment to the metastatic phenotype:
- How early does it occur?
- Can it be reversed?

Progenitor lesions:
- What are the key progenitor lesions?
- What is the efficiency of transition to invasion?
- Are all metastasis precursors clonal?
What is the role of the host?

- Under what conditions does the host drive or suppress the process?
- Does the transition from pre-invasive to invasive lesions require host participation?
- If so what are the molecular and cellular players that are functionally important?
- The circuitry of the tumor host communication may be the key to prevention of invasion.
Physiologic basis of metastasis

• Is metastasis a normal physiologic program which is disregulated or inappropriately activated?
• Does a physiologic motility and invasion program exist for development, angiogenesis morphogenesis and wound healing?
• Is metastasis colony formation a natural ongoing process conducted by stem cells?
What is the driving force?

- Is the metastatic phenotype pre determined within the primary tumor? Within the host microenvironment?
- Are malignant cells a product of adaptation and selection?
- What is the selection factor? If malignant cells are survival of the fittest, then what is the fitness test?
- Is cell survival in a foreign (non home) tissue the ultimate selection factor?
Pre-cellular theory of invasion and metastasis: recognition of malignant tumors and localized versus metastatic disease

**Hippocrates (460–375 B.C.)**

**Galen (131–201 A.D.)**

**Pre-1700:** The Greek physician Hippocrates coined “carcinoma” from *karkinos*, the word for crab.

**LeDran 1757:** Noted that malignant tumors begin as localized disease, then spread to regional lymph nodes and then enter the circulation to subsequently appear in the lung

**Bichat 1801:** Tumors contain both parenchyma and stroma

**Recamier 1829:** Used the term “Metastases”
Validation of the cellular theory of cancer metastasis

**Takahashi: (1915)**

- Spindle cell sarcoma in mouse blood vessel

**1900–1949:** Takahashi found that the cells of various mouse carcinomas and sarcomas produce reproducible patterns of metastases when injected into other mice.

**Tyzzer 1913:** Experimental Metastasis

**1950–1969:** Rygaard and Povlsen showed in 1969 that human tumors can grow in nude mice, which lack a thymus and are T cell deficient. This experimental animal model of human cancer continues to be refined and used today.

**Ziedman and Fidler 1970–80:** Intravenous metastasis models
The organ pattern of metastasis is characteristic of the tumor type and tissue of origin. 50-70% of the metastatic pattern can be predicted by the venous drainage blood flow. The remaining 30-50% may be caused by specific molecular homing mechanisms.

Potential molecular mechanisms:

a) Preferential adhesion in the vessels of the target organ
b) Selective extravasation
c) Organ attractants
d) Organ specific survival and growth
Chemokines regulate leukocyte recirculation and trafficking to sites of inflammation and infection.
Premise: Metastasis homing is dictated by relative abundance of chemokines and cognate receptors on the tumor cell.

Why do the tumor cells express the chemokine receptors in the primary tumor prior to dissemination?

Therapeutic utility is limited because dissemination has already occurred at the time of diagnosis.
Tumor necrosis is a bad prognostic indicator
Hypoxia induces angiogenesis and promotes invasion

Outgrowth of vascular supply  Selection of aggressive cells
Molecular Ecology of the Tumor-Host Microenvironment

Translational Applications

Pathogenic defects in signal pathways extend into the tumor host interface.

- Extracellular signalling networks are the therapeutic target
- Pro survival pathways may be a key selection factor within a given cellular context
As with the other stages of carcinogenesis, metastasis is genetically controlled with the involvement of both enhancing and suppressing modifiers.
## Metastasis Promoting Genes - I

