Chapter 7: Apoptosis and Replicative Senescence in Cancer

- In normal tissue replication is limited by "terminal differentiation"
  - Counterbalanced by apoptosis which leads to cell loss
- Replicative senescence-
  - cell survival but cells exit from cell cycle
  - Occurs after ~ 60 doublings or due to inappropriate proliferation signals

- Apoptosis
  - Use of proteases called – CASPASES
    - Intrinsic Pathway- elicited by strong DNA damage signals or by activated oncogenes
      - » Generates mitochondrial leakage which leads to activation of caspases
    - Extrinsic Pathway- initiated by cell surface receptors (death receptors)
      - » Activated by cytokine ligands and surface proteins of cytotoxic immune cells
      - » Intracellular “death domains” of receptors associate with FADD (Fas-associated death domain protein) adaptor proteins (DISC complex- “Death-inducing signaling complex”) that activates initiator caspases

- Ligands of the Death receptor
  - Members of the tumor necrosis factor family
    - TNF-a , FASL
    - Cause death in both cancer and normal cells
  - Once activated the death receptors activate FADD in the cytoplasm. This complex = DISC
  - DISC triggers the self-cleavage of caspase 8 and 10 (initiator caspases) which then trigger the “executioner caspases 3, 6 and 7)

5 Families of Death Receptors

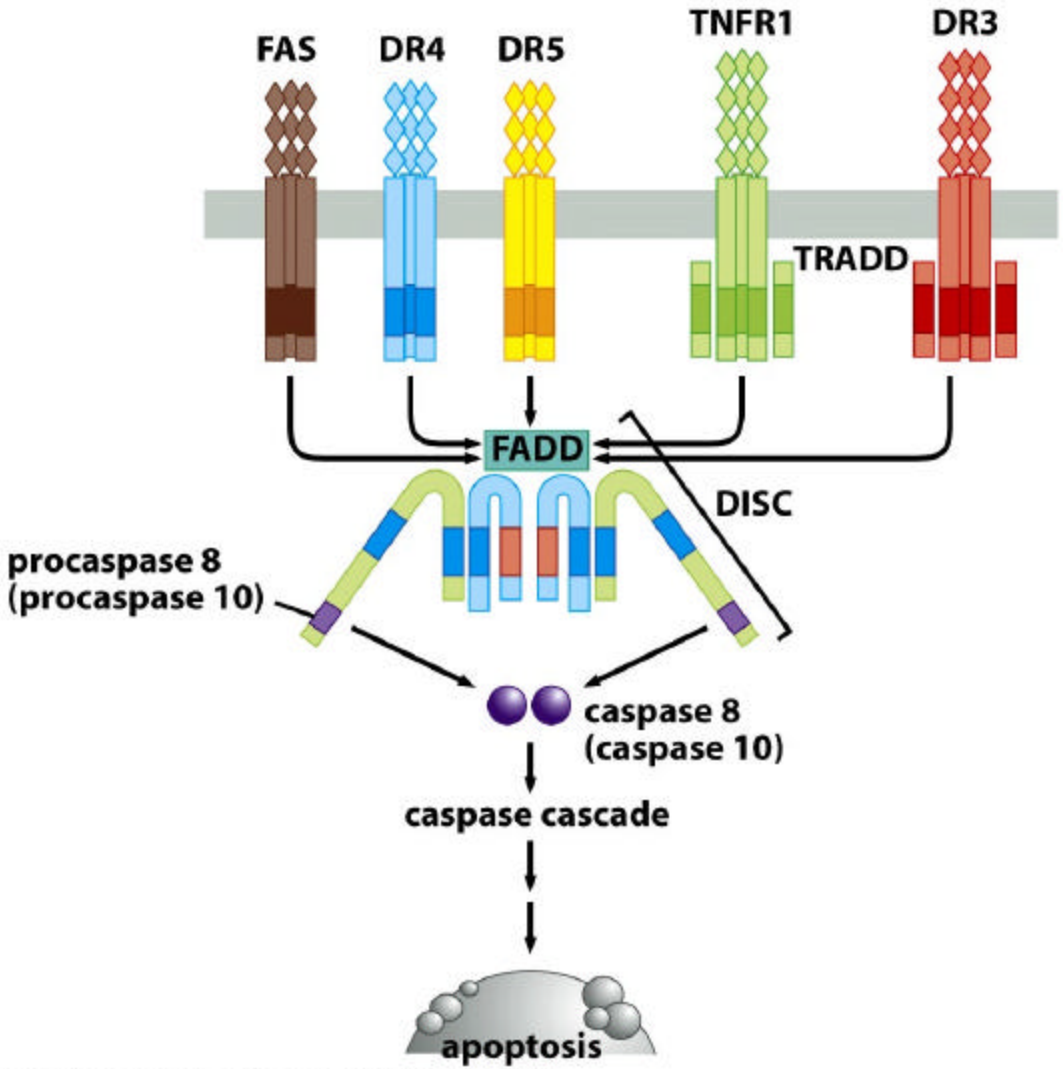

Figure 9-31a The Biology of Cancer (© Garland Science 2007)

