NEOPLASIA DEVELOPMENT

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Angiogenesis

- Even if a solid tumor possesses all of the genetic aberrations that are required for malignant transformation, it cannot enlarge beyond 1 to 2 mm in diameter unless it has the capacity to induce angiogenesis.
- Like normal tissues, tumors require delivery of oxygen and nutrients and removal of waste products;
- Angiogenesis is thus an essential facet of malignancy.

Angiogenesis

- Neovascularization has a dual effect on tumor growth:
- Perfusion supplies needed nutrients and oxygen
- Newly formed endothelial cells stimulate the growth of adjacent tumor cells by secreting growth factors, such as insulin-like growth factors (IGFs) and PDGF.
- The vessels are not normal, however. They are leaky and dilated, and have a haphazard pattern of connection.
- By permitting tumor cells access to these abnormal vessels, angiogenesis also contributes to metastasis.

Angiogenesis

- A relative lack of oxygen due to hypoxia stabilizes HIF1α (hypoxia induction factor)
- Which then activates the transcription of VEGF (vascular endothelial growth factor) and bFGF (fibroblastic growth factor).
- These factors create an angiogenic gradient that stimulates the proliferation of endothelial cells and guides the growth of new vessels toward the tumor.
- VEGF also increases the expression of ligands that activate the Notch signaling pathway
- Which regulates the branching and density of the new vessels.

Angiogenesis

- For example, p53 can stimulate expression of anti-angiogenic molecules, such as thrombospondin-1, and repress expression of pro-angiogenic molecules such as VEGF.
- The transcription of VEGF is also influenced by signals from the RAS-MAP kinase pathway as well as by gain-of-function mutations in RAS or MYC
- Inflammatory cells can release VEGF

Metastatic cascade

- The metastatic cascade is divided into two phases:
- Invasion of the extracellular matrix (ECM)
- Vascular dissemination, homing of tumor cells, and colonization.
- Dissociation of cancer cells from one another is often the result of alterations in intercellular adhesion molecules and is the first step in the process of invasion.
- E-cadherin is generally inhibited.

Metastatic cascade

- Degradation of the basement membrane and interstitial connective tissue is the second step in invasion.
- Cleavage products of collagen and proteoglycans also have chemotactic, angiogenic, and growth-promoting effects.
- MMP9 is a gelatinase that cleaves type IV collagen of the epithelial and vascular basement membrane
- Also stimulates release of VEGF from ECM-sequestered pools

Metastatic cascade

- The third step in invasion involves changes in attachment of tumor cells to ECM proteins.
- Detachment of epithelial cells from basement membranes and from cell-cell interactions can lead to a particular form of cell death called anoikis.
- Tumor-associated macrophages may prevent anoikis by expressing adhesion molecules such as integrins that promote direct physical interactions with tumor cells.

Metastatic cascade

- Stimulated and directed by tumor cell-derived cytokines as well as cleavage products
- Proteases released by inflammatory cells and activated stromal cells degrade adhesion molecules that mediate cell-cell and cell-ECM actions as well as liberate growth factors bound to matrix molecules.

Metastatic cascade

- Stromal cells also produce paracrine effectors of cell motility, such as hepatocyte growth factor/scatter factor, which binds to the receptor tyrosine kinase MET on tumor cells.
- Activate signaling survival pathways in face of DNA damage.
- Tumor associated fibroblasts exhibit altered expression of genes that encode ECM molecules, proteases, protease inhibitors, and various growth factors.

Metastatic cascade

- Locomotion is the final step of invasion, propelling tumor cells through the degraded basement membranes and zones of matrix proteolysis.
- Cells must attach to the matrix at the leading edge, detach from the matrix at the trailing edge, and contract the actin cytoskeleton to ratchet forward

