# Neoplasia
# lecture 6

# Transforming Growth Factor-β Pathway

- **TGF-β, a member of a family of dimeric growth factors that includes bone morphogenetic proteins and activins.**
- **In most normal epithelial, endothelial, and hematopoietic cells, TGF-β is a potent inhibitor of proliferation.**
- **It regulates cellular processes by binding to a complex composed of TGF-β receptors I and II.**
- **Dimerization of the receptor upon ligand binding leads to a cascade of events that result in the transcriptional activation of CDKIs with growth-suppressing activity as well as repression of growth-promoting genes such as *MYC* and *CDK4*.**

- **In many forms of cancer the growth-inhibiting effects of the TGF-β pathways are impaired by mutations affecting TGF-β signaling.**
- **These mutations may alter the type II TGF-β receptor or SMAD molecules that serve to transduce anti-proliferative signals from the receptor to the nucleus.**
- **Mutations affecting the type II receptor are seen in:**

1. **Colon cancer**
2. **Stomach cancer**
3. **Endometrium cancer**
4. **Pancreatic cancer (mutational inactivation of SMAD4)**

- **Loss of TGF-β–mediated growth control occurs at a level downstream of the core TGF-β signaling pathway; for example, there may be loss of p21 expression and/or overexpression of *MYC*.**
- **These tumor cells can then use other elements of the TGF-β induced program, including immune system suppression or promotion of angiogenesis to facilitate tumor progression.**
- **TGF-β can function to prevent or promote tumor growth depending on the state of other genes in the cell.**

# Contact Inhibition, NF2, and APC

- **When cancer cells are grown in the laboratory their proliferation fails to be inhibited when they come in contact with each other.**
- **This is in sharp contrast to nontransformed cells which stop proliferating once they form confluent monolayers.**

- **Cell–cell contacts in many tissues are mediated by homodimeric interactions between transmembrane proteins called *cadherins*.**
- **E-cadherin (E for epithelial) mediates cell–cell contact in epithelial layers.**

- **Two mechanisms have been proposed to explain how E-cadherin maintains contact inhibition:**

**1. One mechanism is mediated by the tumor suppressor gene *NF2*.**

**- Neurofibromin-2 (called *merlin*) acts downstream of E-cadherin in a signaling pathway that helps for maintain contact inhibition.**

**- Homozygous loss of *NF2* is known to cause certain neural tumors as neurofibromatosis type 2.**

2- **E-cadherin may regulate contact inhibition through β-catenin signaling pathway.**

