

CANCER CELL MEMBRANES AND METASTASIS

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CANCER CELL MEMBRANES AND METASTASIS - Lecture Outline

1. Changes at the cancer cell surface
2. Cell surface composition
3. Mucins
4. Cell adhesion
5. Agglutinability of cancer cells
6. Surface proteolytic activity
7. Increased transport
8. Secretion and shedding from cancer cells
9. Epithelial-Mesenchymal Transition and Metastasis

CHANGES IN CANCER CELL MEMBRANES

Changes at the cell surface of cancer cells in vivo are suggested by the tendency of cancer cells to show invasion of surrounding tissues and metastasis to distant sites. The loss of contact inhibition in transformed cells in vitro also suggests cell surface changes. Neoplastic cell proliferation might be related to a number of factors:

1. Loss of feedback control between surface and nucleus
2. Presence of surface proteolytic activity
3. Loss or modification of specific surface glycoproteins and glycolipids
4. Altered nucleotide cyclase activity in the membrane
5. Increased solute transfer of essential nutrients
6. Antigen changes

CELL MEMBRANE COMPOSITION

The chief membrane components are lipids, proteins, glycoproteins, glycolipids and glycosaminoglycans.

There is increased sialic acid in many human tumors but usually less sialic acid in membranes of transformed cultured cells. In many human tumors the content of cholesterol and phospholipid is higher than the corresponding normal tissues. However, mouse and rat leukemic lymphocytes have similar amounts of cholesterol compared to normal lymphocytes. Membrane phospholipids do not vary significantly in many tumor systems compared to normal or untransformed cells, although minor shifts in fatty acid composition have been reported.

CELL MEMBRANE COMPOSITION

Cholesterol decreases the fluidity of membranes. In human leukemia and lymphoma cells there is increased fluidity which has been attributed to the decrease in cholesterol in the membranes or a change in the phospholipid to cholesterol ratio. When cholesterol was added to leukemia cells their membrane viscosity was restored to normal. Animals injected with treated leukemia cells had longer survival times than those injected with untreated leukemic cells. However, many solid tumors show normal membrane fluidity so this change is not a general phenomenon.

CELL MEMBRANE COMPOSITION

In transformed hamster fibroblasts there is

(a) decreased amounts of more complex glycolipids or deletion of terminal saccharide residues

(b) inability of glycolipid synthesis to respond to cell contact with the addition of terminal saccharide sequences

(c) enhanced glycolipid accessibility to antibodies, enzymes and lectins. These types of changes are found in many but not all malignant cells.

MUCINS IN CANCER

Mucins are large extracellular proteins that are heavily glycosylated with complex oligosaccharides.

Cancer cells, especially adenocarcinomas, express aberrant forms or amounts of mucins. The expression of distinct oligosaccharide structures, together with differential glycosylation of mucin core proteins provides an enormous range of potential ligands for interaction with other receptors at the cell surface.

Mucins may contribute to tumor invasion by disrupting interactions between normal cells and establishing new ligands for interaction between invading cells and the adjoining cells.

Two of the most widely used serum diagnostic tests for adenocarcinomas (CA19-9 and CA125) recognize epitopes that are found on mucins. Several clinical trials have targeted mucins that are expressed by adenocarcinomas including monoclonal-antibody-based therapies and tumor vaccines.

Reference: M.A. Hollingsworth and B.J. Swanson, Nature Reviews Cancer 4: 45-60, 2004.

CELL ADHESION AND SIGNALING BY CADHERINS AND IG-CAMS

Cell-adhesion molecules mediate intercellular and cell-matrix interactions. Changes in the expression or function of these molecules can contribute to tumor progression by altering the adhesion status of the cells and by affecting cell signaling.

The function of epithelial (E)-cadherin is decreased in most epithelial tumors during cancer progression. **Loss of E-cadherin function elicits active signals that support tumor-cell migration, invasion and metastasis.** Loss of E-cadherin can be accompanied by increased level of other cadherins such as N-cadherin which promotes tumor cell motility and migration.

NCAM is a member of the Ig-CAM family (immunoglobulin-like cell adhesion molecules). It is markedly down regulated in astrocytomas and in colon and pancreatic cancer and this loss is correlated with poor prognosis.

Reference: U. Cavallaro and G. Christofori, Nature Reviews Cancer 4:118, 2004.

DELETION OF FOCAL ADHESION KINASE

Loss of cellular focal adhesions is crucial to the development and progression of invasive cancer. Specific deletion of focal adhesion kinase has been shown to suppress tumor formation and block malignant progression

Reference: McLean GW et al., Genes Dev. Dec. 2004.