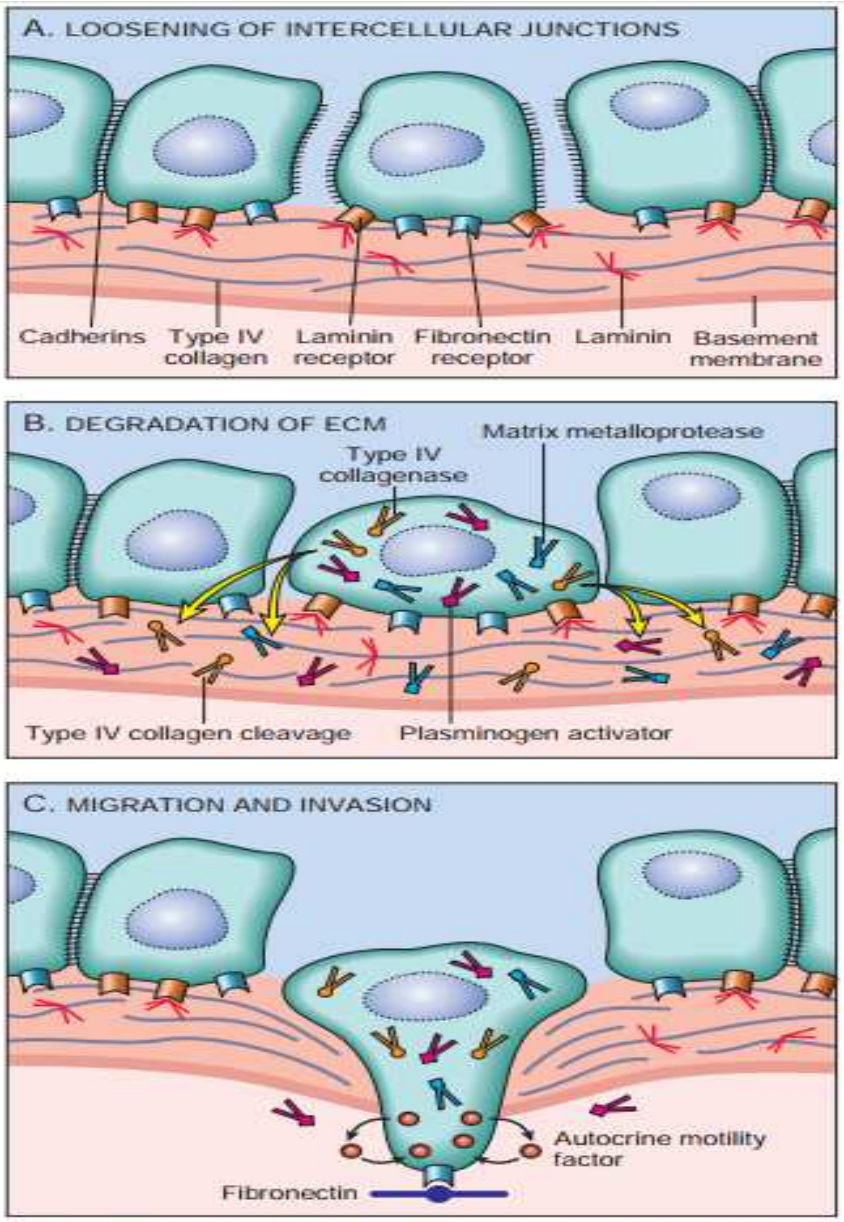

Figure 7-37 Sequence of events in the invasion of epithelial basement membranes by tumor cells. Tumor cells detach from each other because of reduced adhesiveness and attract inflammatory cells. Proteases secreted from tumor cells and inflammatory cells degrade the basement membrane.

Spread

- Within the circulation, tumor cells tend to aggregate in clumps.
- This is favored by homotypic adhesions among tumor cells as well as heterotypic adhesion between tumor cells and platelets.
- Formation of platelet-tumor aggregates may enhance tumor cell survival and implantability.
- Tumor cells may also bind and activate coagulation factors, resulting in the formation of emboli.
- Arrest and extravasation of tumor emboli at distant sites involves adhesion to the endothelium, followed by egress through the basement membrane.

Spread

- Involved in these processes are integrins and laminin receptors as well as proteolytic enzymes.
- The CD44 adhesion molecule, expressed on normal T lymphocytes, is used by T-lymphocytes to migrate to selective sites in lymphoid tissues.
- Such migration is accomplished by the binding of CD44 to hyaluronate on high endothelial venules.
- Solid tumors also often express CD44, which appears to enhance their spread to lymph nodes and other metastatic sites.
- Tumor cells may have other adhesion molecules whose ligands are expressed preferentially on the endothelial cells of the target organ.

