Biology of metastasis

Swarnali Acharyya
Nov 11, 2015
Treatments for primary breast cancer

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

Over 90% survival rate for localized, well treated breast cancers

Better detection and imaging
New surgical options
Targeted therapy

Detection and removal
Dispersion & seeding 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%
- Percent Surviving 5 Years: 89.2%

5-Year Relative Survival

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

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

Target HER2 Protein Trastuzumab

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. Trastuzumab, pertuzumab, and lapatinib. All approved agents</td>
</tr>
<tr>
<td>PIK3CA mutation</td>
<td>PIK3CA-selective inhibitors</td>
<td>2. Phase I BYL719 (18)</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. Phase I BGJ398 (48) and phase I E3800 (47)</td>
</tr>
<tr>
<td>Inherited and somatic BRCA1 and BRCA2 mutation</td>
<td>PARP inhibitors</td>
<td>2. Olaparib [49] and veliparib:NCT01506609†</td>
</tr>
<tr>
<td>Cyclin D1/CDK4/CDK6 amplification or deletion of CDKN1B, CDKN2A, and CDKN2B</td>
<td>CDK4/6 inhibitors</td>
<td>2. PO0032991 (40)</td>
</tr>
<tr>
<td>AKT1-3 gain-of-function mutation/gene fusion via translocation/amplification</td>
<td>AKT inhibitors</td>
<td>3. MK-2206; NCT01277757†</td>
</tr>
<tr>
<td>GATA3 mutation</td>
<td>Aromatase inhibition</td>
<td>3. Retrospective analysis of Z1031 (4)</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. BKM120; NCT01629615‡</td>
</tr>
<tr>
<td>MDM2 amplification in TP53 wild-type tumors</td>
<td>MDM2 inhibitors</td>
<td>3. RO5503781; NCT01462175†</td>
</tr>
<tr>
<td>HER2 mutation</td>
<td>Small-molecule HER2 kinase inhibitors</td>
<td>3. Neratinib; ( NCT01670877)* (50)</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: 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.
Trends in breast cancer incidence and mortality

Source: Surveillance, Epidemiology, and End Results (SEER) Program and the National Center for Health Statistics. Additional statistics and charts are available at the SEER Web site.
**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

*Nature*
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)**

![Diagram of blood vessel and lymph nodes](Modified from www.iopscience.iop.org)

**Lymphatic spread**

![Diagram of lymphatic vessels and organs](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

Overt metastasis

Latency (months to decades)
## Transgenic mouse models of breast cancer

<table>
<thead>
<tr>
<th>Model</th>
<th>Tumor type</th>
<th>latency (weeks)</th>
<th>growth rate</th>
<th>tumor location</th>
<th>references</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMTV-Wnt</td>
<td>Mammary gland</td>
<td>60</td>
<td>b</td>
<td>Lung, LN</td>
<td>[7,16,77]</td>
</tr>
<tr>
<td>MMTV-Neu</td>
<td>Mammary gland</td>
<td>100</td>
<td>6.8a</td>
<td>Lung</td>
<td>[16,102]</td>
</tr>
<tr>
<td>MMTV-Neu activated</td>
<td>Mammary gland</td>
<td>100</td>
<td>3a-5</td>
<td>Lung</td>
<td>[42,44]</td>
</tr>
<tr>
<td>MMTV-Neu (YB)</td>
<td>Mammary gland</td>
<td>100</td>
<td>6a</td>
<td>Lung</td>
<td>[44,67]</td>
</tr>
<tr>
<td>MMTV-Neu (YD)</td>
<td>Mammary gland</td>
<td>100</td>
<td>3.6a</td>
<td>Lung</td>
<td>[44,67]</td>
</tr>
<tr>
<td>MMTV-PyMT</td>
<td>Mammary gland</td>
<td>100</td>
<td>1-6</td>
<td>Lung; LN</td>
<td>[16,45,51]</td>
</tr>
<tr>
<td>MTB-TAN</td>
<td>Mammary gland</td>
<td>100</td>
<td>-</td>
<td>Lung</td>
<td>[16,103]</td>
</tr>
<tr>
<td>MT-Met</td>
<td>Mammary gland</td>
<td>b</td>
<td>10</td>
<td>Lung; LN; kidney; heart; cecum</td>
<td>[16,104]</td>
</tr>
<tr>
<td>C3(1)-Tag</td>
<td>Mammary gland</td>
<td>100</td>
<td>3-6</td>
<td>Lung</td>
<td>[16,105]</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>Sites in patients</th>
<th>METASTASIS SITES</th>
<th>Sites in mice</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical metastasis</strong></td>
<td><strong>ER status</strong></td>
<td><strong>PR status</strong></td>
</tr>
<tr>
<td>Lung</td>
<td>neg</td>
<td>neg</td>
</tr>
<tr>
<td>LN</td>
<td>neg</td>
<td>neg</td>
</tr>
<tr>
<td>LN</td>
<td>pos</td>
<td>pos</td>
</tr>
<tr>
<td>Not detected</td>
<td>neg</td>
<td>neg</td>
</tr>
<tr>
<td>Lung, bone</td>
<td>pos</td>
<td>pos</td>
</tr>
<tr>
<td>Skin, lung</td>
<td>pos</td>
<td>pos</td>
</tr>
<tr>
<td>LN, pancreas, bone, peritoneum</td>
<td>neg</td>
<td>neg</td>
</tr>
<tr>
<td>Lung</td>
<td>neg</td>
<td>not tested</td>
</tr>
<tr>
<td>LN, pleura</td>
<td>pos</td>
<td>pos</td>
</tr>
<tr>
<td>LN, peritoneum</td>
<td>neg</td>
<td>neg</td>
</tr>
</tbody>
</table>
Approach I: Interrogate the end-products