**APC gene mutation and adenomatous polyposis coli**

# Adenomatous Polyposis Coli-β-Catenin Pathway

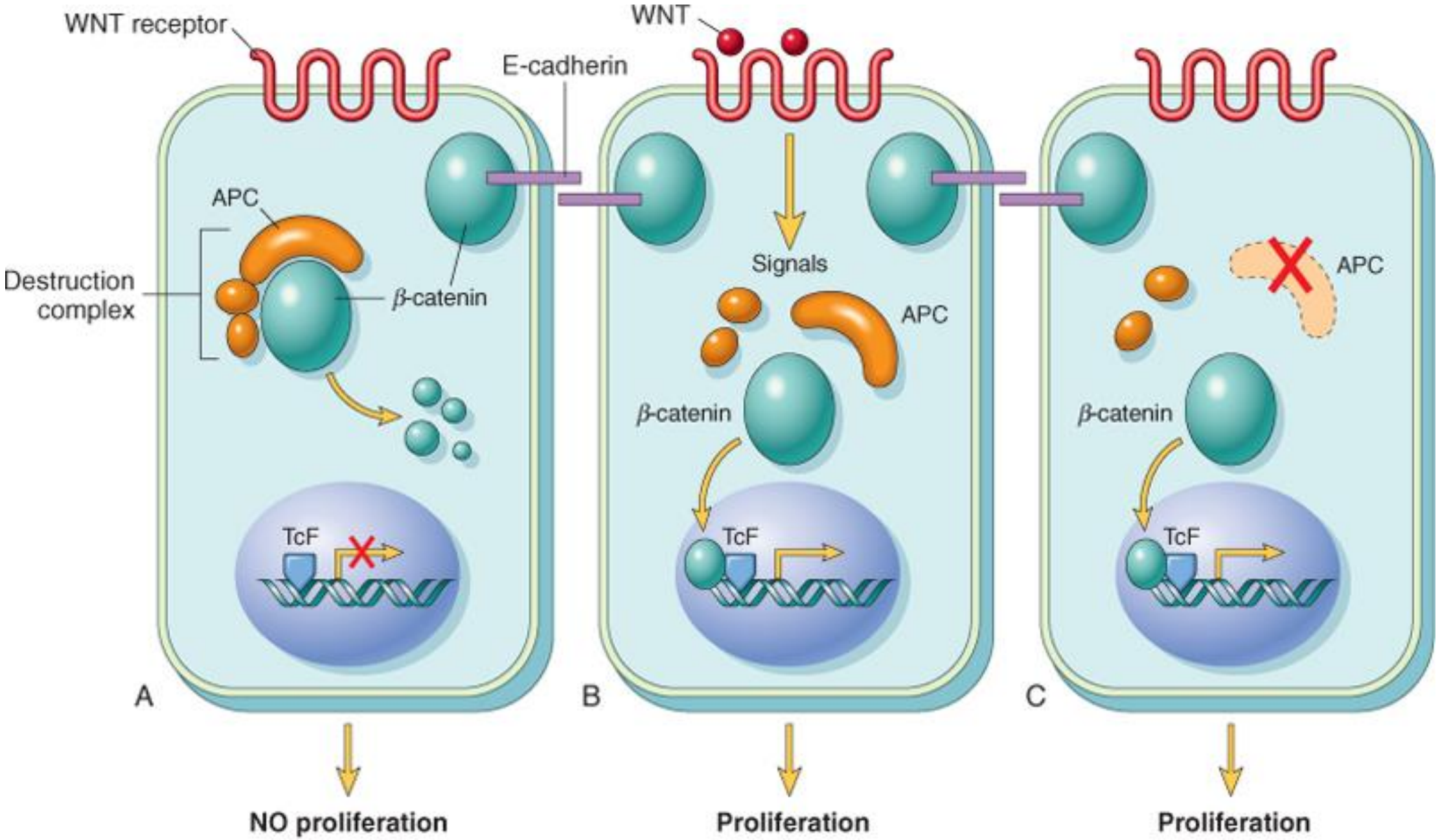
- **The *APC* gene exerts antiproliferative effects in an unusual manner.**
- **It is a cytoplasmic protein whose dominant function is to regulate the intracellular levels of β-catenin,**
- **β-catenin  a protein with many functions :**

**1- β-catenin binds to the cytoplasmic portion of E-cadherin, a cell surface protein that mediates intercellular interactions.**

**2- It can translocate to the nucleus and activate cell proliferation**

- **β-catenin is an important component of the so-called WNT signaling pathway that regulates cell proliferation.**
- **WNT is a soluble factor that can induce cellular proliferation.**
- **It does so by binding to its receptor and transmitting signals that prevent the degradation of β-catenin, allowing it to translocate to the nucleus where it acts as a transcriptional activator in conjunction with another molecule called TcF .**

- **In quiescent cells which are not exposed to WNT, cytoplasmic β-catenin is degraded by a *destruction complex,* of which APC is an integral part**
- **With loss of APC (in malignant cells), β-catenin degradation is prevented and the WNT signaling response is inappropriately activated in the absence of WNT**
- **This leads to transcription of growth-promoting genes such as cyclin D1 and *MYC***



- ***APC*** **behaves as a typical tumor suppressor gene. Individuals born with one mutant allele develop hundreds to thousands of adenomatous polyps in the colon during their teens or 20s which show loss of the other *APC* allele.**
- **Almost invariably one or more polyps undergo malignant transformation upon accumulation of other mutations in the cells within the polyp**