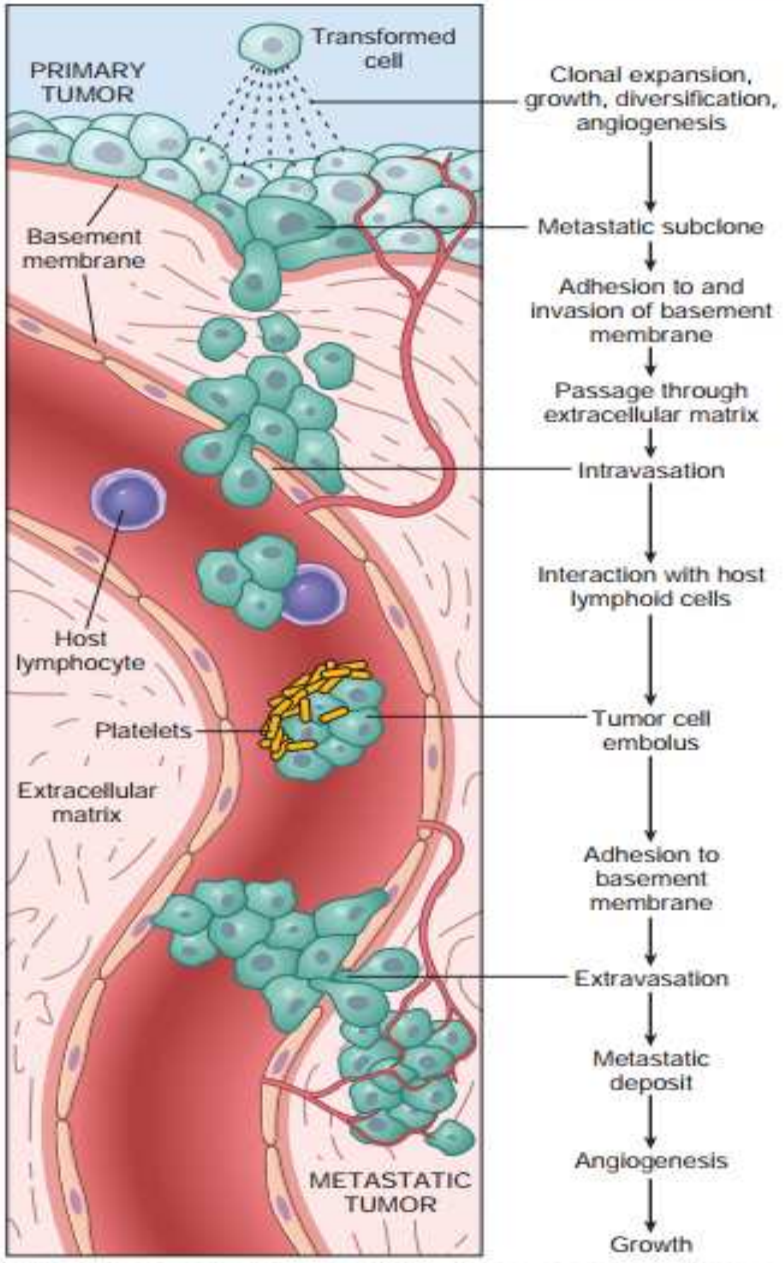

Figure 7-36 The metastatic cascade. Sequential steps involved in the hematogenous spread of a tumor.

Metastasis oncogenes

- SNAIL and TWIST are transcriptional repressors that downregulate E-cadherin expression
- Loss of E-cadherin expression seems to be a key event in epithelial-to-mesenchymal transition (EMT).
- In EMT, carcinoma cells downregulate certain epithelial markers such as E-cadherin and upregulate certain mesenchymal markers such as vimentin and smooth muscle actin.
- These changes are believed to favor the development of a promigratory phenotype that is essential for metastasis.
- EMT has been documented mainly in breast cancers

Metastasis

- The target tissue may be a non-permissive environment for the growth of tumor.
- Although well vascularized, skeletal muscle and spleen are rarely sites of metastasis
- Micrometastases may not progress as the tumor process follows Gompertzian kinetics:
- Growth rates are faster with smaller tumors and slower with larger tumors

Immune surveillance

- Neoplastic transformation results from genetic alterations in proto-oncogenes and tumor suppressor genes
- These mutated genes encode variant proteins that have never been seen by the immune system and are thus recognized as non-self

Immune surveillance

- Cytoplasmic proteins may enter the class I MHC antigen-processing pathway and be recognized by CD8+ T cells.
- In addition, these proteins may enter the class II antigen-processing pathway in antigen presenting cells that have phagocytized dead tumor cells, and thus be recognized by CD4+ T cells also.
- The tumor-specific neoantigens that are recognized by CTLs in patients with cancer are for the most part currently unknown.