INTEGRINS

Integrins are a group of transmembrane proteins that form dimers by the association of alpha and beta subunits. They are involved in signal transduction across the cell membrane. Extracellular matrix components bind to the integrins and initiate an intracellular signaling cascade that results in the formation of a focal adhesion complex consisting of cytoskeletal and signal transduction molecules.

Some integrins are expressed constitutively and others such as $\alpha_v\beta_3$ and $\alpha_5\beta_1$ are expressed by host endothelial cells only when activated as in wound healing and more pathological states such as angiogenesis, solid tumor growth, diabetic retinopathy and rheumatoid arthritis.

Tumor formation and progression can be associated with an altered pattern of integrins.

INTEGRINS

Integrins can act to localize proteases such as matrix metalloproteinases (MMPs) to the leading edge of the cell to support migration and invasion. The majority of MMPs may be produced by fibroblast cells associated with tumor cells. This expression pattern may be related to a tumor cell surface glycoprotein named extracellular matrix metalloproteinase inducer (EMMPRIN). The expression of EMMPRIN in breast cancer correlates with tumor size and staging and is predictive of poor prognosis.

AGGLUTINABILITY

Most tumor or transformed cells agglutinate in the presence of lectins at much lower concentrations than their untransformed counterparts. The major agents studied have been wheat germ agglutinin and concanavalin A. High saturation densities during in vitro cell growth are generally indicative of elevated tumorigenicity and for many cells their lectin agglutination properties directly follow their ability to grow to high densities in vitro. Normal and transformed cells differ not in the number of receptors but in their distribution according to most workers although increased numbers have been reported. Nicolson suggested that lectin binding will be affected by:

- a. saccharide binding constant
- b. number of binding sites
- c. net charge
- d. size.

AGGLUTINABILITY

In normal cells there is less surface mobility of the receptors as shown with ferritin-labeled concanavalin A. There is a greater concentration in cancer cells (capping) as opposed to the random distribution in normal cells. Hynes proposed that outer surface peripheral components such as fibronectin, which is lost or reduced on transformation, may control the mobility of surface glycoproteins and therefore regulate cell agglutination properties. Fibronectin is a glycoprotein and was originally described as a large, external, transformation sensitive (LETS) protein.

Nicolson suggested that a change in one or more of the cell surface restraint systems (microtubules, microfilaments and thick filaments) is the most probable cause of altered mobility of surface components and can be blocked by drugs such as colchicine, and vinblastine.

SURFACE PROTEOLYTIC ACTIVITY

Increased proteolytic activity at the surface of cancer cells does not by itself trigger the neoplastic transformation. During mitosis normal cells decrease in adhesive nature. Surface proteolytic activity by plasminogen activator, a serine protease, favors the conversion of plasminogen to plasmin. A variety of proteolytic enzymes including collagenase have been reported to increase in activity.

The release of several enzymes by tumor cells has been attributed to leaky membranes. These include glycolytic enzymes, cathepsins, collagenases and plasminogen activator. The fibrinolytic activity of tumor cells appears to arise from the plasminogen activator. Viral transformation of chick embryo fibroblasts is accompanied by increased release of plasminogen activator which can be detected before morphologic change. Transformation of rodent fibroblasts also increases fibrinolytic activity. Reich and coworkers showed that the prominent fibrinolysis of neoplastic cells depend upon:

- a. the presence of normal serum plasminogen
- b. release of plasminogen activator from neoplastic cells. SV40-transformed cells show a greater migration rate on a solid medium than normal cells this rate depend strongly on the availability of plasminogen.

SURFACE PROTEOLYTIC ACTIVITY

The plasminogen activators are serine proteases inhibited by diisopropylphosphofluoride with an arginine specificity. When normal cells are co-cultivated with transformed cells, the normal cells take on the morphology of transformed cells by rounding up and multilayering. Exposure of normal cells to plasmin can also cause these changes.

Density dependent modulations of release of plasminogen activator as seen with normal fibroblasts do not occur in transformed cells.

Release of hydrolases by tumor cells may

1. cause destruction of normal tissues and facilitate infiltration
2. release nutrients from normal cells
3. interfere with normal surface recognition
4. alter antigenic properties of cancer cells.

