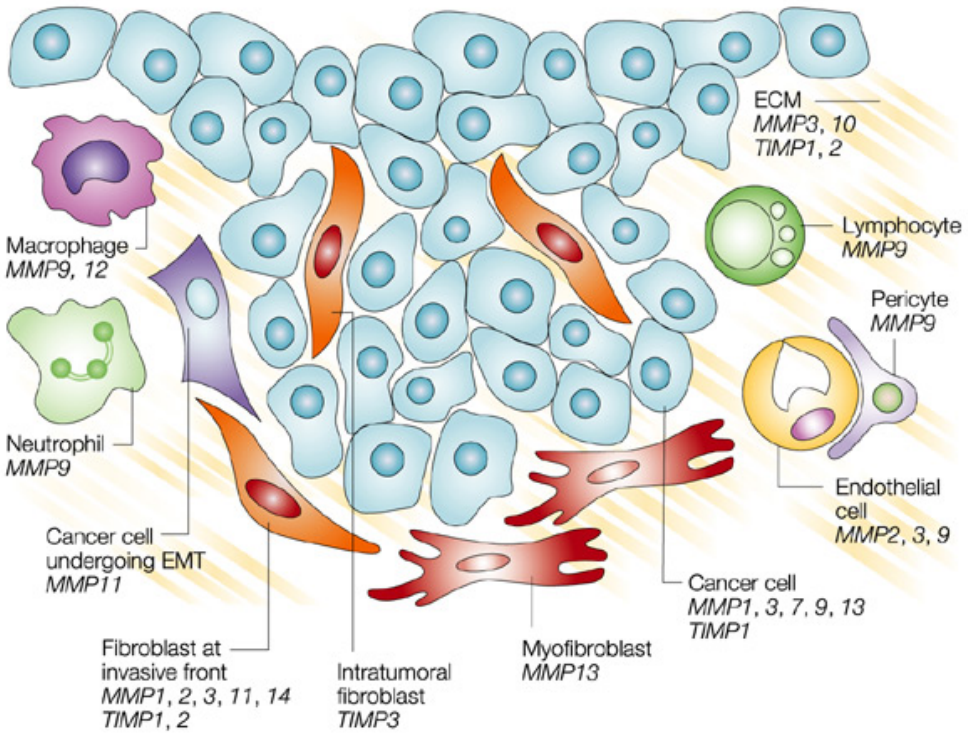
Proteases and Invasion/Metastasis

The ability to invade and metastasize depends on removal of the ECM barrier for cells to "move into" during EMT transition

- MMPs matrix metalloproteinases – degrade extracellular protein of ECM
- ECM includes growth factors and other proteases inactive as pro-hormones/enzymes that are released after degradation
- Stromal cells, macrophages, mast cells, fibroblasts and tumor cells secrete MMPs
- Targets include collagen, fibronextin, tenascin, and proteoglycans

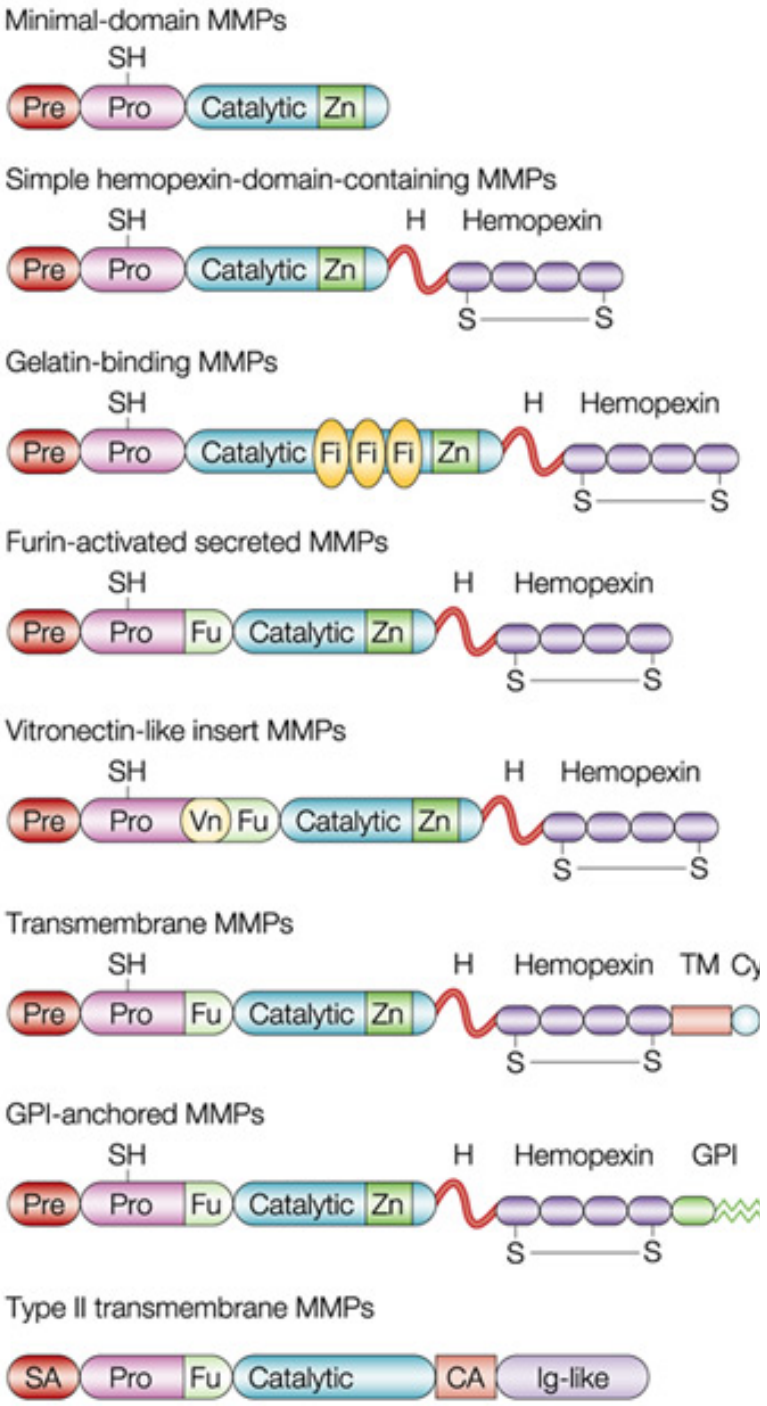


MMPs are a family of pre-pro-enzymes

- 8 functional groups, five are secreted, three are membrane associated

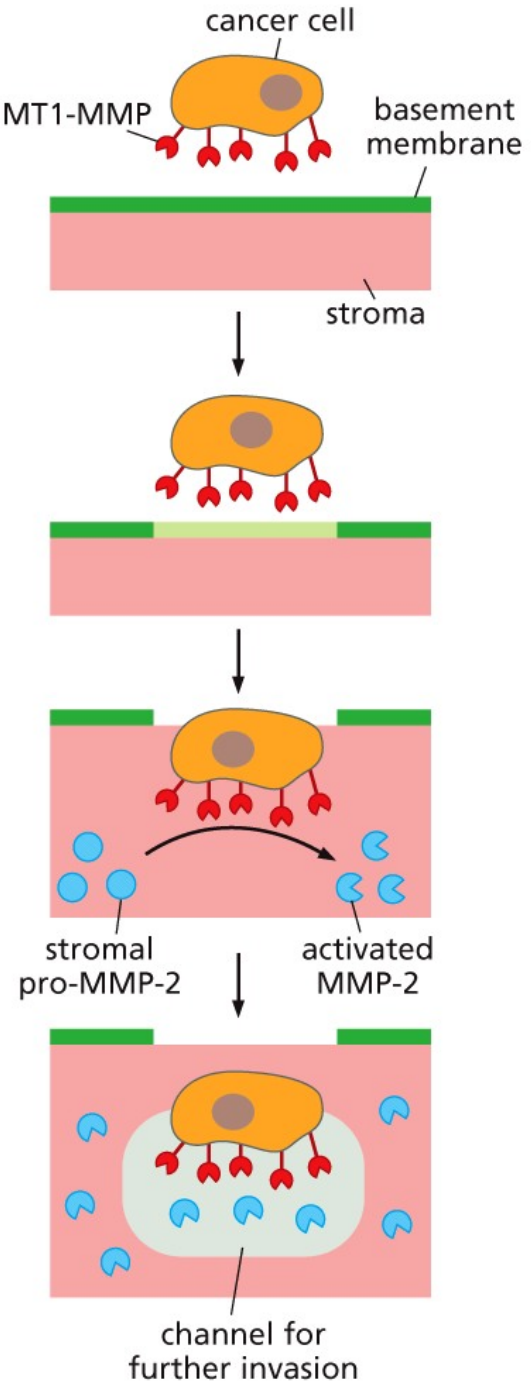
Domains:

- Pre – N term sequence directing protein to ER
- Pro Zn interacting thiol (SH) which is inactive until mature
- Zn catalytic domain
- Hemopexin like domain – mediates interactions of inhibitors of MMP and other molecules