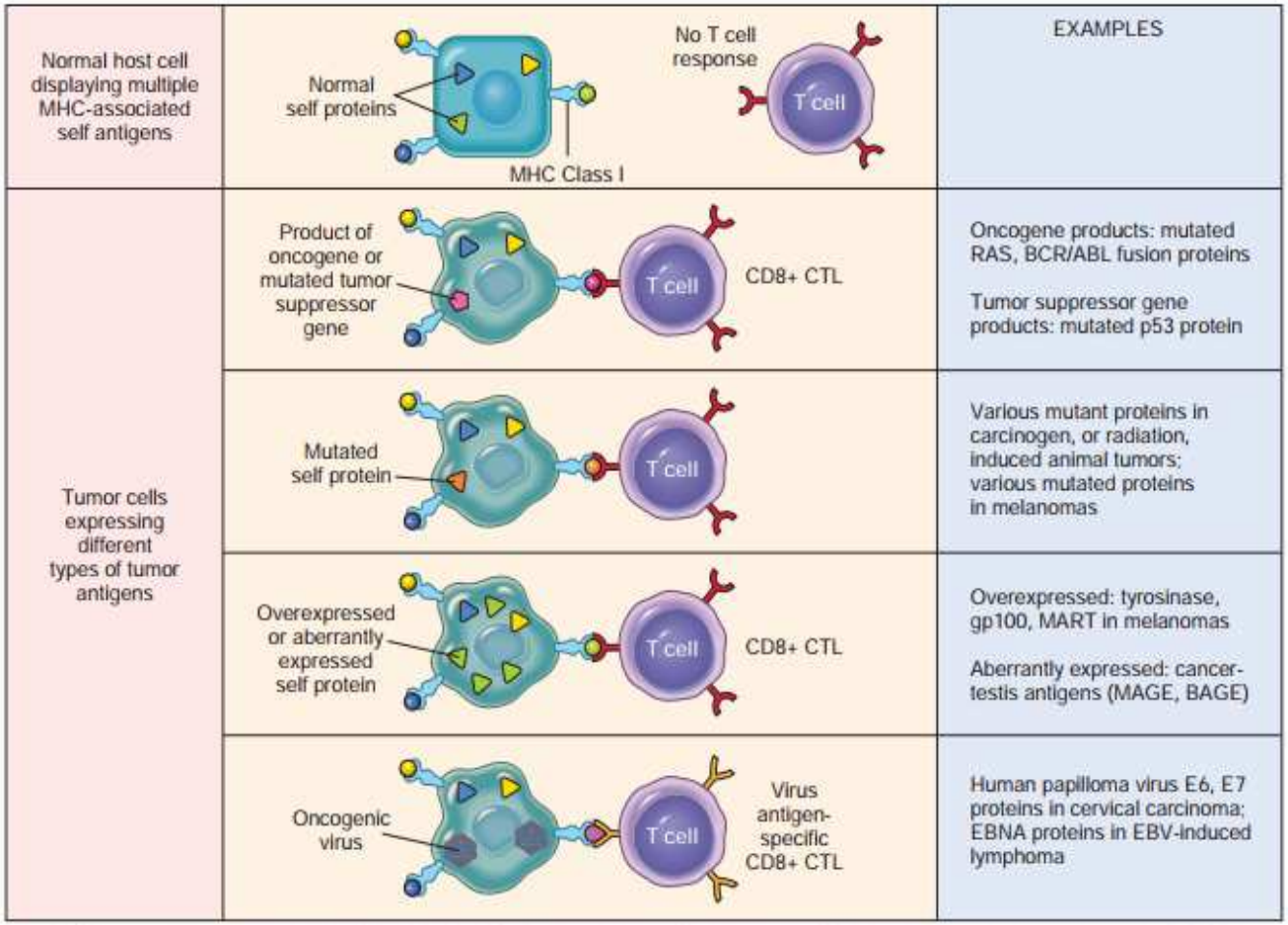

Figure 7-39 Tumor antigens recognized by CD8+ T cells. (Modified from Abbas AK, Lichtman AH: Cellular and Molecular Immunology, 5th ed. Philadelphia, WB Saunders, 2003.)

