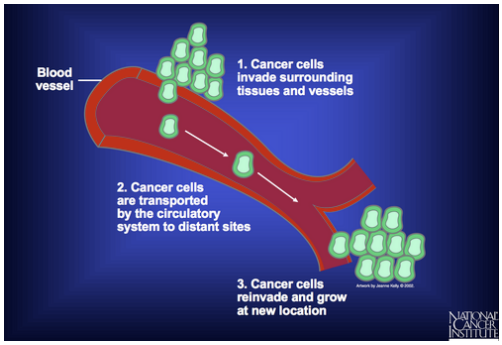
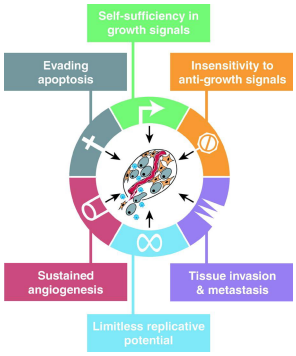
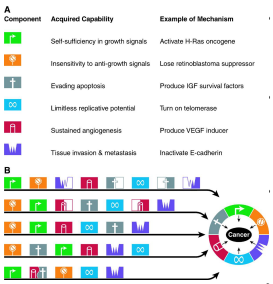
Invasion and Metastasis



6 Hallmarks of Cancer

- Most, if not all cancers have acquired same set of functional capabilities during their development, albeit through various mechanisms
- Invasion and Metastasis most heterogeneous and poorly understood



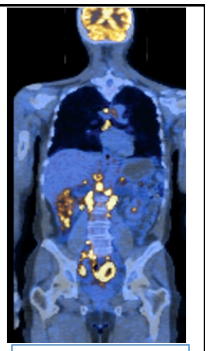


- Means of achieving all six hallmarks will vary significantly, both mechanistically and chronologically
- Thus, the order in which these capabilities are acquired seems likely be quite variable across the spectrum of cancer types and subtypes.
- Moreover, in some tumors, a particular genetic lesion 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

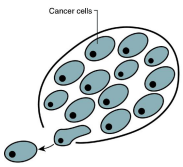
90% of Patient deaths are due to non-primary tumors

- Size of all tumors are difficult to detect
  - Brain vs peritoneal/pleural space
- Compromising vital functions – block passage of product, lung function, bone break
- Seed and soil – metastasis form micrometastasis in distal sites, fertile ground allows tumors to thrive
  - Breast -> brain, liver bones and lungs
  - Colon -> liver
  - Prostate -> bones



F deoxyglucose labeling of metabolically active tissue – brain and tumors – highlighting metastatic disease from non-Hodgkin;s lymphoma

The Reductionist View

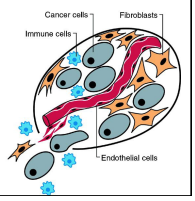


Which View?

- Historical bias of cancer – homogeneous tumor cells break free from basal membrane to form metastasis

A Heterotypic Cell Biology

- Tumors are complex tissues: conscription of normal stromal and inflammation cells to act as collaborators of neoplasmic programing.



Cancer Staging

Staging is used to determine the severity of the cancer – use TMN 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
	Tis	Carcinoma in situ (early cancer that has not spread locally)
	T1, T2, T3, T4	Size and/or extent of tumor
Regional Lymph Nodes (N)	NX	Nodes cannot be evaluated
	N0	No nodal involvement
	N1, N2, N3	Nodal involvement (number/extent of spread)
Distant Metastasis (M)	MX	Cannot be evaluated
	M0	No evidence of metastasis
	M1	Metastasis

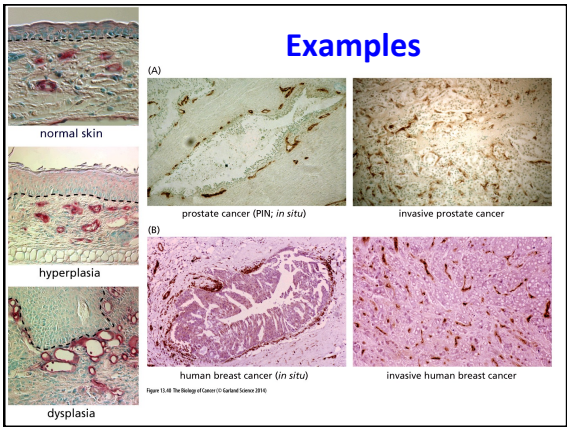
A number may be added to each letter to indicate size or spread of tumor