Release of urokinase plasminogen activator (uPA) and the activation of extracellular proteins

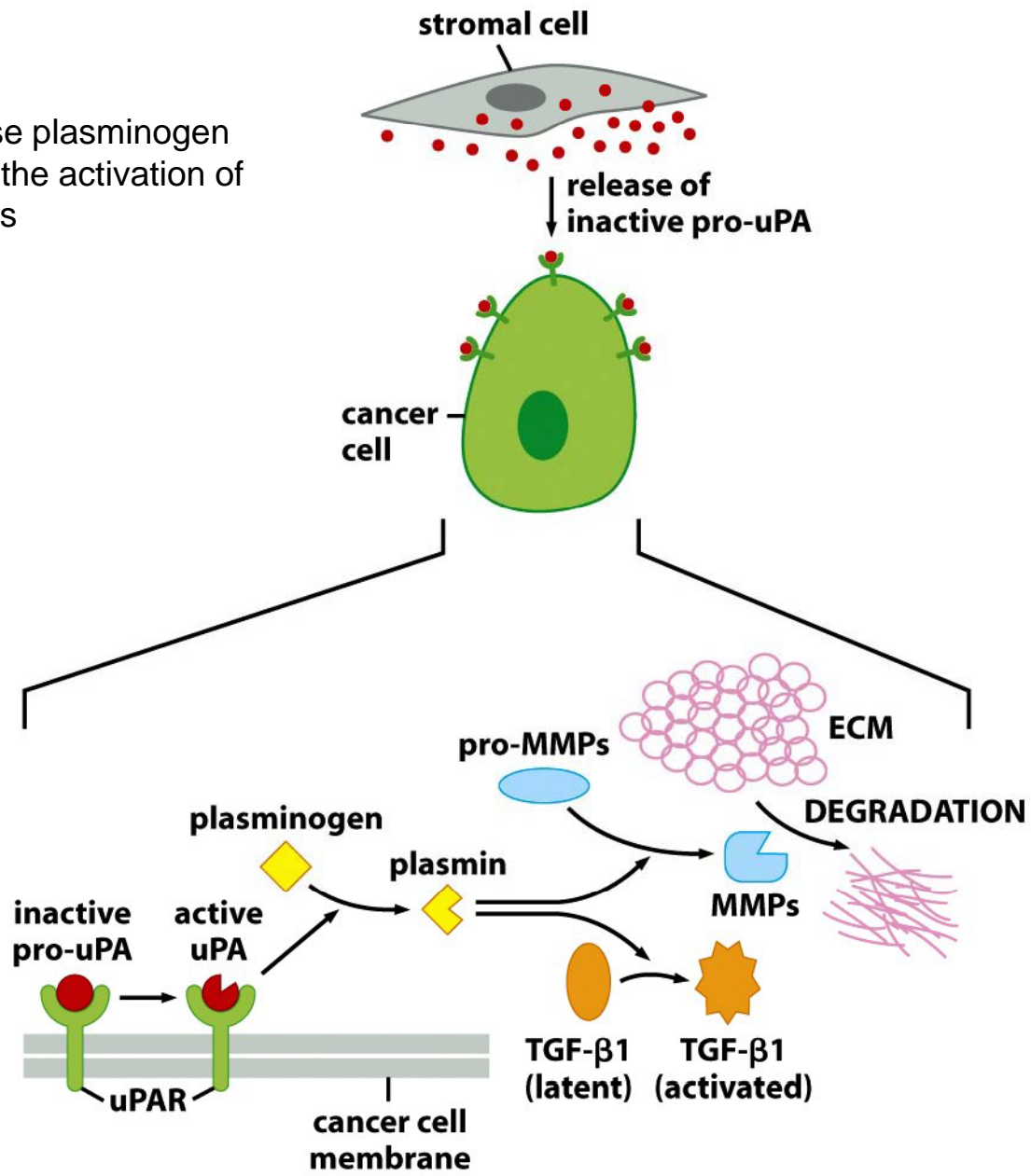


Figure 14-34 The Biology of Cancer (© Garland Science 2007)

INCREASED TRANSPORT

Transformation is often accompanied by increased transport of glucose, amino acids, phosphate and uridine. The best evidence for increased transport of nutrients has been observed with cultured cells.

After transformation of mammalian or avian fibroblasts by oncogenic DNA or RNA viruses there is increased hexose uptake. Studies with temperature sensitive viral mutants indicate that oncogenic conversion rather than the presence of the viral genome is necessary for the changes in metabolite transport. This is further supported by observations that neoplastic transformation by SV40 virus induces alterations in sugar and amino acid transport prior to lysis. More than one mechanism may operate. Oncogenic transformation with DNA viruses caused a three-fold increase in V_{max} for 2-deoxy-D-glucose in chick embryo fibroblasts whereas murine sarcoma viruses produced a 10-20 fold decrease in K_m .