Tumor antigens

- Tumor antigens may also be normal cellular proteins that are abnormally expressed in tumor cells.
- Tyrosinase in melanoma
- Cancer-testis antigen are encoded by genes that are silent in all adult tissues except germ cells in the testis. As they are not expressed on the cell surface, are thought to be tumor specific.
- MAGE-1 expressed in 37% of melanomas

Tumor antigens

- Oncofetal antigens are proteins that are expressed at high levels on cancer cells and in normal developing (fetal) tissues.
- Carcinoembryonic antigen (CEA), α-fetoprotein (AFP)
- Tumors express higher than normal levels and/or abnormal forms of surface glycoproteins and glycolipids
- These altered molecules include gangliosides, blood group antigens, and mucins

Tumor antigens

- Mucins are high-molecular-weight glycoproteins containing numerous O-linked carbohydrate side chains on a core polypeptide.
- Tumors often have dysregulated expression of the enzymes that synthesize these carbohydrate side chains
- Leads to the appearance of tumor-specific epitopes on the carbohydrate side chains or on the abnormally exposed polypeptide core.
- MUC-1 is an integral membrane protein that is normally expressed only on the apical surface of breast ductal epithelium

Tumor cell antigens

- Tumors express molecules that are normally present on the cells of origin.
- These antigens are called differentiation antigens because they are specific for particular lineages or differentiation stages of various cell types.
- Such differentiation antigens are typically normal self-antigens, and therefore they do not induce immune responses in tumor-bearing hosts.
- CD20

Immune defenses

- Cytotoxic T lymphocytes (CTLs)
- Protective role against virus-associated neoplasms
- The number of tumor-infiltrating CD8+ T cells and the presence of a “gene signature” associated with CD8+CTLs correlates with a better prognosis
- NK cells are lymphocytes that are capable of destroying tumor cells without prior sensitization
- After activation with IL-2 and IL-15, NK cells can lyse a wide range of human tumors, including many that seem to be nonimmunogenic for T-cells.

Immune defenses

- T cells, NK cells, and macrophages may collaborate in antitumor reactivity, because interferon-γ, a cytokine secreted by T cells and NK cells, is a potent activator of macrophages.
- Alternatively activated (M2) macrophages are cells induced by cytokines such as IL-4 and IL-13.
- These macrophages produce cytokines that promote angiogenesis, fibroblast proliferation, and collagen deposition.
- In addition, they appear to suppress effective host immune responses to cancer cells

Evasion

- During tumor progression, strongly immunogenic subclones may be eliminated
- Most of these neoplasms are aggressive lymphomas composed of mature B cells.
- X-linked lymphoproliferative syndrome is caused by mutations that encode an adapter protein which participates in NK and T-cell signaling pathways.
- In affected boys, EBV infection evolves into a chronic or sometimes fatal form of infectious mononucleosis or lymphoma.
- Hemophagocytic lymphohistiocytosis is present

Evasion

- Tumor cells may fail to express normal levels of HLA class I molecules
- Escape attack by cytotoxic T cells.
- NK cells may be triggered if the tumor cells express ligands for NK cell activating receptors.
- Tumor cells actively inhibit tumor immunity by engaging normal pathways of immune regulation that serve as “checkpoints” in immune responses.
- Downregulate the expression of co-stimulatory factors on antigen-presenting cells

Evasion

- HLA expression is downregulated
- Dysregulation of NF-kB (silencing)
- Hypermethylation of HLA promoter
- Post-transcription silencing by non-coding RNA
- Down regulation of chaperone proteins
- Diminshed β_2-microglobulin reduces folding and transport of HLA to cell surface

Evasion

- The antigen presenting cells fail to engage the stimulatory receptor CD28 and instead activate the inhibitory receptor CTLA-4 on effector T cells.
- This not only prevents sensitization but also may induce long-lived unresponsiveness in tumor-specific T cells.
- Tumor cells also may upregulate the expression of PD-L1 and PD-L2, cell surface proteins that activate the programmed death-1 (PD-1) receptor on effector T cells.
- PD-1, like CTLA-4, may inhibit T cell activation.

Evasion

- Interferon-γ (IFN-γ) and JAK dysregulation activate STAT axis
- JAK 2 promotes T cell chemotaxis
- IFN-γ enhances T cell killing (MHC II directed)
- STAT diminishes presentation of MHC II complexes
- Upregulates T_{regs} as well as Myeloid Derived Stem Cells (MDSC)
- MDSC consume arginine, tryptophan, depleting availabiltiy for T cell expansion
- Both T_{regs} and MDSC are immunosuppressors

Evasion

- RAF/RAS dysfunction leads to diminished MHC I (HLA) presentation as well as increasing Tregs.
- Also increases PD-L1 expression
- PI_3K dysfunction leads to increased PD-L1 expression
- Increased production of immunosuppressing cytokines IL-6 and IL-10.