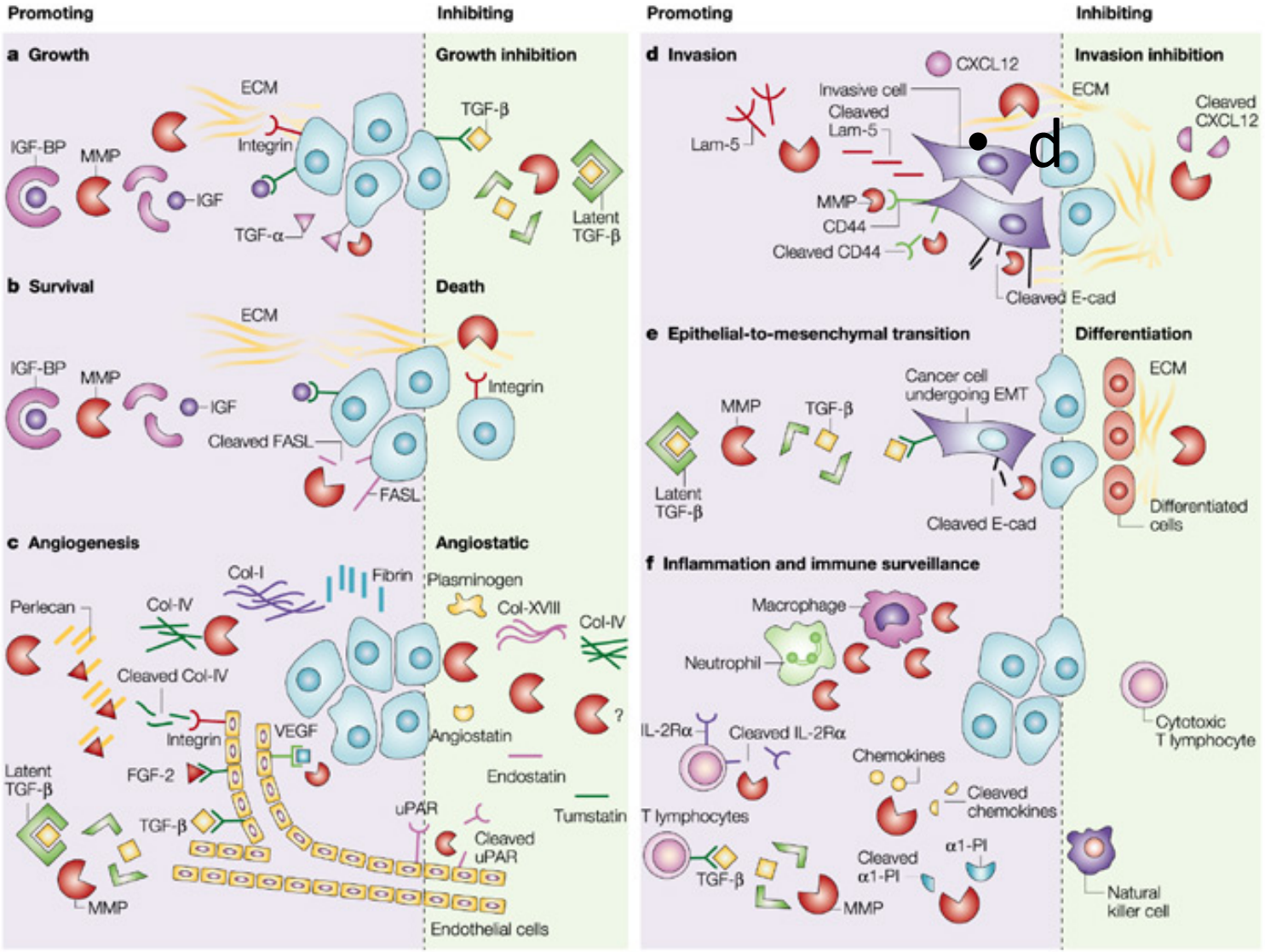


MMPs Produced by tumor/ tumor associated cells

- BM creates network of proteins with small pore size – too small for most cancer cells to fit through
- Invadapodia – regions at leading edge of cell where MMP is secreted or tethered to membrane producing localized BM degradation
- Stromal cells release or activate MMP2/9 to degrade collagen coordinating with tumor cells MT-MMP
- Acidification of ECM by tumor cells activates MMP and supports secretion

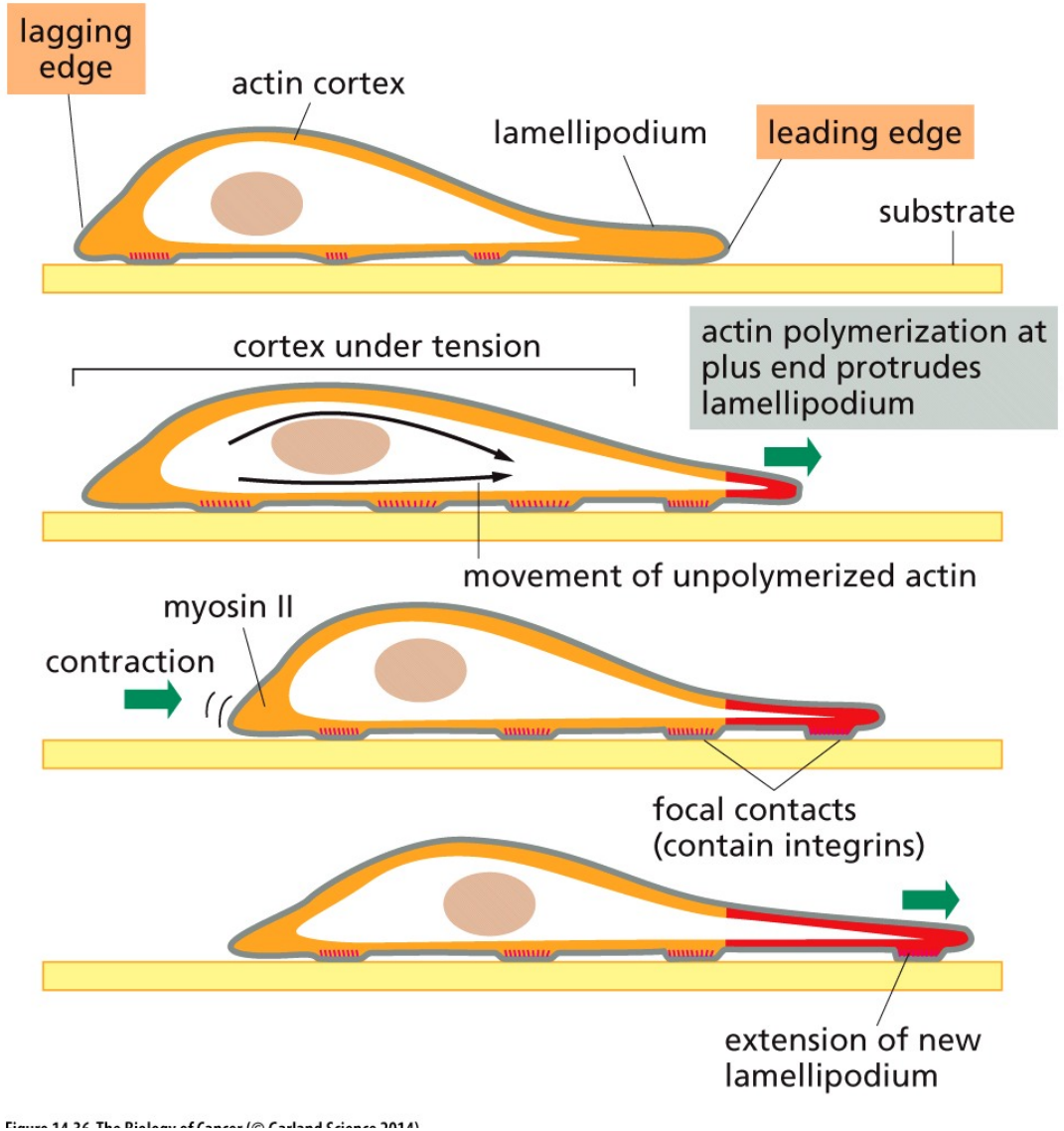


Matrix metalloproteinases (MMPs) have both cancer-promoting and cancer-inhibiting functions, and pathways with opposing effects on cancer progression are sometimes initiated by cleavage of the same substrate



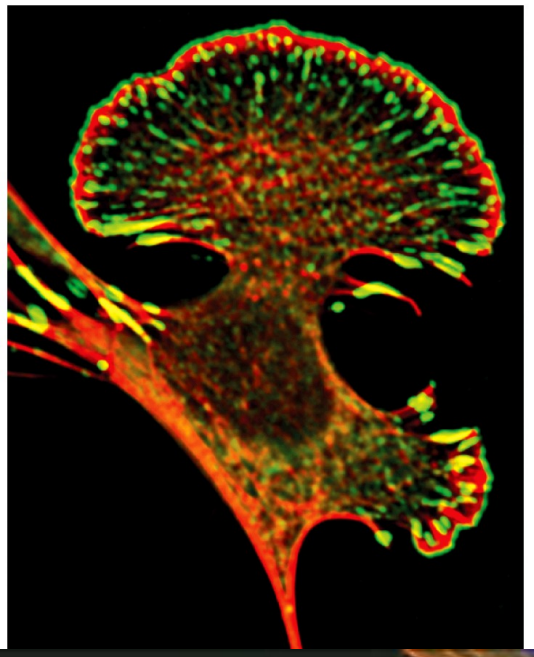
Cell motility requires coordination of many proteins – driven by small G proteins

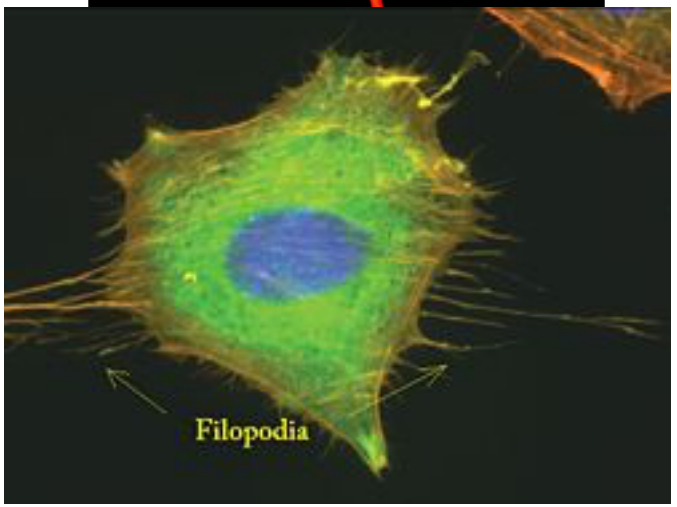
- Cell motility involves cytoskeletal protein at both leading and trailing edges.



