Biology of metastasis

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Treatments for primary breast cancer

Detection and removal, dispersion & seeding of primary tumor

89% survival rate for localized breast cancers
Advances in breast cancer treatment

- Better detection and imaging
- New surgical options
- Targeted and immunotherapy

Detection and removal of primary tumor
Dispersion & seeding of primary tumor
Metastasis is responsible for 90% of cancer deaths

Overt metastasis

Latency (months to decades)
At a Glance

Estimated New Cases in 2014: 232,670
% of All New Cancer Cases: 14.0%
Estimated Deaths in 2014: 40,000
% of All Cancer Deaths: 6.8%

New Cases

Deaths

Year


Percent Surviving 5 Years: 89.2%
2004–2010

5-Year Relative Survival

Percent

Localized 98.5%
Regional 84.6%
Distant 25.0%
Unstaged 49.8%

Small cell lung cancer

Poor prognosis for almost all metastatic cancers

Reference: Nakazawa K et al., Oncol. Lett. 2011
How are primary tumors and metastasis different?

Metastasis are more difficult to treat and are resistant to most drugs.

Cell-autonomous mechanisms: Genetic, epigenetic differences?

Non cell-autonomous mechanisms: Stromal influences
Breast Cancer Genes

Mutations are highly concordant between primary tumor and metastasis
Ref: Colon cancer, Brannon et al., Genome Biology, 2014
Breast cancer subtypes

Luminal A slow growth and **best** prognosis

Luminal ca : ER positive
Express luminal markers (ER alpha, cytokeratins 8/18)

Basal like triple negative cancers: **worst** prognosis
Express basal cell markers (CK 5/6, 14, 17)

Basement membrane

Basal or Myoepithelial cells

Luminal epithelial cells

HER2 positive breast cancer

Modified from Hongkong Breast Cancer Foundation
Signaling downstream of HER family

http://www.nature.com/nrclinonc/journal/v9/n1/fig_tab/nrclinonc.2011.177_F1.html
### Table 2. Examples of mutation-matched therapies for breast cancer

<table>
<thead>
<tr>
<th>Altered genes with predictive biomarker potential</th>
<th>Treatment approach</th>
<th>Strength of hypothesis for somatic alteration-targeted drug match (reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HER2 amplification</td>
<td>HER2-directed antibodies and HER2 kinase inhibitors</td>
<td>1</td>
</tr>
<tr>
<td>PIK3CA mutation</td>
<td>PIK3CA-selective inhibitors</td>
<td>2</td>
</tr>
<tr>
<td>FGFR1 amplification, FGFR3 amplification, other FGF ligands and receptors, and rare receptor mutations</td>
<td>FGFR small-molecule inhibitors and antibodies</td>
<td>2</td>
</tr>
<tr>
<td>Inherited and somatic BRCA1 and BRCA2 mutation</td>
<td>PARP inhibitors</td>
<td>2</td>
</tr>
<tr>
<td>Cyclin D1/CDK4/CDK6 amplification or deletion of CDKN1B, CDKN2A, and CDKN2B</td>
<td>CDK4/6 inhibitors</td>
<td>2</td>
</tr>
<tr>
<td>AKT1-3 gain-of-function mutation/gene fusion via translocation/amplification</td>
<td>AKT inhibitors</td>
<td>3</td>
</tr>
<tr>
<td>GATA3 mutation</td>
<td>Aromatase inhibition</td>
<td>3</td>
</tr>
<tr>
<td>PTEN/INPP4B loss-of-function mutation/deletion/loss of expression in TNBC</td>
<td>Broad-spectrum PI3K pathway inhibitors</td>
<td>3</td>
</tr>
<tr>
<td>MDM2 amplification in TP53 wild-type tumors</td>
<td>MDM2 inhibitors</td>
<td>3</td>
</tr>
<tr>
<td>HER2 mutation</td>
<td>Small-molecule HER2 kinase inhibitors</td>
<td>3</td>
</tr>
<tr>
<td>PIK3R1 loss-of-function mutation</td>
<td>PI3K pathway inhibitors?</td>
<td>4</td>
</tr>
<tr>
<td>MLL family member mutation</td>
<td>HDAC inhibition?</td>
<td>4</td>
</tr>
<tr>
<td>Rare RTK mutations</td>
<td>Various matched inhibitors?</td>
<td>4</td>
</tr>
</tbody>
</table>

**NOTE:** Number 1 indicates approved therapy; 2, early evidence of efficacy; 3, clinical investigations under way; and 4, clinical investigations not yet activated.