LMS: 18-gene signature associated with lung relapse

Lung metastasis-free survival (months)

Probability

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
- KRT7B1
- MAN1A1
- KYNU
- C10orf116
- RARRES3

**COX2**: Vascular permeability

- Epiregulin: Cell motility
- Fascin1: Invadopodia
- MMP1: Collagenase
- ANGPTL4: Endothelial disjunction

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

Nanog$, Oct4$, Sox2$, CD44$^+$CD24$^-$ metastasis-initiating breast cancer cell
Extended survival of breast cancer cells for decades in the bone

Modified from
http://www.myxgeva.com/breast-cancer-bone-metastases.html
The undetectable but present *Latent metastasis*
Zhang et al., Cancer Cell, Jul 7, 2009
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
Failing metastasis

Neuron

Blood capillary

Astrocyte

Plasminogen

PA

Plasmin

sFasL

L1CAM

FADD

Growing metastasis

sFasL

Anti-PA Serpin

PA

L1CAM

Cancer cell survival

Vascular cooption

Tumor re-initiation

Cancer cell death
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**

- **Days after injection**
- **Tumor vol. (mm$^3$)**
- **p < 0.001**

**Lung metastasis**

- **shControl**
- **shCXCL1/2**

- **Lung metastasis**

**Apoptosis**

- **shControl**
- **shCXCL1/2**

- **Cleaved caspase3**

- **Apoptotic cells/FOV**

**MMTV-PyMT**

**MDA231-LM2**

- **Foci number/FOV**
- **p < 0.0001**

**CXCR2**
CXCL1/2 recruit granulocytic CD11b\(^{+}\)Ly6G\(^{+}\) cells in the tumor microenvironment

[Diagram showing flow cytometry data for different cell populations: CD11b\(^{+}\)Ly6G\(^{+}\), CD11b\(^{+}\)Ly6C\(^{+}\), F4/80\(^{+}\), CD11b\(^{+}\)Gr1\(^{+}\), CD31\(^{+}\), and Unsorted. Cxcr2 mRNA levels are depicted on a log scale (Relative units).]
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

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

CXCL1/2 promotes tumor progression via myeloid cell S100A8/9
CXCL1/2 axis mediates tumor growth and metastasis

CXCL1/2
Breast primary

Invasion stress

Survival

Cancer cell

CXCL1/2

CXCR2

CD11b+Gr1+
Myeloid cells

S100A8/9

Metastatic stress

Survival

Metastasis

CXCL1/2
Chemotherapy hyperactivates the CXCL1/2-S100A8/9 cycle.
CXCR2 inhibitor enhances chemotherapy

Implant cancer cells

Days: 0

Cancer cells → Lung metastasis

Gland #4 → Mammary tumor

CXCR2 inhibitor

Chemo

Lung metastasis

Vehicle

i-CXCR2

AC chemo

Two drugs

MDA231-LM2

CN34-LM1

Metastatic cells / FOV

MDA231-LM2

CN34-LM1

p=0.028

p=0.024

Chemo

CXCR2i

+ Chemo

+ Chemo

+ Chemo

+ Chemo

+ Chemo

+ Chemo

+ Chemo

+ Chemo

+ Chemo

+ Chemo

+ Chemo

+ Chemo

+ Chemo

+ Chemo

+ Chemo

+ Chemo

+ Chemo

+ Chemo

+ Chemo

+ Chemo

+ Chemo

+ Chemo

+ Chemo

+ Chemo

+ Chemo

+ Chemo

+ Chemo

+ Chemo

+ Chemo

+ Chemo

+ Chemo

+ Chemo

+ Chemo
Distant metastasis
Brain
Bone
Primary tumor

Normalized Photon Flux

Vehicle  Chemo  CXCR2i  Chemo +CXCR2i
Cytotoxic chemo, shortlived targeted therapy…..immunotherapy promise

http://www.revealtherapies.com/AllPages/Immunotherapy.html
Treatments for primary breast cancer

89% survival rate for localized breast cancers