Invasion and Metastasis

- Most, if not all cancers have acquired same set of functional capabilities during their development, albeit through various mechanisms.
- Invasion and Metastasis are heterogeneous and poorly understood.
- Means of achieving all six hallmarks vary significantly, both mechanistically and chronologically.
- Thus, the order in which being acquired seems likely to be quite variable among the spectrum of cancer types and subtypes.
- Moreover, in some tumors, a particular genetic lesion may confer several capabilities simultaneously, decreasing the number of distinct mutational steps required to complete tumorigenesis.
- In other tumors, a capability may only be acquired through the collaboration of two or more distinct genetic changes, thereby increasing the total number necessary for completion of tumor progression.
- Thus, in the eight-step pathway shown, invasion/metastasis and resistance to apoptosis are each acquired in two steps.

Moving Out

90% of patient deaths are due to non-primary tumors.
- Size of all tumors difficult to detect – how vs peritoneal/pleural space
- Compromising vital functions – block passage of product, lungs, bone break
- Seed and soil – metastasis form micrometastasis in distant sites, fertile ground allows tumors to thrive
  - Breast -> brain, liver bones and lungs
  - Colon -> liver
  - Prostate -> bones

Which View?

- Historical bias of cancer – homogeneous tumor cells break free from basal membrane to form metastasis
- Tumors are complex tissues: conscription of normal stromal and inflammation cells to act as collaborators of neoplastic programing.

Cancer Staging

Staging is used to determine the severity of the cancer – use TNM staging system
- Tumor – size extent and location (0-4)
- Nodes – lymph node involvement (0-3)
- Metastasis – Presence or absence of distant metastasis (0 or 1)

Primary Tumor (T)
- TX: Tumor cannot be evaluated
- T0: No evidence of primary tumor
- T1: Tumor 2 cm or less
- T2: Tumor 2.1-4 cm
- T3: Tumor 4.1-5 cm
- T4: Tumor > 5 cm

Regional Lymph Nodes (N)
- NX: Nodes cannot be evaluated
- N0: No regional lymph node involvement
- N1: Regional lymph node involvement
- N2, N3: More than one lymph node involvement

Distant Metastasis (M)
- MX: Cannot be evaluated
- M0: No evidence of distant metastasis
- M1: Metastasis

A number may be added to each letter to indicate size or extent of tumor or spread of tumor.

A T3N2M0 tumor is large, with local nodes but no evidence of metastasis.
Most cancers can be assigned TNM classification. CNS tumours are classified according to cell type and grade. Lymphomas have a different staging system and and hematological malignancies are considered differently again.

For many cancers, TNM designations correspond to one of five stages. Criteria for stages differ for different types of cancer. For example in bladder cancer T3 N0 M0 is stage III; however, in colon cancer T3 N0 M0 is stage II.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 0</td>
<td>Carcinoma in situ (early cancer that is present only in the layer of cells in which it began)</td>
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<td>Stage I</td>
<td>Higher numbers indicate more extensive disease: greater tumor size, and/or spread of the cancer to nearby lymph nodes and/or organs adjacent to the primary tumor</td>
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<td>The cancer has spread to another organ.</td>
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80% of most aggressive cancers occur in epithelial cells yielding carcinomas. Carcinomas begin on epithelial side of BM and are benign until they breach BM. BM = Extra cellular matrix – produced by fibroblasts and stromal cells. Once crossing BM cells are malignant.

Angiogenesis begins before BM is degraded. But once invading through BM carcinomas gain access to stromal cells, agonists and better involvement with blood vessels and lymphatic system. Loss of BM can predict future course of metastatic disease.

Primary cancer cells entering the blood (or lymph) system is Intravasation.

- After invasion through BM and stromal cells, cancer cells enter by remodeling endothelial lining of blood vessel – between or through cells
- Proteases degrade cadherin and gap junctions inducing cell retraction
- Actin contraction is stimulated by MLC kinase and calcium calmodulin signaling.