*Clinical Trial.gov number. The trials mentioned in this table are examples, and the list is not meant to be comprehensive.
Epithelial-mesenchymal transition (EMT) and metastasis: yes, no, maybe?
Maren Diepenbruck and Gerhard Christofori
EMT and MET
Epithelial-to-mesenchymal transition is dispensable for metastasis but induces chemoresistance in pancreatic cancer

Xiaofeng Zheng, Julienne L. Carstens, Jiha Kim, Matthew Scheible, Judith Kaye, Hikaru Sugimoto, Chia-Chin Wu, Valerie S. LeBleu & Raghu Kalluri

Affiliations | Contributions | Corresponding author


Epithelial-to-mesenchymal transition is not required for lung metastasis but contributes to chemoresistance


Affiliations | Contributions | Corresponding authors

Nature 527, 472–476 (26 November 2015) | doi:10.1038/nature15748
Metastasis: Differences in latencies in cancer types

**Breast adenocarcinoma: Latency and metastatic speciation**

- miR335, miR126
- EREG, COX2, FASCIN1, MMP1, ANGPTL4
- HBEGF, COX2, FASCIN1, MMP1, ANGPTL4, ST6GALNAC5
- IL11, ADAMTS1, MMP1, TGFβ, CTGF, PTHrP

**Lung adenocarcinoma: Rapid metastasis to multiple organs**

- WNT/TCF → LEF1, HOXB9
Evolution of metastasis
Step-wise or parallel?
Timeline of development of metastasis based on mathematical modeling

Luebeck, Nature, 2010
Clonal Evolution of heterogeneous primary tumors giving rise to metastasis
Alternative hypothesis: Parallel evolution of metastasis?

Bone marrow cancer cells genomically different from primary tumor mix
- Spread early and evolve
OR
- Different subpopulation which might not give rise to overt metastasis?

Clinically-optimal to decide treatments based on DTCs or primary tumors?

Reference: Gray, Cancer Cell, 2003
Phenotypically normal untransformed cells can persist in the lungs for months

Podsypanina et al., Science, 2008
Delayed oncogene induction still forms tumors

When normal mammary cells have reached the distant site in the absence of transformation at the primary site.
Seed or the soil: which matters in metastasis?

4 million cells/g of primary tumor shed 0.01% successful!

Ref: Fidler, Nat Rev Cancer, 2003
Understanding the Metastatic Process

1858 - R. Virchow - Tumor Dissemination is determined by mechanical factors

1889 - S. Paget - "Seed to Soil" hypothesis for organs with disseminated cancer

1915 - First murine model of metastasis

1929 - J. Ewing - Metastasis determined by anatomy of channels draining primary tumor

Computational modeling is trying to expand upon the theories of metastatic dissemination including recent work using a Markov Chain model [pubmed][Prezi]

1944 - Role of cellular adhesiveness in metastasis

1952 - Organ specificity of tumor growth after IV Injection of tumor cells

1952 - Transpulmonary passage of tumor cell emboli results in metastases on arterial side

1955 - Cells adapted to ascites growth preexist in partental tumor, have increased malignant phenotype

1962 - Enzymatic manipulations of cell surfaces affects metastatic potential

1965 - Radioactive labeling of tumor cells (Chromium) is used to trace disseminating tumor cells

1970 - Metastasis is shown to result from the survival of a few tumor cells

1973 - Human tumors metastasize in thymic-deficient nude mice

1973 - In Vivo selection of tumor cells for enhanced metastatic potential

1975 - The "Metastatic Cascade" proposed for sequential events in cancer dissemination.