Grab on, Let go, Get away

- At the leading edge of a motile cell – actin polymerizes (stress fibers) to form subcell structures and form focal adhesions.
- MMPs lead the edge
- Membrane proteins anchor stress fibers by linker proteins
  - Often exchange proteins
  - Linker proteins ERM family
- Lamellipodia – broad sheetlike protrusion (ruffles)
- Filopodia are small spikes from lamellipodia





Actin Stress Fibers – regulated by SMG

- RhoA kinase activates machinery to grow the barbed end

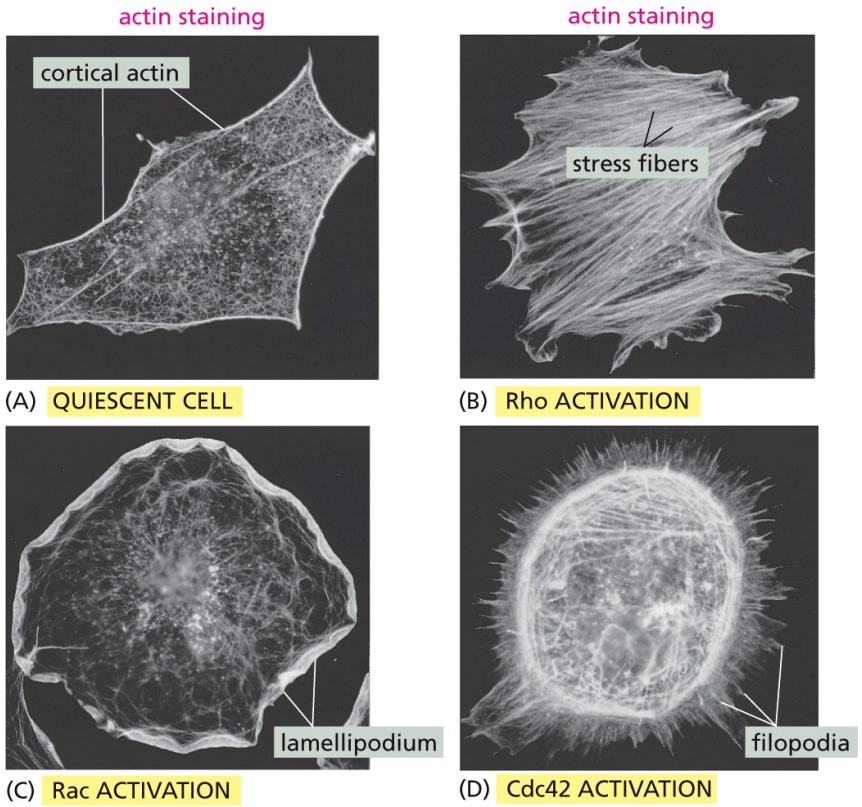


Figure 14.39 The Biology of Cancer (© Garland Science 2014)

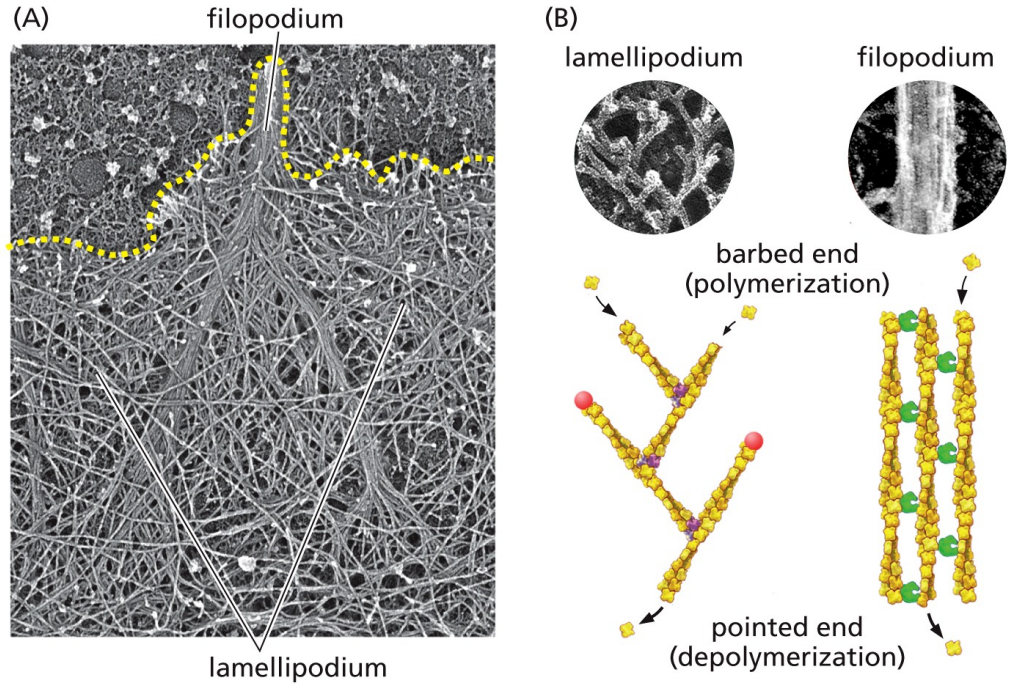


Figure 14.38 The Biology of Cancer (© Garland Science 2014)