**Invasion – Metastasis Cascade**

**Intravasation**

**Examples**

**Stage Definitions**

- **Stage** 0: Carcinoma in situ (early cancer that is present only in the layer of cells in which it began).
- **Stage** I: Higher numbers indicate more extensive disease: greater tumor size, and/or spread of the cancer to nearby lymph nodes and/or organs adjacent to the primary tumor.
- **Stage** IV: The cancer has spread to another organ.

**Invasion and Metastasis: Breaching the BM**

- Angiogenesis begins before BM is degraded.
- But once invading through BM carcinomas gain access to stromal cells, agonists and better involvement with blood vessels and lymphatic system.
- Loss of BM can predict future course of metastatic disease.

**Intravasation**

- After invasion through BM and stromal cells, cancer cells enter by remodeling endothelial lining of blood vessel – between or through cells.
- Proteases degrade cadherin and gap junctions inducing cell retraction.
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**Rough Travels**

- Lack of contact (many tumor cells maintain some requirement), loss of stromal cell factors, sheer factors and size of vessels (cancer cells > 20µM while capillaries ~3-8µM) create hostile environment for most escaping tumor cells.
- Most cells die by anoikis (form of apoptosis).
- Circulating tumor cells often become trapped in lungs – but many do not stay as they find through to tissue capillaries or move within tissues.
- Complicating passage is many tumor cells recruit and macrophages/platelets – found as multicellular aggregate.

**Time to leave - Extravasation**

- Several mechanisms – different than leukocytes (can travel between endothelial cells by a process called diapedesis).
  - Use a similar mechanism as intravasation.
  - Can proliferate to obliterate adjacent vessel wall.
  - Helped by macrophages recruited while reroute.

**Epithelial-Mesenchyme Transition (EMT)** is part of maturation as cancer cells begin before/during intravasation.

- Epithelial cells are highly attached to neighboring cells (via gap junctions and E-cadherin) and are poorly motile. Shift from epithelial to fibroblast appearance and acquire many new mesenchymal phenotypes.

**Driving Miss Daisy**

- Cancer genomes are typically altered at multiple points (hundreds) in a single tumor.
- Key somatic mutations which significantly contribute to tumor progression are "Driver Mutations" Confer growth advantage and positive selection in microenvironment.
- "Passenger or Neutral Mutations" may not help tumor formation and progression and are simply carried along and the result of mutational environment (lack of repair...)
- EMT Drivers include transcription factors altering E-cadherin. (Snail, Slug, Zeb1 and Twist) and E-cadherin itself.
E and N Cadherin

- E Cadherin binds and signals in epithelial cells
- Tethered to actin E Cadherin provides tensile strength to cell sheets and signal via Wnt
- Methylation and point mutation of EC drives EMT
- N Cadherin increases stromal/cancer cell interaction and inhibits sheets of cells
- Bonds between NC are weaker than EC favoring motility and mesenchyme like behavior

Pancreatic Cancer - EMT

- Increased expression of EMT markers
- Inhibition of EMT markers

TrkB + BDNF

% mice with metastatic lesions on lung surface

- sh-EGF #1
- sh-EGF #2
- sh-Zeb1 #1
- sh-Zeb1 #2

breast cancer

- Low Snai
- High Snai

melanoma

- Low Twist
- High Twist

Pancreatic Cancer

- EMT markers
- Inhibition of EMT markers
Stromal Cell impact and EMT-MET

Colonization – the new beginning

- Micrometastases – colonization by small clumps of metastasizing cancer (and associated) cells
- Most foreign tissues do not contain proper mix of stromal cells/signaling factors for continued proliferation
- > 30% of breast cancer patients harbor 100-1000s of micrometastases but only half will show development of these metastatic nascent tumors
- In mice primary tumors (1g ~10⁹ cells) seed more than 1 mil cells per day into blood/lymph but less than 5 will survive to form metastasis.

Proteases and Invasion/Metastasis

- Genetic heterogeneity of tumors may help evolution of micrometastasis
  - Only those cells that can colonize will form secondary tumors
  - Evolution of such tumors are the reason for clinical relapse from “cancer-free” patients years later