A T3N2M0 tumor is large, with local nodes but no evidence of metastasis

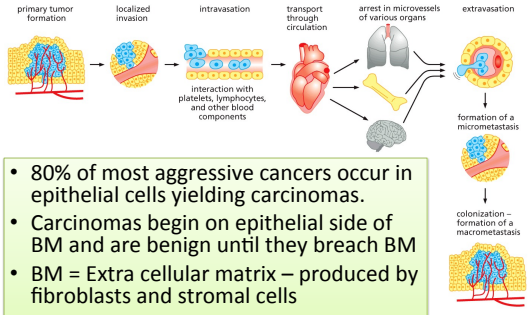
- Most cancers can be assigned TNM classification. CNS tumours are classified according to cell type and grade, lymphomas have a different staging system and and hematological malignancies are considered differently again.
- For many cancers, TNM designations correspond to one of five stages. Criteria 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.

Stage	Definition
Stage 0	Carcinoma in situ (early cancer that is present only in the layer of cells in which it began)
Stage I Stage II Stage III	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.

Examples



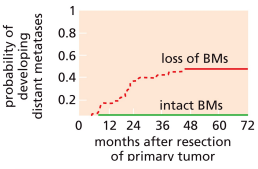
Invasion – Metastasis Cascade



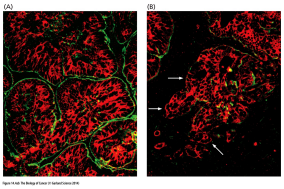
- 80% of most aggressive cancers occur in epithelial cells yielding carcinomas.
- Carcinomas begin on epithelial side of BM and are benign until they breach BM
- BM = Extra cellular matrix – produced by fibroblasts and stromal cells
- Once crossing BM cells are malignant

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

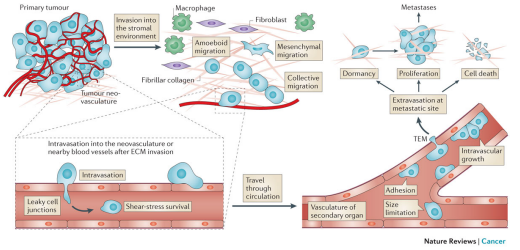


High grade carcinomas T3 and correlation with BM loss. Survival after surgical removal of primary tumor



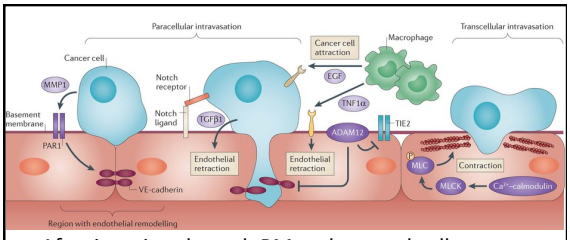
A) Less aggressive/differentiated colorectal carcinoma islands (red anti cytokeratin Ab) and BM (green laminin Ab). Stromal cells are not labeled (black)
B) Less differentiated aggressive carcinoma staining – arrows highlight loss of BM and stromal/carcinoma interaction

Intravasation



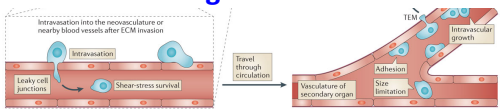
- Primary cancer cells entering the blood (or lymph) system is Intravasation.

Crossing the endothelial barrier during metastasis
Nicolas Reymond, Bárbara Borda d'Água & Anne J. Ridley
Nature Reviews Cancer **13**, 858–870 (2013) doi:10.1038/nrc3628



- 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

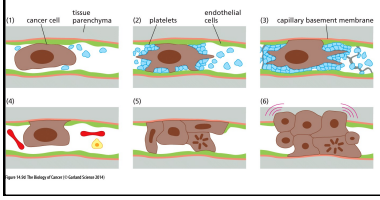
Rough Travels



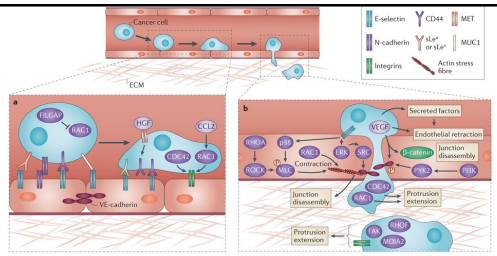
- Lack of contact (many tumor cells maintain some requirement), loss of stromal cell factors, sheer factors and size of vessels (cancer cells > 20μM while capillaries ~3-8μM) create hostile environment for most escaping tumor cells
- Most cells die by anoikis (form of apoptosis)
- Circulating tumor cells 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