- Rac and CDC42 both differently impact cell structure

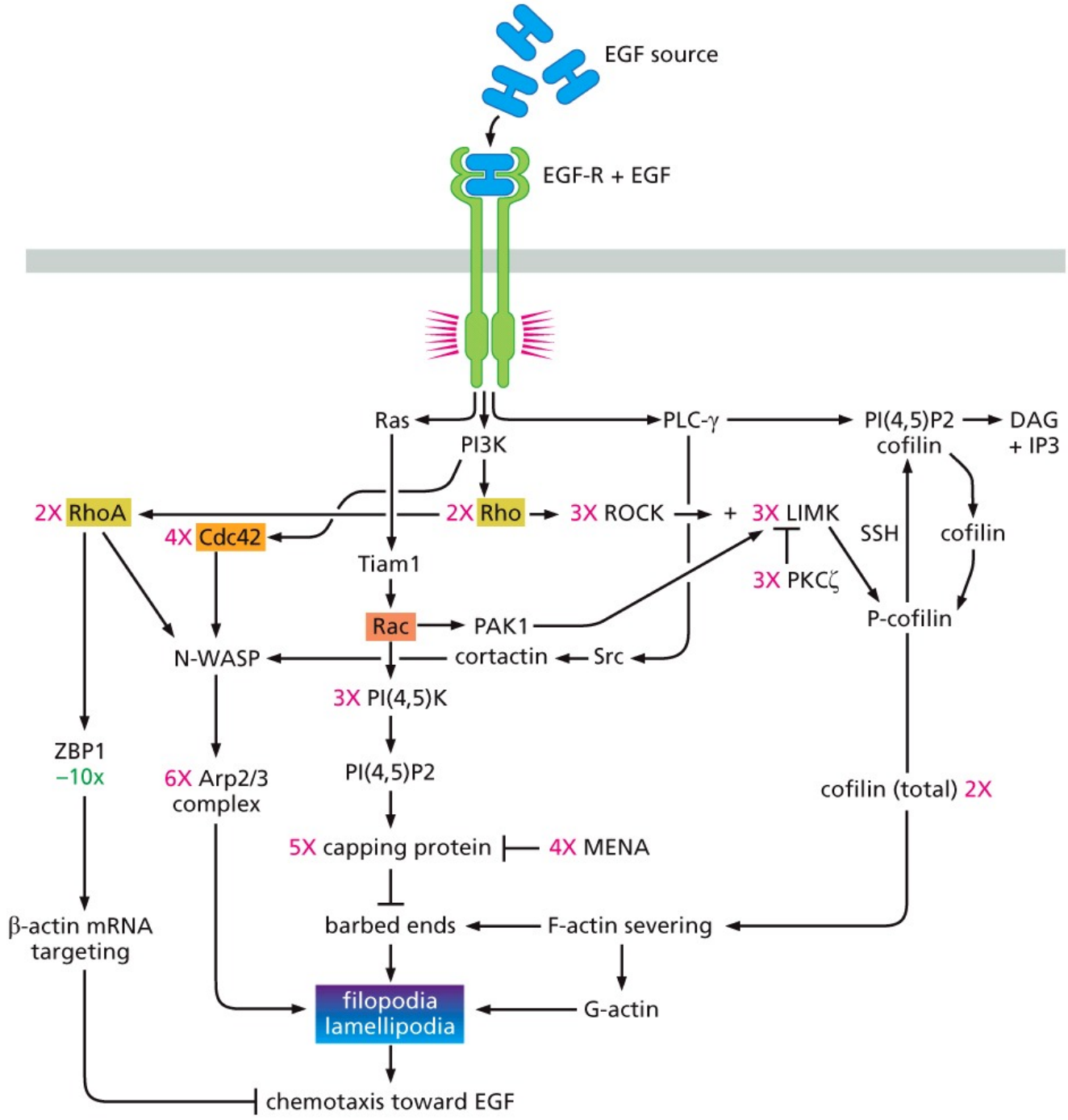
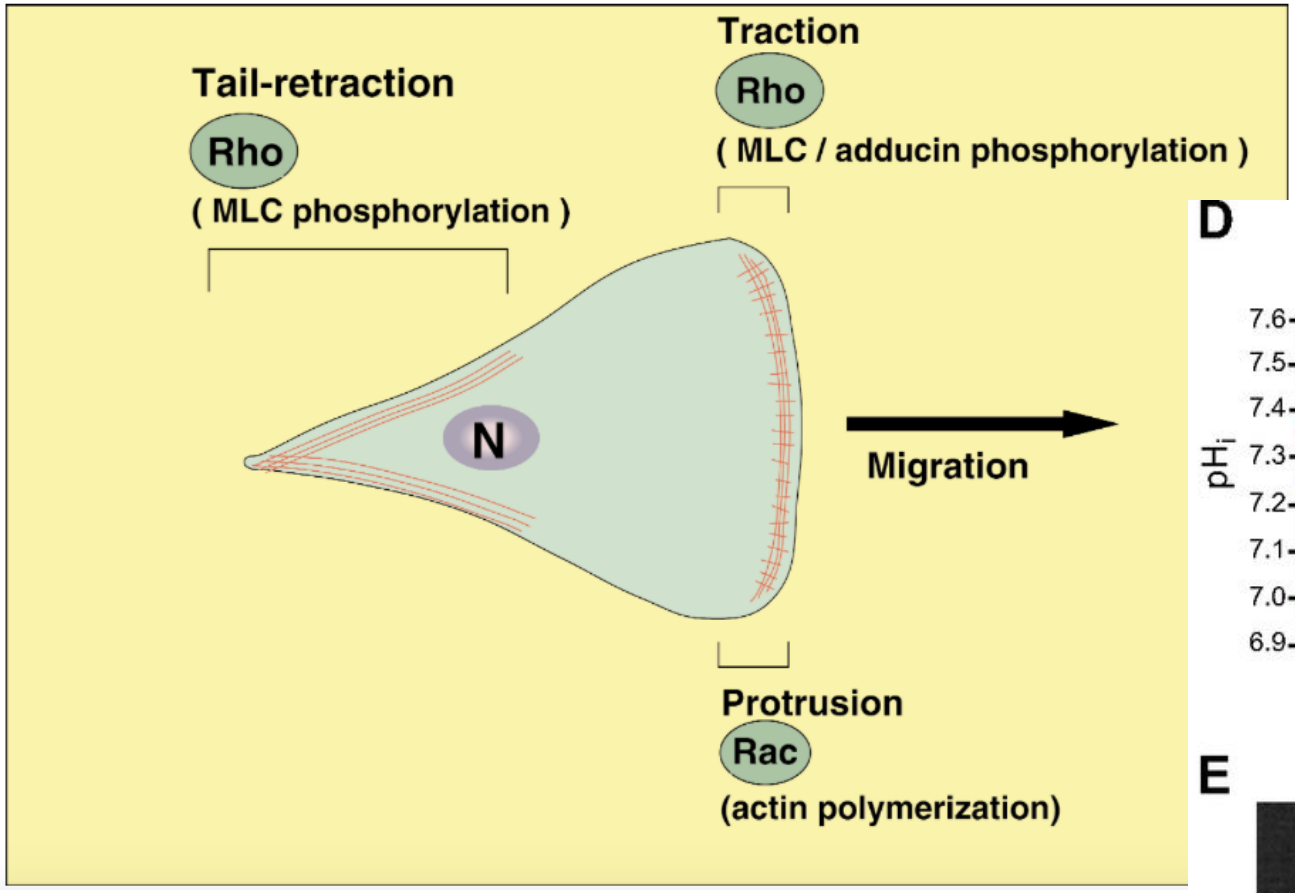
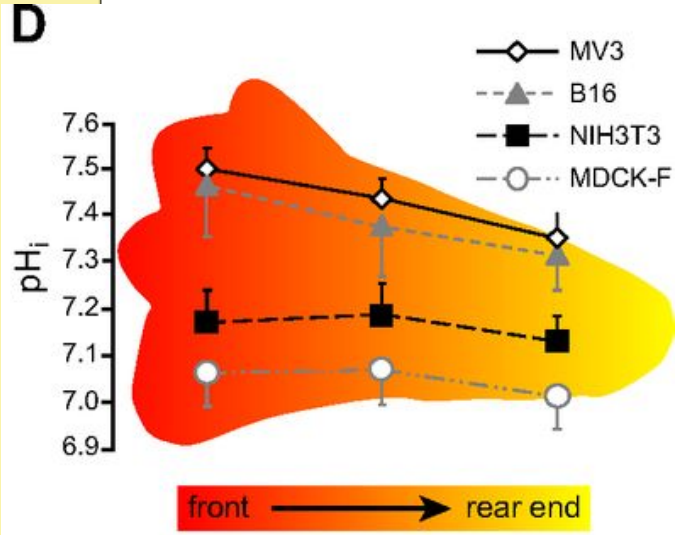
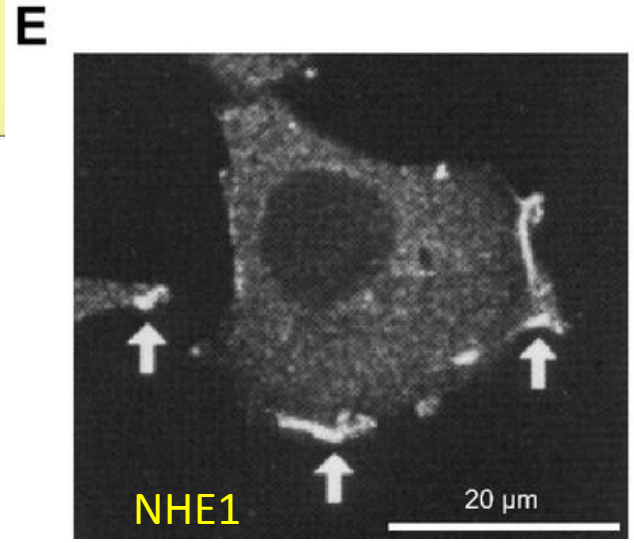


Figure 14.40 The Biology of Cancer (© Garland Science 2014)