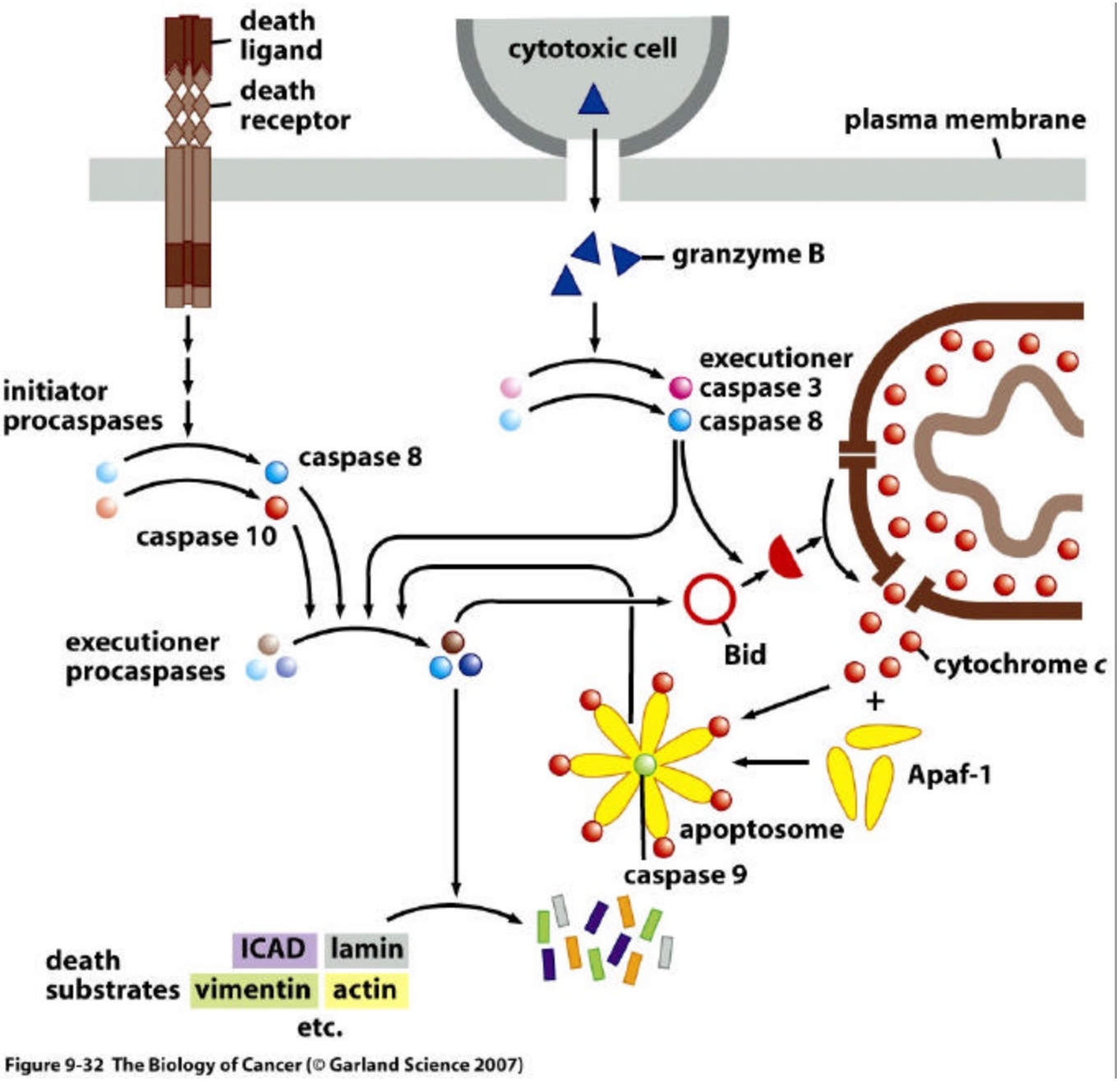

Figure 9-32 The Biology of Cancer (© Garland Science 2007)

- Interaction of extrinsic and intrinsic pathways

- $T_{cytotoxic}$ cells contain FASL when activated
  - These bind to FAS receptors on tumor cells
  - TC cells inject granzmes into cancer cells which cleave and activate procaspases