Evasion

- Tumor immunoglobulin and mucin binding domain 3 (TIM3) overexpressed (and regulated) on dysfunctional or exhausted CD8+ T cells as well as dendritic cells.
- A checkpoint receptor.
- Hepatitis A origin
- Chronic antigen stimulation leads to increased Lymphocyte Activation Gene 3 (LAG3) activity.
- Blocks binding of MHC II to T-cell receptor.

Evasion

- TGF-β is secreted in large quantities by many tumors
- TGF-β increase leads to diminished MHC I (HLA) presentation as well as diminishing CD8+ T cells
- Other tumors secrete sugar-rich lectin-like factors (galectins) that skew T-cell responses so as to favor immunosuppression.
- Favor the recruitment of immunosuppressive T regulatory cells or suppress the function of CD8+ cytotoxic T cells

Evasion

- Other soluble factors that may inhibit the host immune response include:
- IL-10, prostaglandin E2, tryptophan metabolites and VEGF
- VEGF can inhibit the diapedesis of T-cells from the vasculature into the tumor bed.
- Fibrosis may also limit entry of Tumor Infiltrating Lymphocytes (reacting to immunogenicity of tumor)

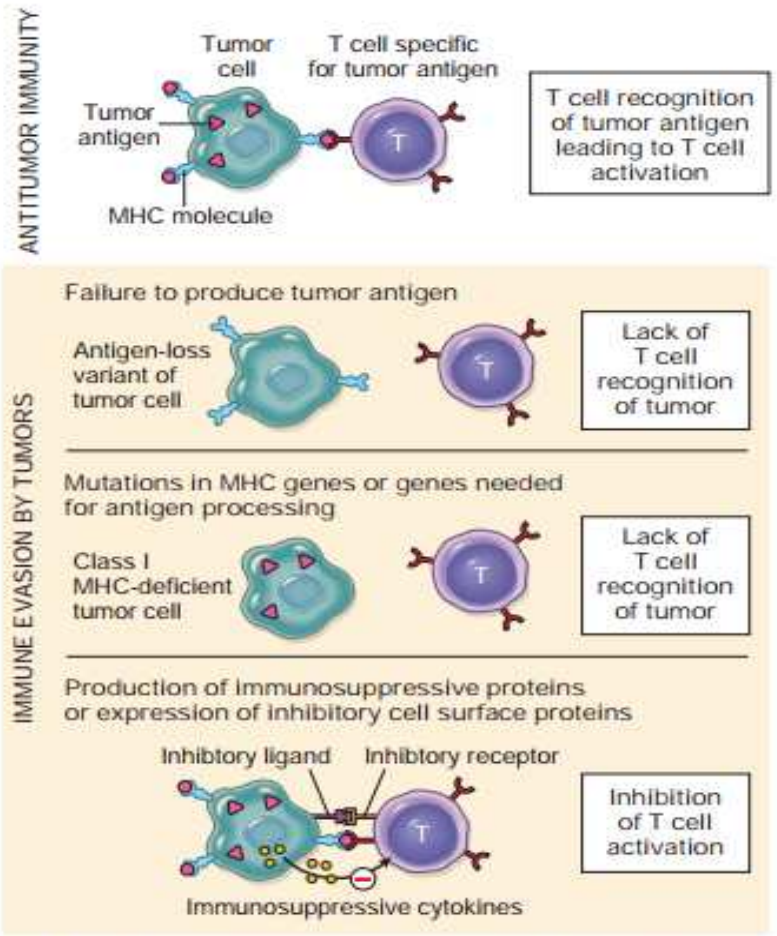

Figure 7-40 Mechanisms by which tumors evade the immune system. Tumors may evade immune responses by losing expression of antigens or major histocompatibility complex (MHC) molecules or by producing immunosuppressive cytokines or ligands such as PD-L1 for inhibitory receptors on T cells. (Reprinted from Abbas AK, Lichtman AH, Pillai S: Cellular and Molecular Immunology, 7th ed. Philadelphia, WB Saunders, 2012.)