INCREASED TRANSPORT

Amino acid transport has been studied most extensively with the non-metabolizable amino acid analog alpha-aminoisobutyrate and cycloleucine. Neoplastic transformation of mouse or hamster fibroblasts by DNA viruses increased the V_{max} by a factor of 2-3. No change in K_m was observed. Changes in K_m would be more significant for amino acid uptake than for glucose. The circulating glucose concentration (about 5 mM) is considerably above the K_m for glucose transport in both normal and transformed cells. However, the plasma concentrations of essential amino acids (0.02-0.2 mM) are well below the K_m values for amino acid transport into cells.

Not all changes in neoplasia indicate increased uptake of metabolites. Kelley and Potter found primary hepatomas in rats can have decreased uptake of alpha-aminoisobutyrate. Decreased orotate uptake occurs in liver and kidney tumors. In the case of amino acids it is the A system for small neutral amino acids which tends to show increased activity while the L system is unchanged.

If cancer cells had typical junctions with normal cells the relative advantage of increased metabolite uptake would be diminished. It has been suggested that there maybe a failure in junctional communication in cancer cells that permits levels of metabolites to be attained which act as a signal for cell division.

SECRETION AND SHEDDING FROM CANCER CELL SURFACES

It has long been suggested that cancer cells may secrete growth promoting polypeptides. In addition to being mitogens these polypeptides might cause morphological changes and anchorage-independent growth and the loss of density-dependent inhibition of growth. Receptors for the polypeptides would be on the cell surface.

Sporn and Todaro proposed the term autocrine secretion for this type of self stimulation. They called the polypeptides “transforming growth factors” (TGFs). TGFs have been isolated from the medium of cultured mouse and human tumor cell lines. They are of relatively low molecular weight (6,000 to 20,000 daltons). They are acid stable and inactivated by dithiothreitol. More recent work has shown that oncogene products can be growth factors or modified growth factor receptors.

SECRETION AND SHEDDING FROM CANCER CELL SURFACES

Secretion implies exocytosis but many cell-surface components can be lost by simple shedding. It has been suggested that the release from malignant cells of molecules such as fibronectin or certain glycosaminoglycans may be responsible for the loss of adhesion which characterizes cancer cells.

In some animal models, cells from the most malignant and most rapidly metastasizing tumors had the least amount of glycocalyx due to continuous shedding. Furthermore the release of cell-surface tumor antigens may reduce the cell's vulnerability to immune attack.

Events in the metastatic process

In about two thirds of patients cancer has already metastasized at the time of diagnosis. Metastasis requires a series of events:

1. Disruption of the basement membrane
2. Cell detachment
3. Cell motility
4. Invasion
5. Penetration of the vascular system
6. Survival in the circulation
7. Arrest (stasis)
8. Extravasation and proliferation

Reference: McKinnell et al., The Biological Basis of Cancer, p. 53 (1998)

Table 10-1. Metastasis Facts (Holland-Frei, 2010)

- Up to 60% of patients with invasive cancer have overt or occult metastases at diagnosis.
- Acquisition of the invasive phenotype is an early event in cancer progression.
- Millions of tumor cells are shed daily into the circulation.
- Less than 0.01% of circulating tumor cells successfully initiate a metastatic focus.
- Angiogenesis is a ubiquitous and early event that is necessary for and promotes metastatic dissemination.
- Invasion and angiogenesis use the same signal transduction programs and gene expression cassettes.
- Circulating tumor cells can be detected in patients who do not develop overt metastatic disease.
- Metastases may be as susceptible to anticancer therapy as their primary tumors.
- Therapeutic intervention against targets of invasion and metastasis may alter both the metastatic process and angiogenesis.