<table>
<thead>
<tr>
<th>Gene</th>
<th>Tissue Site</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARM-1</td>
<td>Lymphoma</td>
<td>Promotes adhesion of tumor cells to the endothelium</td>
</tr>
<tr>
<td>ATX</td>
<td>Breast, Liver, Lung, Melanoma, Teratocarcinoma</td>
<td>Cytoskeletal reorganization and motility; G-protein coupled receptor activation</td>
</tr>
<tr>
<td>CD44</td>
<td>Multiple sites</td>
<td>Cell-cell interactions; activates HGF/c-Met pathway</td>
</tr>
<tr>
<td>Cox2</td>
<td>Breast, Colorectal, Gastric</td>
<td>Prostaglandin synthase; induces VEGF</td>
</tr>
<tr>
<td>Cyr61</td>
<td>Breast</td>
<td>Mediates adhesion; Erb-B2/3/4 pathway</td>
</tr>
<tr>
<td>Ezrin</td>
<td>Liver, Ovary, Pancreas, Prostate, Uterus</td>
<td>Membrane-cytoskeletal linker; RHO and RAC interactions</td>
</tr>
<tr>
<td>HMG-I(Y)</td>
<td>Breast, Cervical, Colorectal, Prostate, Skin, Thyroid, Uterus</td>
<td>Regulated by EGF and MMP-9</td>
</tr>
<tr>
<td>Laminin-5</td>
<td>Multiple sites</td>
<td>EGF and TGF-α induce expression of laminin subunits; cell adhesion, motility</td>
</tr>
<tr>
<td>c-Met</td>
<td>Multiple sites</td>
<td>Activated by HGF; Modulates Ras and PI3 kinase</td>
</tr>
<tr>
<td>Gene</td>
<td>Tissue Site</td>
<td>Function</td>
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<tr>
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<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>MTA1</strong></td>
<td>Breast, Cervix, Melanoma, Ovary</td>
<td>Neucleosome remodeling; histone deacetylase complex</td>
</tr>
<tr>
<td><strong>Oncostatin M</strong></td>
<td>Lung</td>
<td>Activates PKA-dependent pathway</td>
</tr>
<tr>
<td><strong>PP2A</strong></td>
<td>Not determined</td>
<td>Activated by p38/MAPK; inhibits MEK1, MEK2, and MMP-1</td>
</tr>
<tr>
<td><strong>RAGE</strong></td>
<td>Gastric, Lung, Pancreatic, Renal</td>
<td>transmembrane receptor; activates p21, MAPKs, NF-6B, cdc42/rac</td>
</tr>
<tr>
<td><strong>S100A4</strong></td>
<td>Breast, Colorectal, Prostate</td>
<td>Calcium-binding protein; activates c-erbB-2</td>
</tr>
<tr>
<td><strong>S100A9</strong></td>
<td>Colon, Gastric, Skin</td>
<td>Calcium-binding protein; Modulates Mac-1 integrin receptor through G-protein</td>
</tr>
<tr>
<td><strong>Semaphorins</strong></td>
<td>Gastric, Leukemia, Lung, Skin</td>
<td>cell-cell interactions; Receptor crosstalk with c-Met binding semaphorin receptor, plexin</td>
</tr>
<tr>
<td><strong>Thymosin-β15</strong></td>
<td>Prostate</td>
<td>actin binding; motility</td>
</tr>
<tr>
<td><strong>Wnt-5a</strong></td>
<td>Breast, Colon, Lung, Melanoma, Pancreas, Prostate</td>
<td>PKC activation with associated changes in cytoskeleton, cell adhesion, and motility</td>
</tr>
</tbody>
</table>
Cellular Phenotypes Modulated by IGF1

- Growth
- Apoptosis
- Invasion
- Metastasis
- Angiogenesis
- Response to chemotherapy
IGF-1/IGF-R as Positive Regulators of Metastasis

- Mutant IGF-R (soluble receptor) blocks metastasis but not tumor growth of breast cancer cells (Dunn/Barrett).
- Serum IGF-1 levels influence metastasis of colon cells (Wu/LeRoith).
- IGF-R overexpression accelerates metastatic progression in RIP-Tag mice (Lopez/Hanahan).
Evidence for Genetic Influences on Metastatic Potential