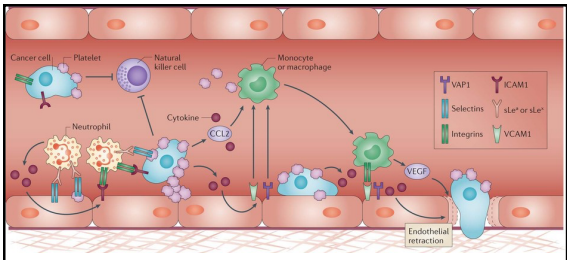
- Several mechanisms – different than leukocytes (can travel between endothelial cells by a process called diapedesis)
  - Use a similar mechanism as intravasation
  - Can proliferate to obliterate adjacent vessel wall.
  - Helped by macrophages recruited while reroute



Trapped cells (cancer and platelets) form microthrombus. Protease and mechanical reduction of endothelial cells leave capillary BM to invade.
- Proliferation within the lumen allows invasion into surrounding parenchyma tissue



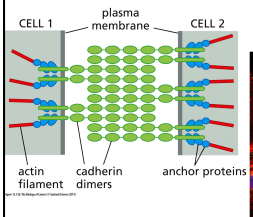
- Receptor mediated attachment of cancer cell to endothelial cells induce small G proteins (Ras, Rac, Rho) to re-arrange cancer cell shape and allow protrusion and tail retraction
- Receptors also induce expression and secretion of factors (integrin) and proteases to allow for junction disassembly of endothelial cells.



- Cancer cell interaction with platelets protect tumor cells against immune lysis by natural/hunter killer cells.
- Platelets secrete factors and help recruit neutrophils which secrete pro-inflammatory cytokines and help adhesion.
- Cancer cells help the process by release of cytokines which drive endothelial retraction and extravasation by VEGEF and other factors

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



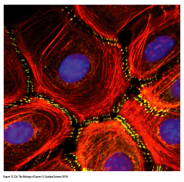


Table 14.2 Cellular changes associated with an epithelial–mesenchymal transition

Loss of
Cytokeratin (intermediate filament) expression
Tight junctions and epithelial adherens junctions involving E-cadherin
Epithelial cell polarity
Epithelial gene expression program
Acquisition of
Fibroblast-like shape
Motility
Invasiveness
Increased resistance to apoptosis
Mesenchymal gene expression program including EMT-inducing transcription factors
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
Stem cell-like traits

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

Table 14.3 Transcription factors orchestrating an EMT

Name	Where first identified	Type of transcription factor	Cancer association
Snail (SNAI1)	mesoderm induction in *Drosophila*; neural crest migration in vertebrates	C2H2-type zinc finger	invasive ductal carcinoma
Slug (SNAI2)	delamination of the neural crest and early mesoderm in chicken	C2H2-type zinc finger	breast cancer cell lines, melanoma
Twist	mesoderm induction in *Drosophila*; emigration from neural crest	bHLH	various carcinomas, high-grade melanoma, neuroblastoma
Goosecoid	gastrulation in frog	paired homeodomain	various carcinomas
FOXC2	mesenchyme formation	winged helix/forkhead	basal-like breast cancer
ZEB1 (δEF1)	postgastrulation mesodermal tissue formation	2-handed zinc finger/ homeodomain	wide variety of cancers
ZEB2 (SIP1)	neurogenesis	2-handed zinc finger/ homeodomain	ovarian, breast, liver carcinomas
E12/E47 (Tcf3)[a]	associated with E-cadherin promoter	bHLH	gastric cancer

[a]It remains unclear whether E12/E47 can function on its own to induce an EMT, or whether this bHLH functions as a subunit of a heterodimeric TF complex formed with other well-validated EMT-TF proteins such as Twist.

Table 14.3 The Biology of Cancer (© Garland Science 2014)

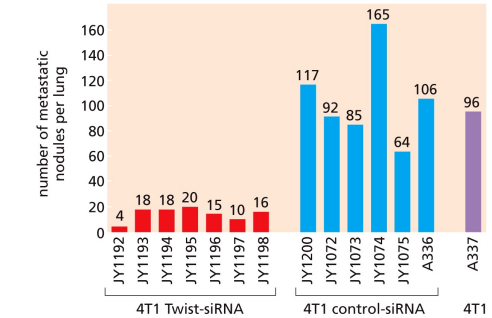


Figure 14.30a The Biology of Cancer (© Garland Science 2014)