1975 - Organ specificity of metastasis determined by cell adhesion

1976 - Clonal Evolution of tumor cell populations

1976 - Invasion and metastasis linked to metastatic cells producing proteolytic enzymes

1977 - metastatic heterogeneity of neoplasms
Metastatic cells spread through lymphatics or blood?

Blood (hematogenous spread)

Lymphatic spread

Modified from www.iopscience.iop.org

Modified from www.cancer.org
Lymphatic spread of breast cancer
Lymphatics more accessible to cancer cells?

- Might depend on physical restrictions on invasive tumors.
- Easier to survive in passive, low shear flow.
- Lymphatics more leaky, lacking tight interendothelial junctions (higher chances of intravasation).

Peritumoral lymphatics matter more than intratumoral lymphatics

Wong and Hynes, Cell Cycle, 2006
Potential pathways of reaching circulation from the primary tumor

In prostate cancer, 84% with lymph node spread have hematogenous spread.

Wong and Hynes, Cell Cycle, 2006
Drug resistance of metastases: yet another problem to solve
Challenges to target drug resistant clones

Kuczynski et al., Nat Rev Clin Oncol., 2013
Animal models to study the complex process of metastasis

Latency (months to decades)

Overt metastasis
<table>
<thead>
<tr>
<th>Transgenic mouse models of breast cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMTV-Wnt1</td>
</tr>
<tr>
<td>MMTV-Neu</td>
</tr>
<tr>
<td>MMTV-Neu activated</td>
</tr>
<tr>
<td>MMTV-Neu (YB)</td>
</tr>
<tr>
<td>MMTV-Neu (YD)</td>
</tr>
<tr>
<td>MMTV-PyMT</td>
</tr>
<tr>
<td>MTB-TAN</td>
</tr>
<tr>
<td>MT-Met</td>
</tr>
<tr>
<td>C3(1)-Tag</td>
</tr>
</tbody>
</table>
Development of Patient derived xenografts

Kopetz et al., Clinical Cancer research, 2012
Fresh tumor implantation in immunocompromised mice
Tumor grafts in mice resemble the donor patient tumors by histology, genomics, growth.
<table>
<thead>
<tr>
<th>Clinical metastasis</th>
<th>ER status</th>
<th>PR status</th>
<th>HER2 status</th>
<th>Estrogen Dependence</th>
<th>Metastasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>n/a</td>
<td>Lung, LN</td>
</tr>
<tr>
<td>LN</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>n/a</td>
<td>LN</td>
</tr>
<tr>
<td>LN</td>
<td>pos</td>
<td>pos</td>
<td>neg</td>
<td>Yes</td>
<td>Lung, LN</td>
</tr>
<tr>
<td>Not detected</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>n/a</td>
<td>Not detected</td>
</tr>
<tr>
<td>Lung, bone</td>
<td>pos</td>
<td>pos</td>
<td>pos</td>
<td>Yes</td>
<td>Lung, LN, peritoneum</td>
</tr>
<tr>
<td>pos</td>
<td>pos</td>
<td>not tested</td>
<td>not tested</td>
<td>not tested</td>
<td>Lung, peritoneum, LN</td>
</tr>
<tr>
<td>pos</td>
<td>pos</td>
<td>not tested</td>
<td>not tested</td>
<td>not tested</td>
<td>Lung, LN, bone</td>
</tr>
<tr>
<td>Skin, lung</td>
<td>neg</td>
<td>neg</td>
<td>pos</td>
<td>n/a</td>
<td>Lung, LN</td>
</tr>
<tr>
<td>LN, pancreas bone, peritoneum</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
<td>n/a</td>
<td>Lung, peritoneum, LN</td>
</tr>
<tr>
<td>Lung</td>
<td>neg</td>
<td>borderline</td>
<td>neg</td>
<td>n/a</td>
<td>Lung, LN</td>
</tr>
<tr>
<td>LN, pleura</td>
<td>pos</td>
<td>pos</td>
<td>neg</td>
<td>no, but estrogen stimulated</td>
<td>Lung, LN, bone</td>
</tr>
<tr>
<td>LN, pericardium</td>
<td>neg</td>
<td>neg</td>
<td>pos</td>
<td>n/a</td>
<td>LN, thymus</td>
</tr>
</tbody>
</table>
- Lack of spontaneous metastasis from many PDX primary tumors (3 in 144 observed by the Kerbel group).
- Mouse thymomas arising in aged mice
Approach I: Interrogate the end-products