- Metastasis formation (independent of tumor initiation and growth) in mice is dependent on the genetic background of the mouse and map to multiple loci (Kent Hunter, NCI).
- Hybrids between metastatic cells and non-metastatic cells are suppressed for metastasis independent of tumor forming ability.
- Specific genes can control metastasis independent of tumorigenesis.
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<tr>
<td>Annexin7</td>
<td>Prostate</td>
<td>calcium-dependent GTPase; substrate for PKC and other kinases associated with proliferation</td>
</tr>
<tr>
<td>BRMS1</td>
<td>Breast, Melanoma</td>
<td>gap-junctional communication</td>
</tr>
<tr>
<td>CC3</td>
<td>Colon, Lung</td>
<td>serine/threonine kinase</td>
</tr>
<tr>
<td>CEACAM1-4S</td>
<td>Breast, Colon</td>
<td>Bax pathway</td>
</tr>
<tr>
<td>CRSP3</td>
<td>Melanoma</td>
<td>transcriptional co-activator</td>
</tr>
<tr>
<td>DAP-kinase</td>
<td>Multiple sites</td>
<td>calcium/calmodulin-dependent serine/threonine kinase; pro-apoptotic pathway</td>
</tr>
<tr>
<td>E-cadherin</td>
<td>Multiple sites</td>
<td>Wnt signaling; cytoskeleton; cell-cell adhesion</td>
</tr>
<tr>
<td>HEPSIN</td>
<td>Ovarian, Prostate, Renal</td>
<td>transmembrane serine protease</td>
</tr>
<tr>
<td>HP1^{HSα}</td>
<td>Breast</td>
<td>non-histone heterochromatin-associated protein</td>
</tr>
<tr>
<td>KAI-1</td>
<td>Breast, Prostate</td>
<td>Transmembrane tetraspondin; role in adhesion, motility, growth regulation, and differentiation; integrin interaction</td>
</tr>
<tr>
<td>KiSS1</td>
<td>Breast, Melanoma</td>
<td>Modulates Rho, Rac, and MAPK signaling</td>
</tr>
<tr>
<td>Maspin</td>
<td>Breast, Colon, Oral Squamous Cell, Prostate</td>
<td>Serine protease inhibitor; binds collagen and can modulate integrins</td>
</tr>
<tr>
<td>Melastatin</td>
<td>Melanoma</td>
<td>Calcium channel protein</td>
</tr>
<tr>
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</tr>
<tr>
<td>MKK4</td>
<td>Ovary, Prostate</td>
<td>MAPK; phosphorylates and activates p38 and JNK kinases</td>
</tr>
<tr>
<td>NESH</td>
<td>Lung, Prostate</td>
<td>src homology 3 adapter protein; down regulates p21 pathway</td>
</tr>
<tr>
<td>NM23-H1</td>
<td>Breast, Colon, Melanoma, Oral Squamous Cell</td>
<td>histidine kinase; phosphorylates KSR, which might reduce ERK 1/2 activation</td>
</tr>
<tr>
<td>PTEN</td>
<td>Multiple sites</td>
<td>phosphatase; growth regulation, cell motility</td>
</tr>
<tr>
<td>RhoGD12</td>
<td>Bladder</td>
<td>Inhibits GTP binding; regulates RHO and RAC</td>
</tr>
<tr>
<td>SFRP1</td>
<td>Breast, Colorectal</td>
<td>Modulates Wnt signaling pathway</td>
</tr>
<tr>
<td>SHPS-1</td>
<td>Breast, Leukemia</td>
<td>glycoprotein; may regulate RAS-MAPK signaling; suppresses anchorage independent growth</td>
</tr>
<tr>
<td>Syk</td>
<td>Breast, Colon, Pancreas, Skin</td>
<td>Tyrosine kinase; inhibits PI3 kinase; necessary for MAPK activation</td>
</tr>
<tr>
<td>TSP-1</td>
<td>Multiple sites</td>
<td>inhibits endothelial cell proliferation and migration; c-Myc expression inhibits TSP-1</td>
</tr>
<tr>
<td>tropomyosins</td>
<td>Breast</td>
<td>interacts with e-cadherin/catenin complex</td>
</tr>
<tr>
<td>VDUP1</td>
<td>Melanoma</td>
<td>Thioredoxin inhibitor; upregulates KiSS1; interacts with CRSPs</td>
</tr>
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Lessons Learned from Studies of Genes Involved in Metastasis

- Both positive and negative regulation of metastasis are involved in cancers.
- Metastasis suppressor genes can be lost early in the development of cancers.
- Multiple mechanisms are involved in metastasis.
- Interactions and possible pathways of proteins involved in metastasis are observed.
- Negative regulators of metastasis often exhibit epigenetic silencing rather than mutations in cancers.
- Negative regulators of metastasis exhibit plasitcity of expression and function.
Prostatic Metastasis Suppressor Gene

KAI-1

抗癌 Kang-ai ---- Anti Cancer
KAI1 / CD 82

Names: KAI1 / CD82, (C33, R2, IA4)
Gender: Transmembrane Glycoprotein
Ligands?: Signal Pathways?:

Biological Function:
\- motility
\- invasiveness
\- cell-cell interactions

Particularity

\- Member of the tetraspanin or transmembrane 4 superfamily (TM4SF)
\- Contains an internalization sequence at its C-terminus (YSKV)

Current Address:

\- Cell membrane (lymphocytes, epithelial cells)
\- Lysosomes
\- Vesicles
High level of KAI1/CD82 is a good prognosis factor or associated with low grade histology:

- prostate
- lung
- pancreas
- carcinoma
- colon

KAI1/CD82 expression is inversely related to the metastatic potential:

- prostate
- lung carcinoma
- colon
- hepatoma
- breast
- lung (non-small-cell carcinoma)
- bladder cancer
- ovary
- melanoma

Transfection of tetraspanin reduces metastatic potential:

- melanoma
  - B16
  - MDA-MB-435 *
- prostate
  - AT6.1, AT6.3
- breast
  - MDA-MB-231

(from Boucheix & Rubinstein, 2001)
Loss of KAI-1 Expression in Prostate Cancer
Figure 6. Immunohistochemistry of KAI1 in Prostatic Intraepithelial Neoplasia (PIN)