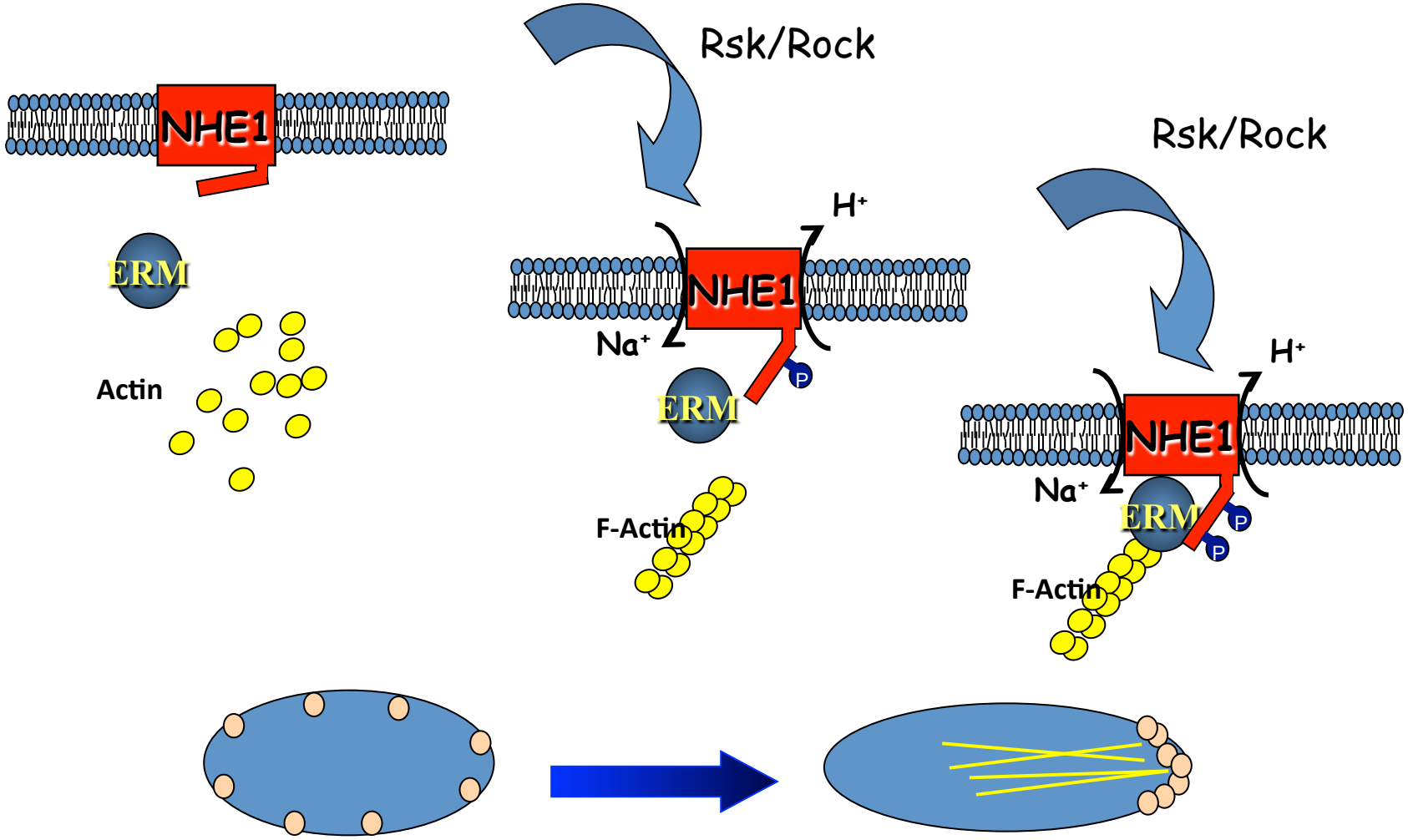
Cell Polarity and Motility

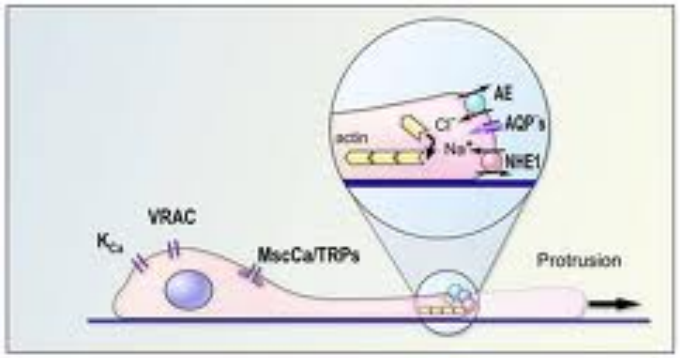


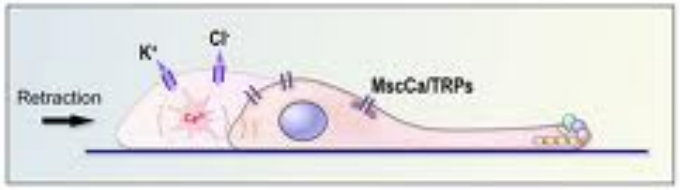




NHE and Migration

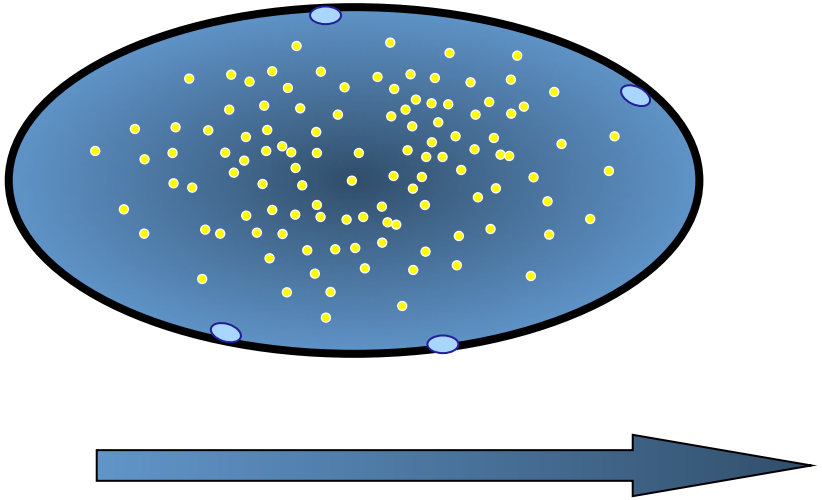






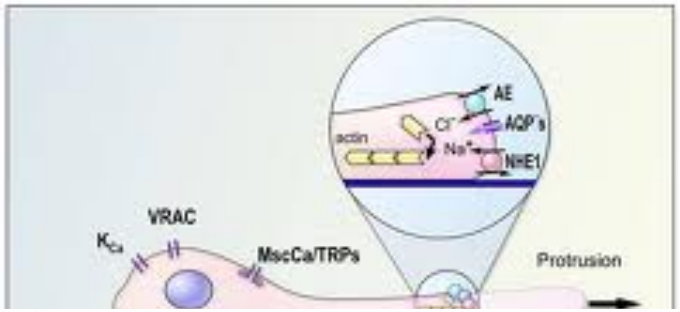
Focal Adhesions and Stress Fibers - Invadapodia

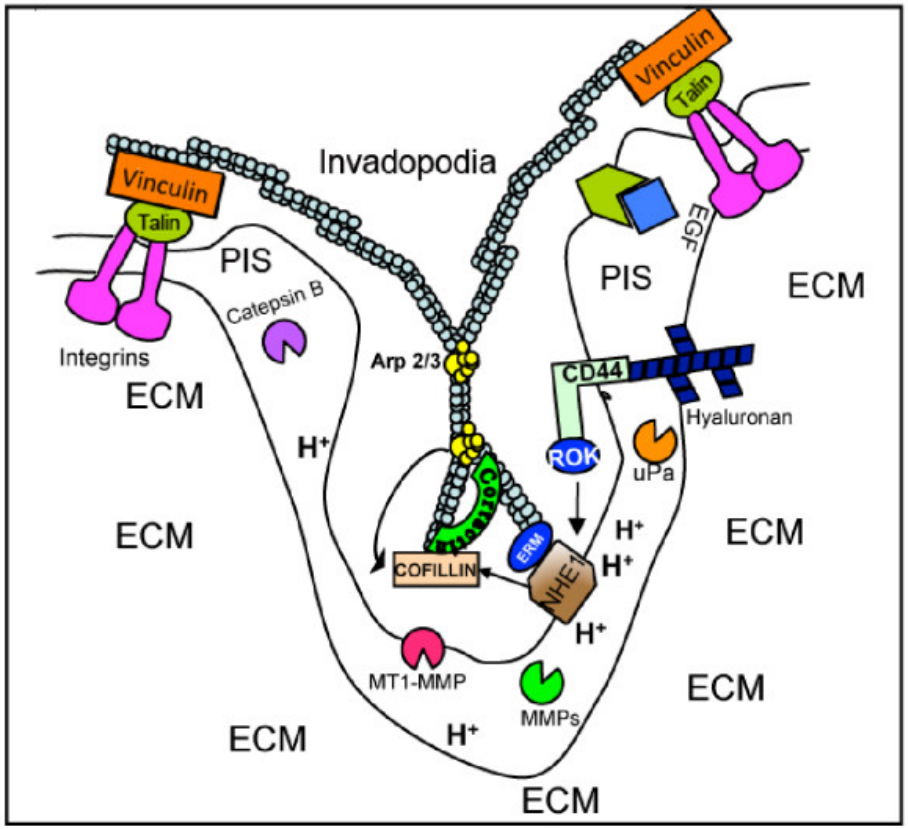
- NHE1 collects at the leading edge of migrating cells
  - ✓ Activates actin polymerization
  - ✓ Binds ERM proteins
    - Ezrin, radixin, moesin
  - ✓ ERM binds actin filaments driving cell migration
- NHE1 is a major participant supporting the hallmarks of cancer

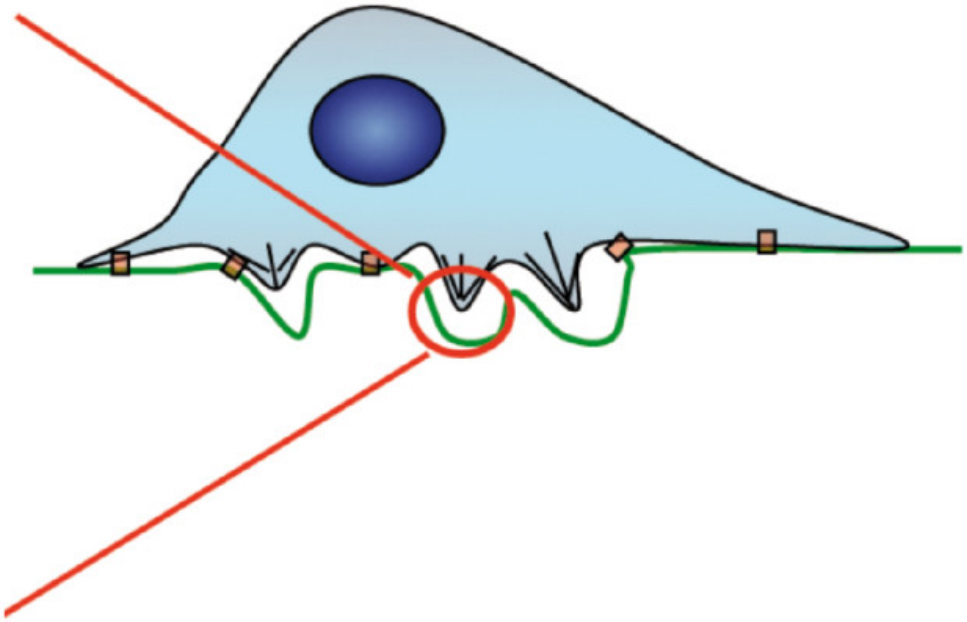


Direction of Migration

Focal Adhesions and Stress Fibers - Invadapodia







Direction of Migration

- NHE1 is a major participant supporting the hallmarks of cancer

Now that we are moving… where to?