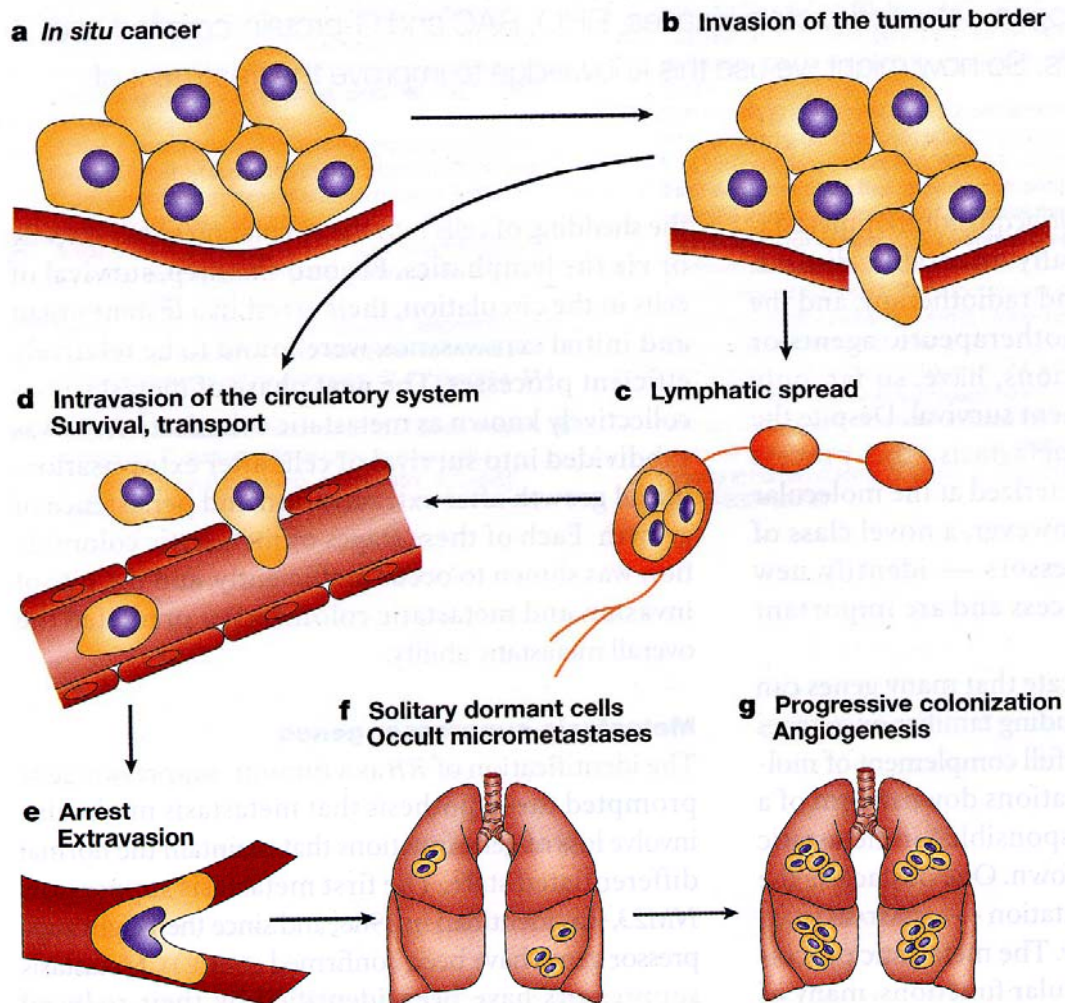


Figure 1 | **Metastasis is a complex, multistep process.** A schematic of the metastatic process, beginning with **a** | an *in situ* cancer surrounded by an intact basement membrane. **b** | Invasion requires reversible changes in cell–cell and cell–extracellular-matrix adherence, destruction of proteins in the matrix and stroma, and motility. Metastasizing cells can **c** | enter via the lymphatics, or **d** | directly enter the circulation. **e** | Survival and arrest of tumour cells, and extravasation of the circulatory system follows. **f** | Metastatic colonization of the distant site progresses through single cells, which might remain dormant for years, to occult micrometastases and **g** | progressively growing, angiogenic metastases.

Metastasis may require a change of phenotype known as the Epithelial-Mesenchymal Transition

Table 14.2 Cellular changes associated with the epithelial–mesenchymal transition

Loss of

- Cytokeratin (intermediate filament) expression**
- Epithelial adherens junction protein (E-cadherin)**
- Epithelial cell polarity**

Acquisition of

- Fibroblast-like shape**
- Motility**
- Invasiveness**
- Mesenchymal gene expression program**
- Mesenchymal adherens junction protein (N-cadherin)**
- Protease secretion (MMP-2, MMP-9)**
- Vimentin (intermediate filament) expression**
- Fibronectin secretion**
- PDGF receptor expression**
- α v β 6 integrin expression**