→ normal appearing epithelial cells, KAI1 +

→ high-grade PIN, KAI1 -
Normal Colon and Tumor KAI1 Scores by Stage

<table>
<thead>
<tr>
<th>Stage</th>
<th>Normal</th>
<th>Tumor</th>
</tr>
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<tbody>
<tr>
<td>Stage I</td>
<td></td>
<td></td>
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<tr>
<td>Stage II</td>
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<tr>
<td>Stage III</td>
<td></td>
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<tr>
<td>Stage IV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metastasis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ (Promotes)</td>
<td>− (Inhibits)</td>
<td></td>
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<td>-------------</td>
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<td></td>
</tr>
<tr>
<td>Cell Aggregability</td>
<td>Invasion</td>
<td></td>
</tr>
<tr>
<td>Cell Adhesion</td>
<td>Motility</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Metastasis</td>
<td></td>
</tr>
</tbody>
</table>
The Key Question:

How does KAI1 exert its effect on the ability of cancer cells to invade?
KAI-1 as a Molecular Facilitator
Association of KAI1 / CD82 with other cell surface molecules

**TM4SF members** \{ the tetraspanin web \}

**β integrins**

\[ \alpha_3\beta_1, \quad \alpha_6\beta_1, \quad \alpha_4\beta_1, \quad \alpha_1\beta_2 \]

**Molecules of the immune system**

HLA-DR, MHC class I, CD4, CD8, CD19, CD46,

**Others**

CD9P-1, ProHB-EGF, γ-glutamyl transpeptidase,

EGF-R

DARC

E-Cadherin
Confocal microscopy

MCF-7

KAI1

E-Cadherin

merge

MCF-7 - Kai1-9

objective x100
**EGF-R**

**Names:** EGF-R (erb-1)

**Gender:** Receptor Tyrosine Kinase (RTK)

**Ligands:** EGF, TGFβ

**Signal Pathways:** MAPK, PI3K

**Biological Function:**
- Morphogenesis
- Growth regulation

**Oncogenic effects:**
- Initiation of DNA synthesis
- Enhanced cell growth
- Invasion
- Metastasis

**Current Address:**
- Cell membrane (membrane microdomains)
- Vesicles
- Nucleus (?)
Why KAI-1 and EGF-R pathways?

Attenuation of EGF receptor signaling by a metastasis suppressor, the tetraspanin CD82/KAI1

Facilitation of ligand-induced endocytosis of the EGF-R and its subsequent desensitization by CD82/KAI1

Opposite effects of KAI1 and EGF-R pathway

Selective enrichment of Tetraspan proteins on the internal vesicles of multivesicular endosomes and on exosomes secreted by human B- lymphocytes

“Co-localisation” in endosomes of KAI1 and EGF-R
Possible Mechanisms for Loss of KAI-1 Function

- Mutation – never observed
- Down regulation of mRNA – common in many cancers
- DNA methylation of promoter – not observed
- Posttranslational modification – glycosylation differences observed in some tumors
- Loss of function in KAI-1 partners or downstream effectors – not fully tested
Regulators of KAI-1 expression in cancer cells

- 5-AzadC
- Phorbol esters
- Nerve growth factor
- TNF/NFkB
A Metastasis Suppression Pathway

DRIP130

Other Genes

VDUP1

TRX

Transcription

KISS1

GPR54

ASK1/MAPKKK5

Motility

Invasion

Other Genes

Metastasis
Metastasis Suppressor Pathways

- **β-Catenin**
- **RhoGDI2**
- **Ras**
- **KISS1**
- **NFκB**
- **HIF**
- **TXNIP**
- **BAD**
- **PTEN**
- **AKT**
- **PI3K**
- **PLC**
- **KSR**
- **RAF**
- **MEK**
- **ERK**
- **NRG1**
- **GPR54**
- **Rho**
- **Rac**

Pathways:
- **Apoptosis**
- **Inflammation**
- **Differentiation**
- **Survival**
- **Angiogenesis**
- **Motility**
- **Invasion**
- **Growth**
- **Differentiation**
Lessons Learned from Studies of Genes Involved in Metastasis

• Both positive and negative regulation of metastasis are involved in cancers
• Multiple mechanisms are involved in metastasis
• Interactions and possible pathways of genes involved in metastasis are observed.
• Negative regulators of metastasis often exhibit epigenetic silencing in cancers.
• Negative regulators of metastasis exhibit plasticity of expression and function
Hard Clinical Truths About Metastasis

1. Upwards of 70% of patients may have overt or occult metastases at diagnosis.

2. Acquisition of the invasive and metastatic phenotype is an early event in cancer progression.

3. Millions of tumor cells are shed daily into the circulation.

4. Angiogenesis is a ubiquitous and early event that is necessary for and promotes metastatic dissemination.
Lucky Clinical Truths About Metastasis

1. Both malignant invasion and angiogenesis use the same “hardware” and “software” programs.

2. Less than 0.01% of circulating tumor cells successfully initiate a metastatic focus.

3. Circulating tumor cells can be detected in patients who do not develop overt metastatic disease.

4. Metastases may be as susceptible to anti-cancer therapy as primary tumors.