- Colonization of micromatastasis is very inefficient where most never survive or remain in stasis.
- Tumors seem to target organs with highly specific stromal cell/ supportive environment.
  - Endothelilial cells exert an influence on ability of carinoma cells to form petastasis in specific organs
  - Some tissues require fewer changes for metastasis to adapt
  - Seed and Soil Hypothesis

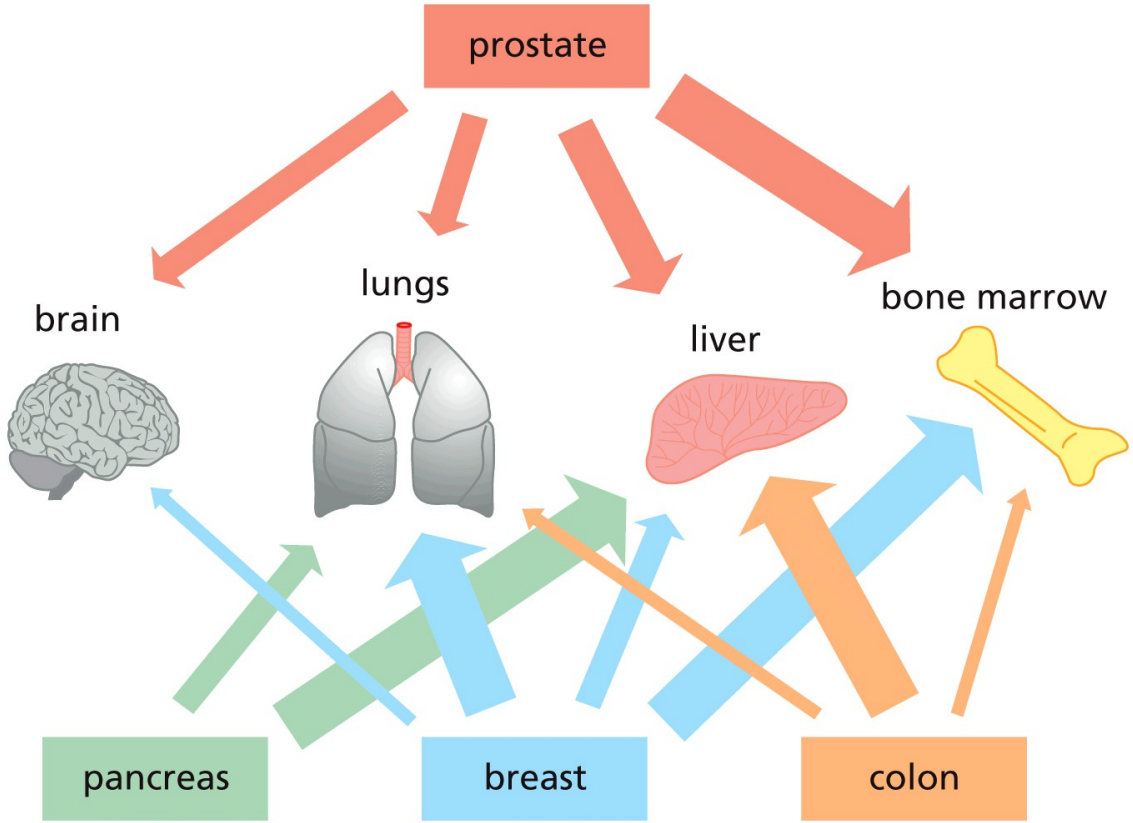
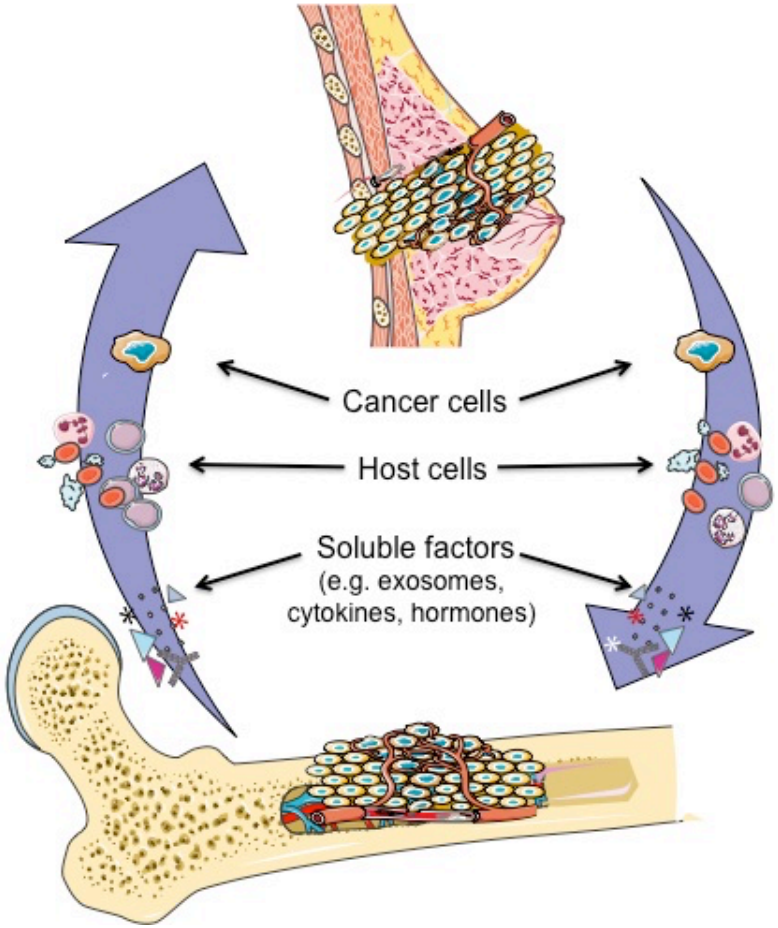
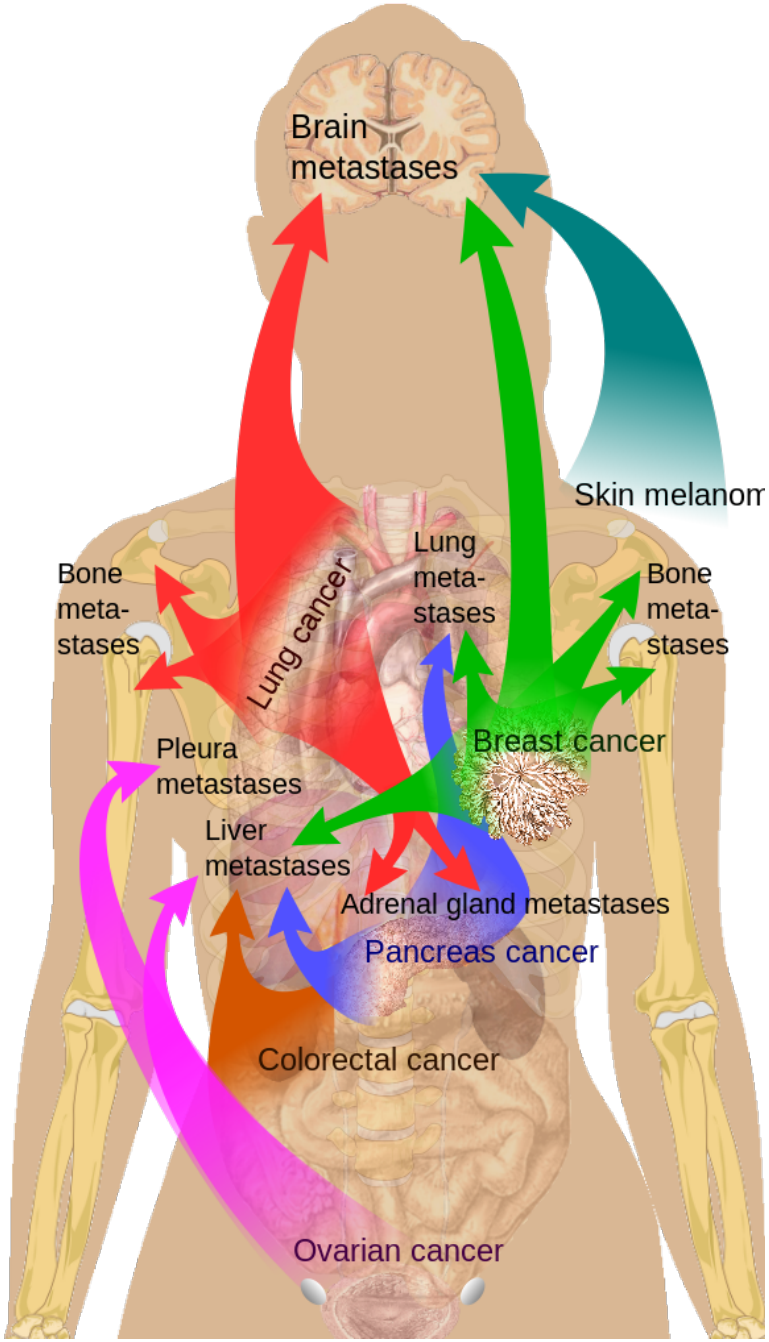