Regulation of Apoptosis

  - BCL2 prohibits the pro-apoptotic proteins BAX and BAK from acting at mitochondria
  - FLIP (FLICE-inhibitory protein) inhibits the extrinsic pathway at the receptors by binding to the death domains
  - IAPs act at the “apoptosome” (this activates caspases) and inhibit activation of caspases
  - SMAC/Diablo is a protein complex that inhibits IAPs

- Cancer cells must overcome the barriers set up by apoptosis and replicative senescence in order to grow

Table 9.5 Examples of anti-apoptotic alterations found in human tumor cells

Alteration	Mechanism of anti-apoptotic action	Types of tumors
CASP8 promoter methylation	inactivation of extrinsic cascade	SCLC, pediatric tumors
CASP3 repression	inactivation of executioner caspase	breast carcinomas
Survivin overexpression[a]	caspase inhibitor	mesotheliomas, melanomas, many carcinomas
ERK activation	repression of caspase-8 expression	many types
ERK activation	protection of Bcl-2 from degradation	many types
Raf activation	sequestration of Bad by 14-3-3 proteins	many types
PI3K mutation/activation	activation of Akt/PKB	gastrointestinal
NF-κB constitutive activation[b]	induction of anti-apoptotic genes	many types
p53 mutation	loss of ability to induce pro-apoptotic genes	many types
$p14^{ARF}$ gene inactivation	suppression of p53 levels	many types
Mdm2 overexpression	suppression of p53 levels	sarcomas
IAP-1 gene amplification	antagonist of caspases-3 and 7	esophageal, cervical
APAF1 methylation	loss of caspase-9 activation by cytochrome *c*	melanomas
BAX mutation	loss of pro-apoptotic protein	colon carcinomas
Bcl-2 overexpression	closes mitochondrial channel	~ of human tumors
PTEN inactivation	hyperactivity of Akt/PKB kinase	glioblastoma, prostate carcinoma, endometrial carcinoma
IGF-1/2 overexpression	activates PI3K	many types
IGFBP repression	loss of anti-apoptotic IGF-1/2 antagonist	many types
Casein kinase II	activation of NF-κB	many types
TNFR1 methylation	repressed expression of death receptor	Wilms tumor
FLIP overexpression	inhibition of caspase-8 activation by death receptors	melanomas, many others
Akt/PKB activation	phosphorylation and inactivation of pro-apoptotic Bcl-2-like proteins	many types
Stat3 activation	induces expression of Bcl-X_L	several types
TRAIL-R1 repression	loss of responsiveness to death ligand	small-cell lung carcinoma
FAP-1 overexpression	inhibition of Fas receptor signaling	pancreatic carcinoma
XAF1 methylation[c]	loss of inhibition of anti-apoptotic XIAP	gastric carcinoma
Wip1 overexpression[d]	suppression of p53 activation	breast and ovarian carcinomas, neuroblastoma

[a]Survivin is an inhibitor of apoptosis (IAP) in gastric, lung, and bladder cancer and melanoma in addition to the mesotheliomas indicated here. The expression of a number of IAP genes is directly induced by the NF-κB TFs.
[b]Induces synthesis of c-IAPs, XIAP, Bcl-X_L, and other anti-apoptotic proteins.
[c]XAF1 (XIAP-associated factor 1) normally binds and blocks the anti-apoptotic actions of XIAP, the most potent of the IAPs.
[d]Wip1 is a phosphatase that inactivates p38 MAPK, which otherwise would phosphorylate and stimulate the pro-apoptotic actions of p53.

Diminished Apoptosis

- Caused by….
  - Downregulation of death receptors
  - Secretion fo “decoy” receptors
  - Use of FAS/FASL receptors to kill immune system cells instead of the other way around
  - Mutational inactivation of P53
  - PI3K or NF?B pathways increasing survival of cells
  - Overexpression of inhibitory proteins (BCL2 or survivin

Replicative senescence

- Induced by:
  - > 50-60 cell doublings
  - Telomere length reduced to below minimum size
    - Consist of repeats of hexanucleotide TTAGGG (> 100 of these repeats)
    - Telomeres shorten with each division
    - Many cancers express telomerase (enzyme that makes these repeats) and stabilize length

Limits to Cell Proliferation

- Number of cells in a tissue limited by:
  - Cell growth vs cell death
    - Cell death by....
      - » apoptosis- rapid, cell blebbing, nuclear fragmentation, loss of cell connections & matrix alterations (“DNA laddering” on agarose gels)
      - » Purpose of apoptosis is to shape tissues during development, to eliminate autoreactive immune cells, to destroy cells infected by virus,
      - » necrosis- cells burst after swelling, followed by inflammation
      - » Occurs in hypoxic areas of tissue/tumor (no energy)