LMS: 18-gene signature associated with lung relapse

Probability

Lung metastasis-free survival (months)

LMS+ TβRS+

p < 0.0001

Kang et al  Cancer Cell 2003
Minn et al  Nature 2005
Tavazoie et al  Nature 2008
Bos et al  Nature 2009
Breast Cancer Lung Metastasis Signature (LMS)

- EREG
- COX2
- MMP1
- ANGPTL4
- FSCN1
- NEDD9
- ID1
- CXCL1
- TNC
- VCAM1
- CXCR4
- LTBP1
- ROBO1
- KRTHB1
- MAN1A1
- KNYU
- C10orf116
- RARRES3

COX2: vascular permeability
Epiregulin: cell motility
Fascin1: invadopodia
MMP1: collagenase
ANGPTL4: endothelial disjunction

Tenascin-C
VCAM-1

Padua et al *Cell* 2008
Kim et al *Cell* 2009
Tavazoie et al *Nature* 2008
Oskarsson et al *JACS* 2010
Tumor microenvironment interactions that support tumor progression

Quail and Joyce,, Nat Med, 2013
In transit
Role of platelets in the early hours of metastatic colonization

Reference Labelle et al., PNAS, 2014
At the distant site (lung)
Role of VCAM1 in establishing lung metastasis

Chen and Massague, Clinical Cancer Research, 2012
Fighting for survival
Role of extracellular matrix molecules in metastasis

Tenascin C at the invasive edge of tumors

Ref: Oskarsson et al., Nature Medicine, 2011
Depleting Tenascin-C in cancer cells reduces lung metastasis
Role of Tnc in outgrowth of micrometastasis

TNC interaction with cancer cells at the invasive front enhances NOTCH and WNT signaling

Enhances fitness of metastasis

Initiating cells
Extended survival of breast cancer cells for decades in the bone

<table>
<thead>
<tr>
<th>Bone Without Metastases</th>
<th>Beginning of Metastases</th>
<th>Bone with Fully Developed Metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer Cells</td>
<td>When they reach bone, the cells begin to form <strong>bone metastases</strong>.</td>
<td>Breakdown caused by bone metastases may lead to bone problems, such as <strong>fracture</strong>.</td>
</tr>
</tbody>
</table>

Cancer cells travel from the primary tumor.

Modified from
http://www.myxgeva.com/breast-cancer-bone-metastases.html
The undetectable but present Latent metastasis
Role of Src signaling in the survival of bone metastasis

- No role in homing to lungs or bone.
- If these cells reach, then Src signaling becomes critical for their outgrowth.
Src enhances survival and outgrowth of latent metastatic cells in the bone marrow.
Brain metastasis from breast cancer

Modified from www.braintumors.in
Plasmin-reactive brain stroma for protection of host

Valiente et al., Cell, 2014
Tumor microenvironment components

Macrophage/microglia microenvironment targeting in brain tumors

Pyonteck et al., Nat Med, 2013
T regulatory cells are present in growing tumors and metastases

Poor prognosis in breast cancers
Treg cells ablated from established tumors can reduce metastatic growth

Myeloid derived suppressor cells in cancer
- Another group of poor prognosis immunosuppressive cells

Modified from Wynn, Nat Immunology, 14(3), 197-99
CXCL1/2 mediates mammary tumor progression