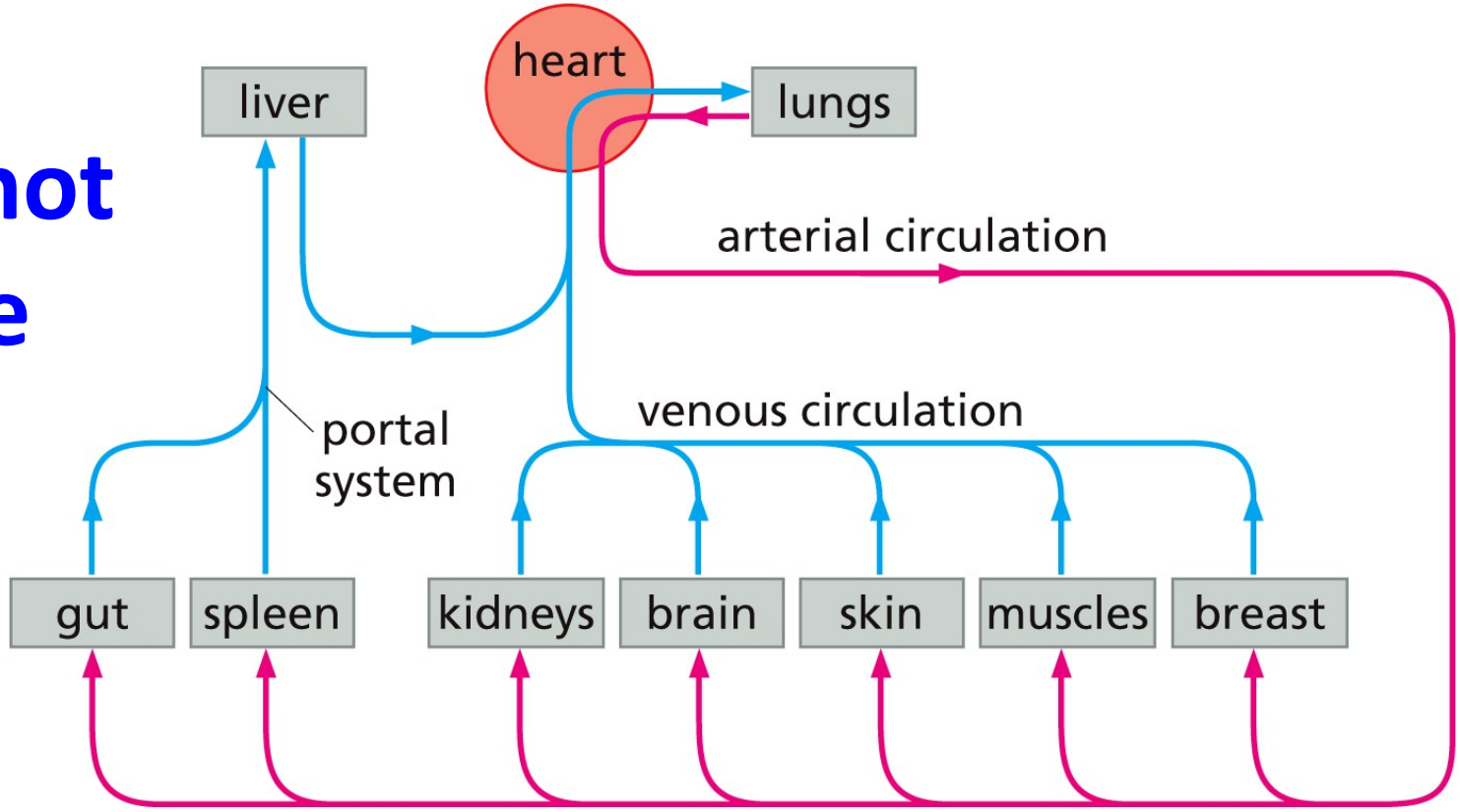
Figure 14.43 The Biology of Cancer (© Garland Science 2014)



- Hypothesis is that patterns of metastasis formation in patients could not be explained by random scattering or by patterns of cells through circulation
- A plant goes to seed, its seeds are carried in all directions; but they can only live and grow if they fall on congenial soil



…but not quite



- This seems to work for several (like breast) cancers but not all.
- Colon cancers may settle in liver simply due to proximity via portal vein – trapped in small capillary beds – sheer numbers of cells influence ability to form distal tumor sites
- Secondary breast tumor development in lungs is poor but happens due to frequency of implantation/extravasion
- Gives rise to a “filter and flow” design to cell location
- Secondary issue is more likely metastatic tropism – which involves both filter and microenvironment creating a permissivee site for colonization

Metastasis to bone – an example of seed and soil

- Osteotropic cancers: ~50-70% of patients with breast and prostate cancer metastasize to bone (lung and kidney cancer at high levels too)
- Once bone is colonized, metastasis are incurable and result in morbidity (rate of sickness or symptoms of disease) prior to patient death.
- Bone metastases leads to significant pathological fractures, pain, nerve compression and hypercalcemia.
- Treatments are mostly palliative (treat pain not disease)

Bone remodeling is a constant event – bone (collagen matrix with calcium phosphate and metal ppt – hydroxyapatite) is dissolved and replaced by two sets of cells – up 10% of skeletal mass is dissolved and replaced each year

Osteoclasts – derived from precoursor phagocytes and are responsible for bone resorption

Osteoblasts – specialized cell from stromal lineage responsible for laying down new matrix (collagen and other matrix)

Bone Remodeling

- RANK Ligand (Receptor activator of NFkB or RANKL) is a protein ligand on surface of osteoblasts which bind receptors on osteoclast precursors leading to osteoclast maturation
- This is mediated by another osteoblast receptor - osteoprotegerin (OPG), which binds and blocks RANKL signaling
- Balance of signaling between RANKL and OPG (along with other factors control balance of osteoblast and osteoclast production and activity

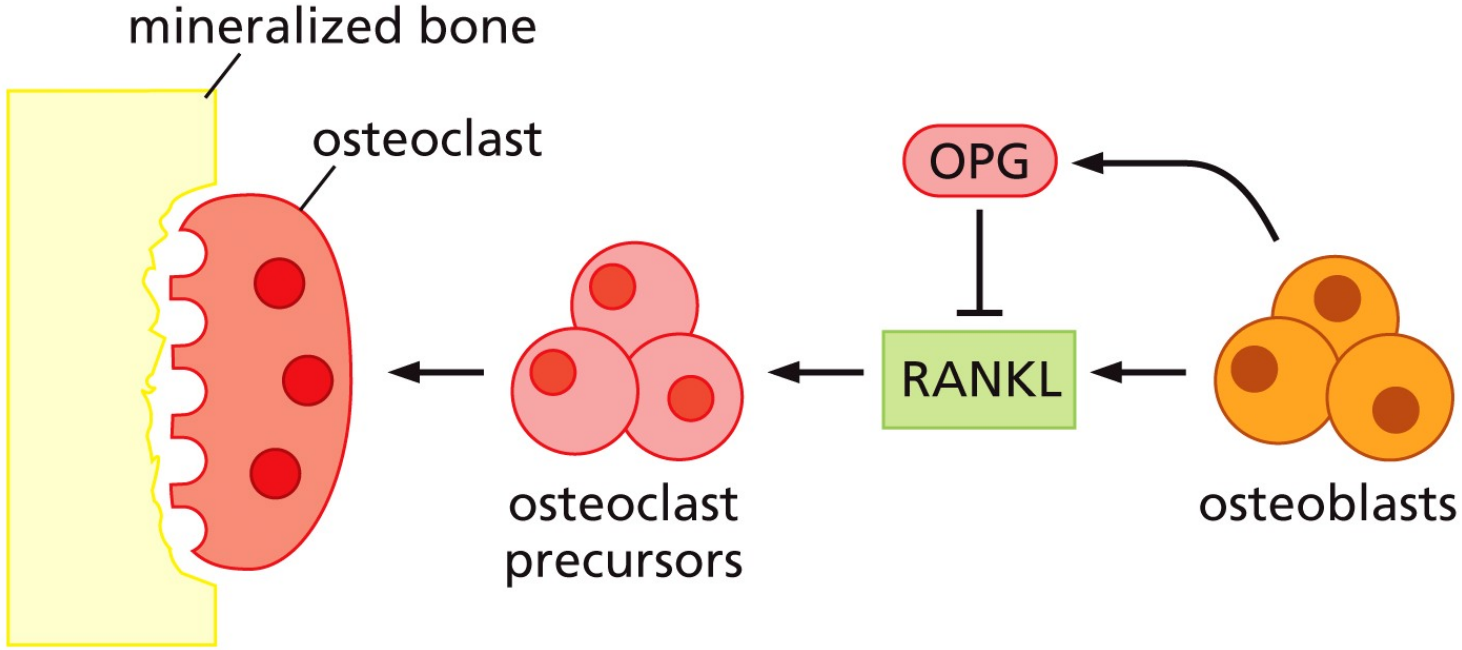
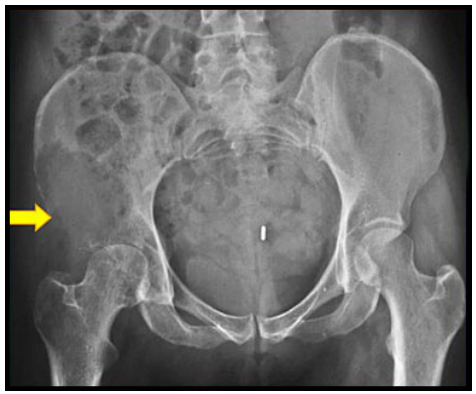
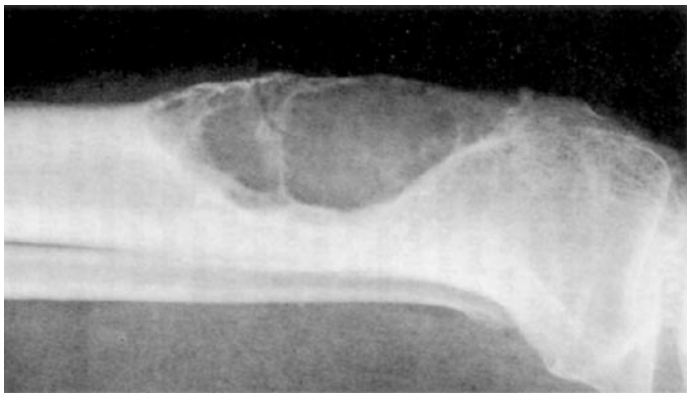


Figure 14.48a The Biology of Cancer (© Garland Science 2014)