- Also limited by number of cells active in cell cycle
  - Cells removed from cell cycle proliferation by ….
    - » Replicative senescence and terminal differentiation
- Decreased rate of terminal differentiation required for tumor cell growth
  - In some tumors, proteins that are involved in terminal differentiation NOT found in tumor cells
  - Cells in germ line do NOT undergo replicative senescence
- Cells that have lost replicative senescence are term “immortal” (SV40 infection and large T-antigen induction do this)

Mechanisms of Apoptosis

- Initiation
  - Intrinsic (cell damage), mitochondrial involvement
  - extrinsic pathways (T_C cells)
    - Most important regulators are the BCL2 members
      - » 20 different proteins- some are pro-apoptotic and others are anti-apoptotic
      - » BCL-2 found in B cell lymphoma cells as a translocation (14:18)- over-expression of BCL2 prevents apoptosis
      - » Induction of apoptosis requires inactivation of BCL2 and other proteins located at the mitochondrial membrane
- Execution
- Removal

Caspases

- Cysteine proteases
  - Cleave peptide bond following an Asp in the concensus sequence QAD\RG
  - 14 caspases known
    - Initiator caspases (caspase 9)
    - Executor caspases (caspase 3)
    - Inflammatory caspases (caspase ??)- process cytokines
  - Activation of caspases induced by release of AIF protein by mitochondria
    - Must overcome action of IAPs (Inhibitors of apoptosis)
      - » XIAP and survivin
  - SMAC/Diablo protein released from mitochondria binds to and sequesters IAPs to promote apoptosis
    - Several viruses may express their own IAPs to prevent apoptosis

Extrinsic Pathway

- Initiated when surface receptors attach specific ligands
  - “death receptors” belong to TNF receptor “super family”
    - TNFa secreted by monocytes/macrophages
  - Following ligand-receptor binding complexes trimerize and receptor death domains in cytoplasm binds FADD
    - FADD undergoes steric alterations and bind to pro-caspases to initiate apoptotic pathway (8 or 10). This complex = DISC (death inducing signaling complex)
    - These initiate caspase 3 (executioner caspase)
    - FLIP protein acts as an inhibitor of extrinsic pathway by interfering with initiator caspase activation

- The multiple biochemical and morphological alterations during the execution phase are brought about by:
  - Proteolytic cleavage of > 300 cellular proteins by caspase 3 and caspase 6
    - Substrates include regulators of the cell cycle, repair enzymes, cytoskeletal proteins, DNAases (CAD= caspase activated DNAase), adhesion proteins

Diminished apoptosis in cancer

- 1. Desensitization of death receptor
- 2. Counterattack (avoidance of death receptor signaling)
- 3. Loss of TP53 function
- 4. Inactivation of the intrinsic pathway
- 5. Overexpression of IAPs
- 6. Activation of anti-apoptotic pathways

Replicative Senescence-Telomeres

- Two mechanisms…
  - Short telomeres
  - CDK inhibitors
  - Telomeres
    - 5-30 kb long
    - Made up of 1000-5000 repeats of TTAGGG hexamers
    - Most of telomere consists of ds DNA, but has single strand length of 75-150 nucleotides
    - Has loop at end to make ds (T-loop)
    - Loop DNA wrapped around nucleosome

- With each cell replication the telomere shortens
  - Has to do with DNA replication process that RNA primer has to be removed and there is no template to copy the rest of the strand
- Telomerase (enzyme) prevents shortening in germ-line cells
  - Is a specialized reverse transcriptase that uses an RNA template (AAUCCC) to elongate telomeres using the hTERC subunit
    - » Catalytic subunit hTERT subunit present in germ-line cells, tissue stem cells, and memory immune cells
    - » The promoter of the TERT gene is a target for *myc* proteins
- Shortening of telomeres to below a certain length causes replicative senescence
    - » Telomerase shortening leads to chromosome stability

CDK Inhibitor Proteins: Limitation of life-span of cells

- Inhibitor proteins include:
  - $p16^{INK4A}$- strongly induced by p53. May be responsible for arrest of cell cycle after telomere shortening
  - $p21^{CIP1}$- accumulates in cells that proliferate continuously- independent of p53 (does this allow cell proliferation or is it there because of cell proliferation????)
  - $p57^{KIP2}$- p53 independent, induced by E2F

- Cell cycle inhibitors and their respective accumulation in the cell appear to be due to not only the number of cycles but primarily due to how quickly they follow each other

- Use of rodents as models for aging and cancer
  - 2 year old mouse approaching old age vs 70 year old human
  - Humans may require additional protective mechanisms that prevent cancer progression than mouse
    - » More difficult to transform human cells than rodent cells