**Tumor growth**

- **sh Control**
- **sh CXCL1/2**

Tumor volume (mm³) vs. Days after injection

**Lung metastasis**

- **sh Control**
- **sh CXCL1/2**

Foci number/FOV

**Apoptosis**

- **sh Control**
- **sh CXCL1/2**

Cleaved caspase3

Apoptotic cells/FOV

**MMTV-PyMT**

**MDA231-LM2**

*p < 0.0001*
Granulocytic CD11b^+Ly6G^+ cells

CXCL1/2 recruit granulocytic CD11b^+Ly6G^+ cells in the tumor microenvironment

Granulocytic CD11b^+Ly6G^+ cells

sh Control

shCXCL1/2
MDSC levels correlate with accelerated disease progression and poor survival in patients

- Colorectal Cancer (Solito et al, 2011)
- Lymphoma (Montero., 2012)
- Gastrointestinal Cancer (Gabitass et al., 2011)

**MDSC**

- Suppress Anti-tumor Immunity
  (Gabrilovich, Ostrand-Rosenberg and Bronte, 2012)
- Tumor Angiogenesis
  (Yang et al, 2004, Shojaei et al, 2007)
- Blood vessel remodelling and tumor cell extravasation
  (Yan et al, 2010)
CXCL1/2 promotes tumor progression via myeloid cell S100A8/9

S100a9+/+ and S100a9−/− bone marrow reconstituted mice as recipients

Donor bone marrow → Cancer cells to gland #4 → Tumor growth

**Graph:**
- Tumor volume (mm³) over days after injection
- S100a9+/+bm vs. S100a9−/− bm, p=0.001

**Images:**
- Vimentin expression in S100a9+/+ and S100a9−/−
- Bar graphs showing:
  - Mammary tumors: S100a9+/+, S100a9−/−, p=0.002
  - Lungs: S100a9+/+, S100a9−/−, p<0.0001

**Bar graph:**
- Metastatic cells/FOV
- S100a9+/+ vs. S100a9−/−, p<0.0001
CXCL1/2 axis mediates tumor growth and metastasis

Breast primary

Invasion stress

Survival

Cancer cell

CXCL1/2

Metastasis

Survival

Metastatic stress

S100A8/9

CXCL1/2

CXCR2

CD11b+Gr1+ Myeloid cells

S100A8/9
Chemotherapy hyperactivates the CXCL1/2-S100A8/9 cycle

Patient breast tumor
Before chemo
After chemo

S100A8/9 score
Before chemo:
Score 0
Score 1
Score 2
Score 3

p<0.0001
**CXCR2 inhibitor enhances chemotherapy**

**Cancer cells**
- Implant cancer cells into Gland #4
- CXCR2 inhibitor
- Chemo

**Lung metastasis**
- Vehicle
- i-CXCR2
- AC chemo
- Two drugs

**Graphs**
- MDA231-LM2
  - CXCR2i + Chemo vs. Chemo
  - Days: 0, 26, 34, 40, 46, 53, 60
  - Metastatic cells/FOV
  - p = 0.028

- CN34-LM1
  - Chemo vs. CXCR2i + Chemo
  - Metastatic cells/FOV
  - p = 0.024
Cytotoxic chemo, short-lived targeted therapy.....immunotherapy promise

Use Your Own Immune System to Defeat Cancer

IMMUNOTHERAPY

“TRAC-EM™” combination
1. Apherisis, or “Subtractive Therapy™”
2. Macrophage Activation
3. Dendritic Cell Response

Cancer Cells

Macrophage Activation

Apheresis

Dendritic Cell Response

http://www.revealtherapies.com/AllPages/Immunotherapy.html
23% REDUCTION IN CANCER DEATH RATE

1.7 million lives saved.

U.S. adults who smoke are 25 times more likely to develop lung cancer than those who do not; but those who quit, cut their chance of dying from lung cancer in half within 10 years.

http://clincancerres.aacrjournals.org

AACR Cancer Progress Report 2016
Newer anti-cancer agents:
Immunotherapy, vaccines, oncolytic
Viruses, mutation specific therapies.

Metastatic cancer still claims the majority of lives