Two types of bone cancer

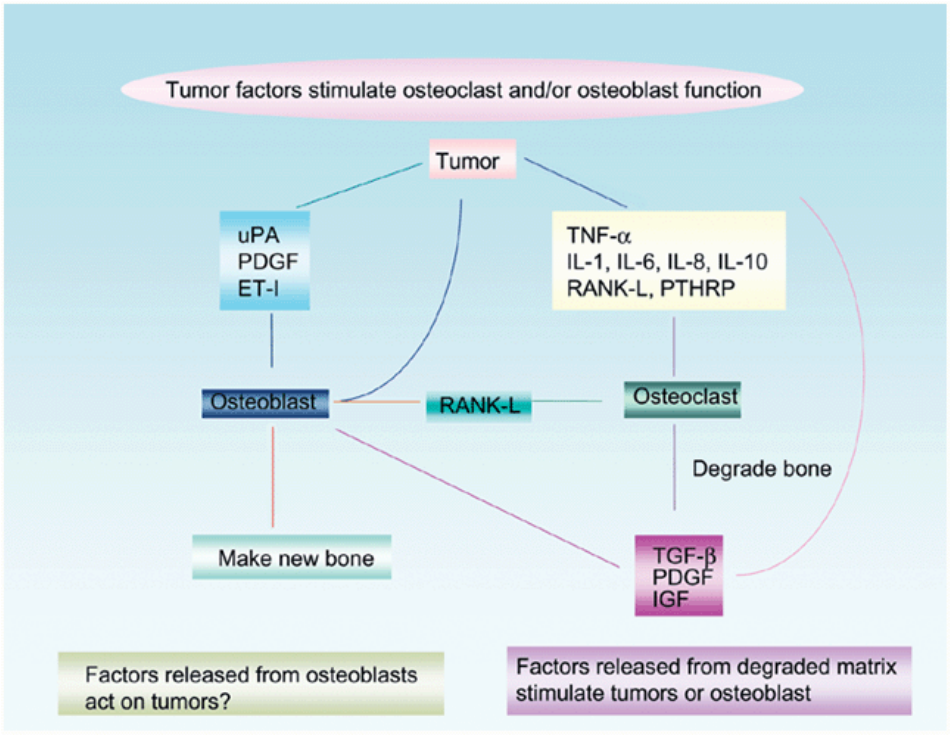
- Osteolytic -(most common) leads to bone destruction (osteoclastic)
- Osteosclerotic – leads to bone growth/formation (osteoblastic)
  - Prostate cancer are usually osteoblastic while breast and lung are osteolytic



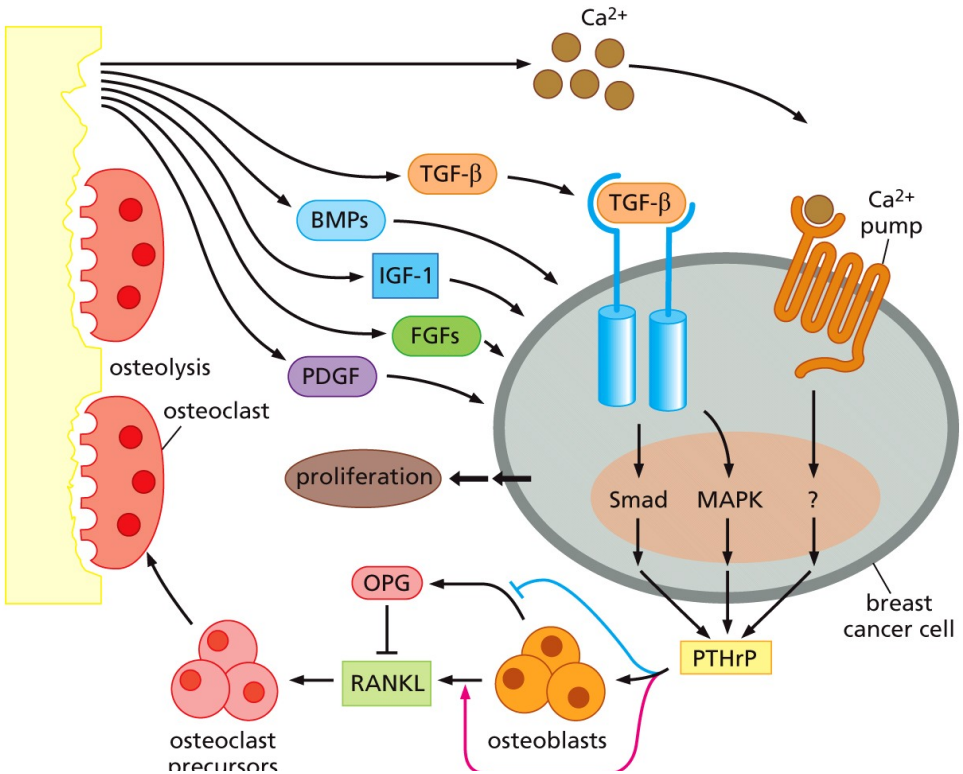


- Most *in vivo* studies indicate that osteolysis is caused by osteoclast stimulation, not by the direct effects of cancer cells on bone. Osteolytic metastases are associated with increased osteoclast activity and reduced osteoblast activity that is uncoupled from bone resorption

Components that contribute to bone metastasis growth – a vicious cycle



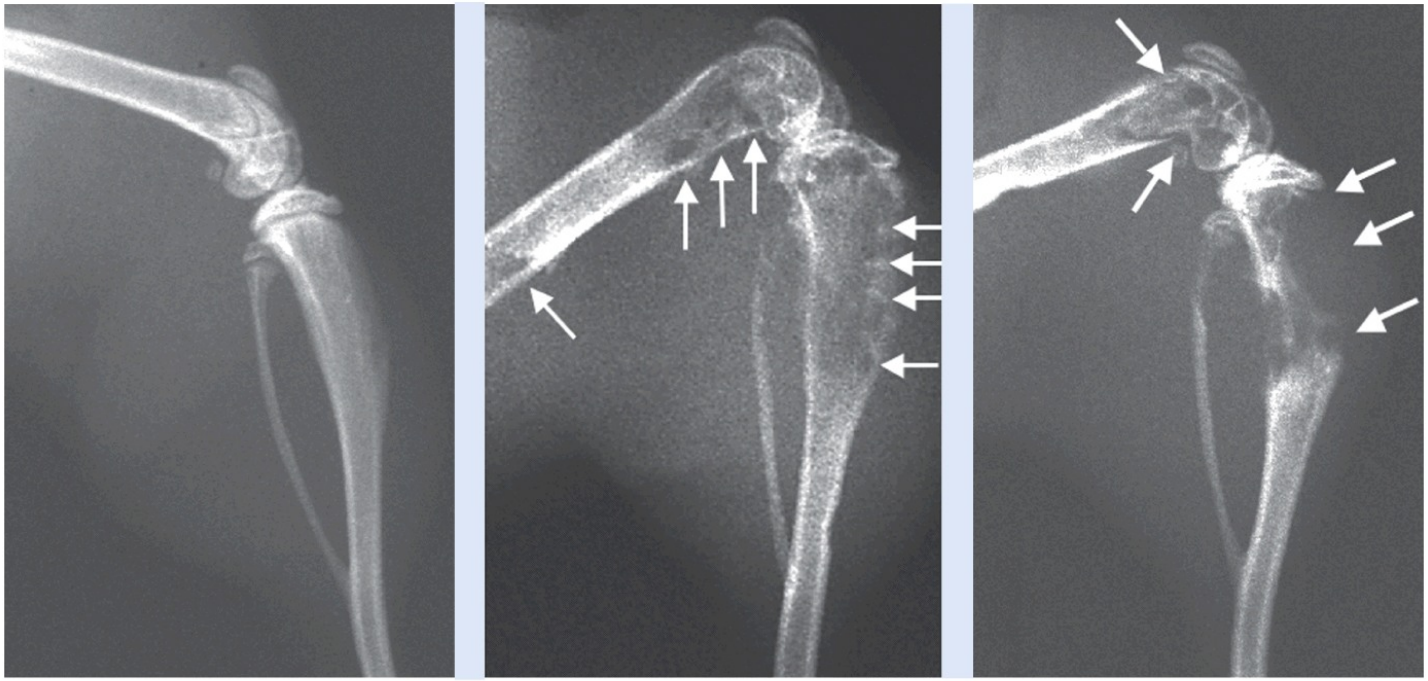
- It is becoming evident that osteolytic bone metastases are derived from a complex cycle of progressive interactions between tumor cells and the bone microenvironment, which has been called a "viscous cycle"
- Characteristics that allow tumor cells to localize to bone, tumor derived-factors that stimulate responses from osteoclasts and osteoblasts, and stored factors within the bone matrix contribute to this cycle



- Bone ECM contains several growth and tropic factors for several carcinoma survival
- Removal of bone minerals, exposing ECM allows carcinoma cells to gain access to substrate and growth medium
- Carcinomas reach bone stromal cells via feeding blood vessels

- Breast cancer cells relaste parathyroid homone-related peptide (PHTHrP) which cause osteoblast to release RANKL – free RANKL in turn binds and activates formation of osteoclasts – normal method of achieving calcium in breast cells – hyper-driven in cancer
- Growth factors released by osteoclasts include FGF, BMP, TGF and others which stimulate further tumor growth inducing more PHTHrP release “vicious cycle”

MDA-MB-231cells transfected with:



dn TGF-βRII

dn TGF-βRII
+ ca TGF-βRI

dn TGF-βRII
+ PTHrP

- Human breast cancer cells injected in mice produce osteylytic tumors
- Dominant negative TGF receptor expression in these cells blocks tumor formation indicating the importance of growth factor stimulation in cycle
- Replacement (exogenous expression) with constitutively active receptor restores lytic tumor potential
- Loss of TGF receptor plus expression of parathyroide hormone drives lytic tumors indicating the PTHrP is downstream or supplemental to TGF and that TFG may stimulate PTHrP release…