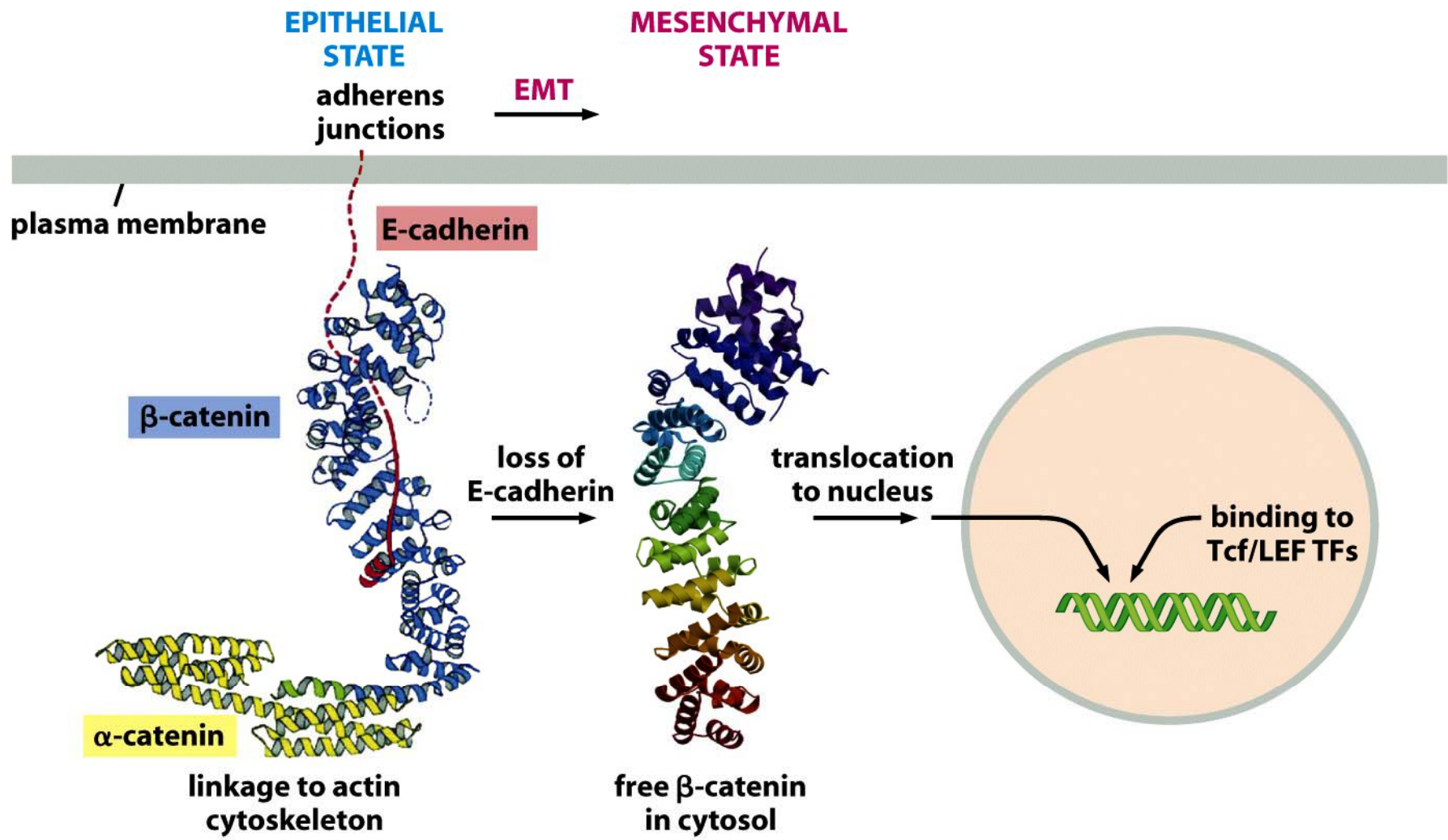


Figure 14-14c The Biology of Cancer (© Garland Science 2007)