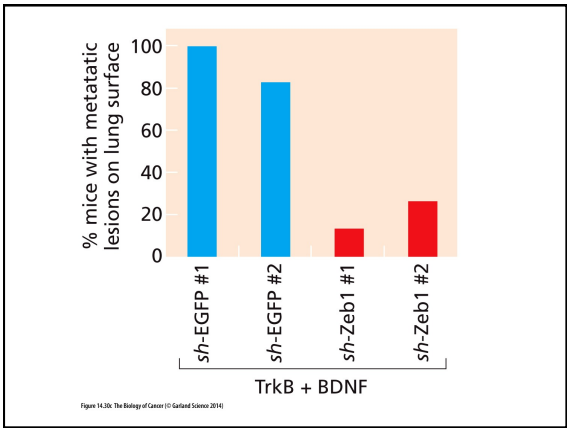
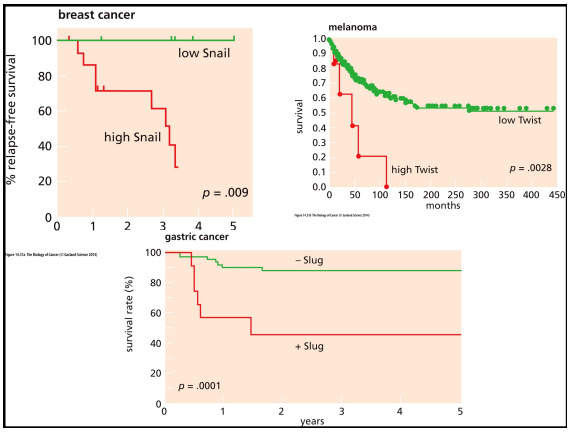
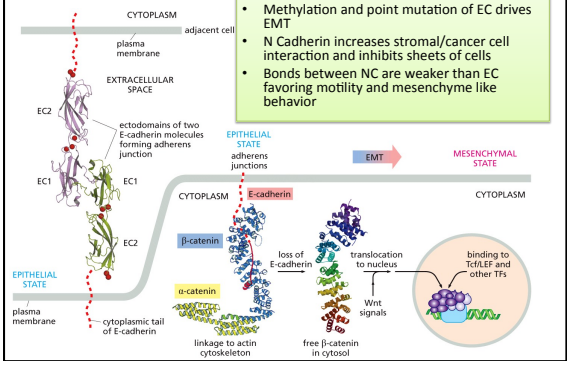


Figure 14.30c The Biology of Cancer (© Garland Science 2014)

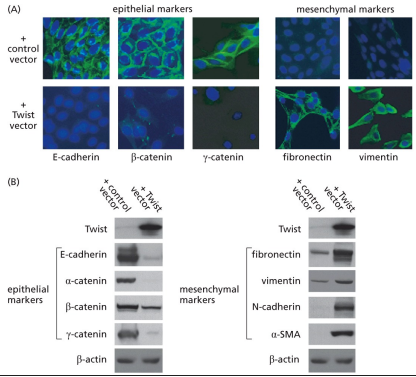


E and N Cadherin

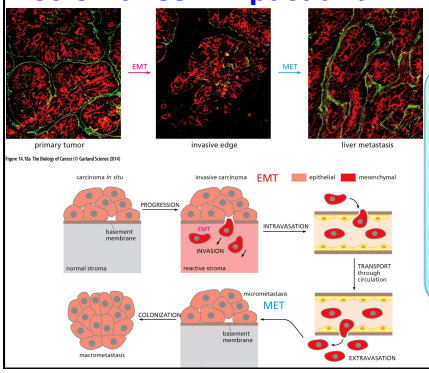
- E Cadherin binds and signals in epithelial cells
- Tethered to actin E Cadherin provides tensile strength to cell sheets and signal via Wnt
- Methylation and point mutation of EC drives EMT
- N Cadherin increases stromal/cancer cell interaction and inhibits sheets of cells
- Bonds between NC are weaker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



Pancreatic Cancer - EMT



Stromal Cell impact and EMT-MET



Post extravasation EMT is reversed and colon tumor cells begin to revert to epithelial cells resembling primary tumors

Much is driven by change in stromal cell support between original and distal site

Colonization – the new begining

- 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 theses metastatic nascent tumors
- In mice primary tumors (1g ~10^9 cells) seed more than 1 mil cells per day into blood/lymph but less than 5 will survive to form metastasis.

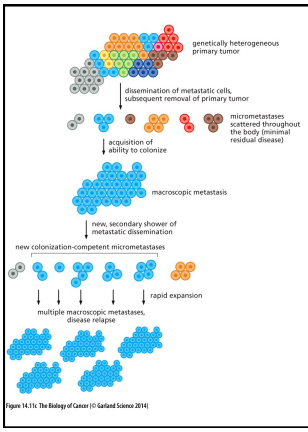


Figure 14.11c The Biology of Cancer (© Garland Science 2014)

- Genetic heterogenaity 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

Proteases and Invasion/Metastasis