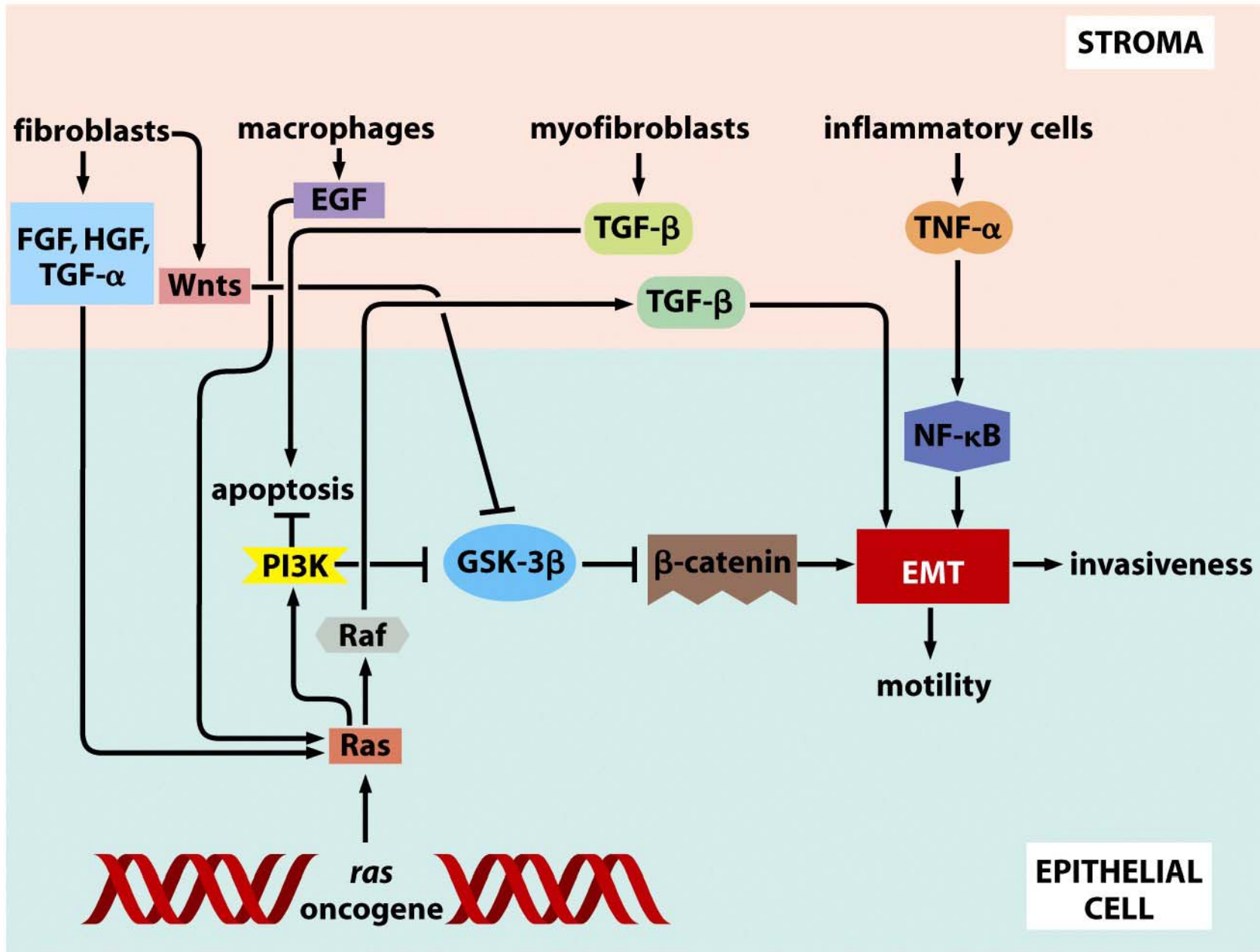


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Nm23 GENE

Some types of cancer such as melanoma are highly metastatic. Others, such as ovarian cancer have a much lower tendency to metastasize.

Expression of the nm23 gene has been found to be highly expressed in normal tissues but has an inverse relationship with the tendency to metastasize in melanoma cell lines.

Reference: McKinnel et al., The Biological Basis of Cancer, p.68-70 (1998)

Metastasis-associated proteins (MTAs)

MTA1 is one of the most commonly overexpressed gene products in human cancers.

The mechanism involved has been proposed to be either transcriptional regulation, postranslational modification or both.

Overexpression of MTA1 was able to confer an anchorage-independent phenotype to a noninvasive breast cancer cell line.

Some actions of MTA1 are believed to arise because it is a component of the nucleosome remodeling and histone deacetylase (NuRD) complex.

MTA1 regulates multiple oncogenes and may a “regulator of regulators” of transformation.

Reference: D-Q. Li et al., Metastasis-associated protein 1/nucleosome remodeling and histone deacetylase complex in cancer. *Cancer res.* 72: 387-394, 2012

METASTASIS

Tumor sublines can be selected with preferred target organs for metastasis. Three mouse melanoma lines derived from B16 tumor cells, which preferentially colonized lung, brain and the ovaries, had a different pattern of surface proteins. In general the amount (or the accessibility) of these specific surface proteins increased with the cell's metastatic potential. Oncogene and tumor suppressor genes have been identified that either increase or decrease metastasis, respectively. Thus the nm23 protein is an inhibitor of metastasis and low levels of the protein in breast cancer have been associated with metastasis and poor survival.

METASTASIS

The matrix metalloproteinases (MMPs) appear to exert a number of roles from regulation of tumor growth through intravasation and extravasation and growth in a metastatic site. These proteases include three major groups: collagenases degrading fibrillar collagen, stromelysins acting on proteoglycans and glycoproteins and gelatinases degrading nonfibrillar and denatured collagens. There are tissue inhibitors of metalloproteinases (TIMPs) whose activities inhibit metastasis.

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Primary tumors and their metastatic tropisms

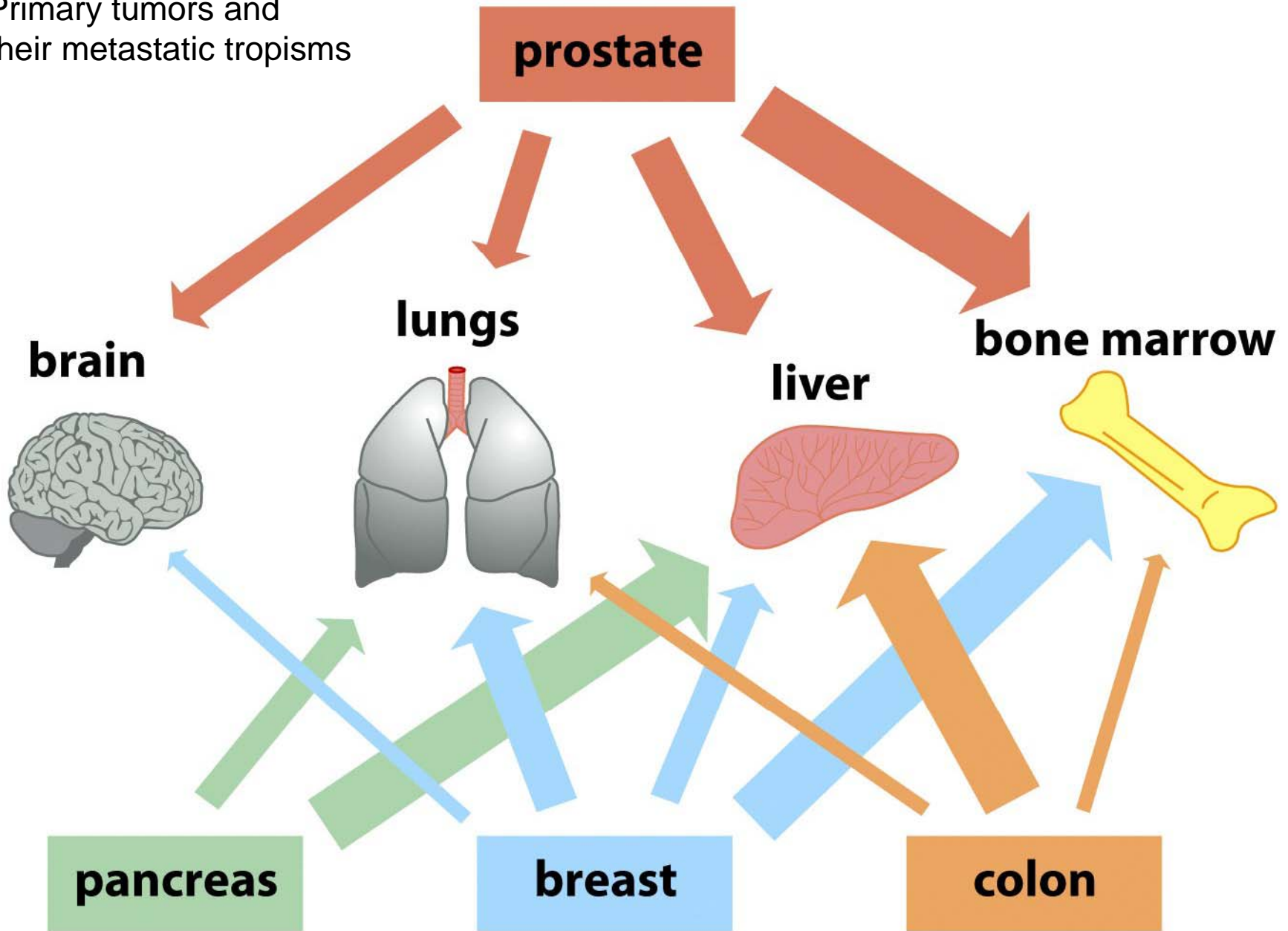


Figure 14-42 The Biology of Cancer (© Garland Science 2007)

CANCER CELL MEMBRANES AND METASTASIS - SUGGESTED READING

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