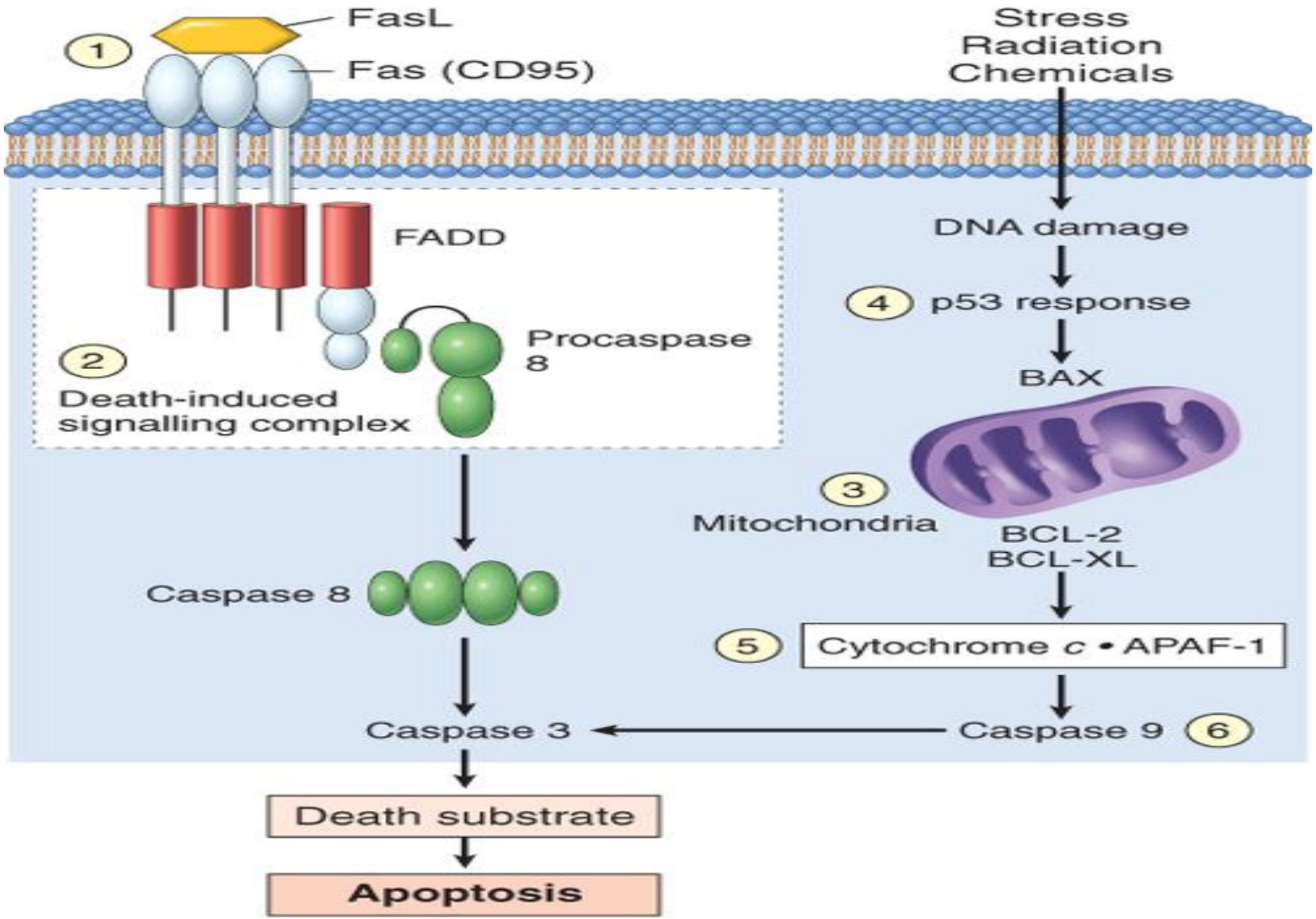
- ***APC*** **mutations are seen in 70-80% of sporadic colon cancers.**
- **Colonic cancers that have normal *APC* genes show activating mutations of β-catenin that render them refractory to the degrading action of APC.**

# Evasion of Apoptosis

- **There are two distinct programs that activate apoptosis:**

**1- The extrinsic pathway (death receptor CD95/Fas ).**

**2- The intrinsic pathway (DNA damage ).**



- **The extrinsic pathway is initiated when CD95 is bound to its ligand, CD95L → trimerization of the receptor and thus its cytoplasmic *death domains α* → attract the intracellular adaptor protein FADD → recruits procaspase 8 to form the death-inducing signaling complex.**

- **Procaspase 8 is activated by cleavage into smaller subunits generating caspase 8.**
- **Caspase 8 then activates down-stream caspases such as caspase 3 a typical executioner caspase that cleaves DNA and other substrates to cause cell death.**

- **The intrinsic pathway of apoptosis is triggered by a variety of stimuli, including:**
- **1- Withdrawal of survival factors.**
- **2- Stress.**
- **3- Injury.**

- **Activation of this pathway leads to permeabilization of mitochondrial outer membrane, with resultant release of molecules such as cytochrome *c* that initiate apoptosis.**

- **The integrity of the mitochondrial outer membrane is regulated by pro-apoptotic and anti-apoptotic members of the BCL2 family of proteins.**
- **The pro-apoptotic proteins, BAX and BAK, are required for apoptosis and directly promote mitochondrial permeabilization.**

- **Their action is inhibited by the anti-apoptotic members of this family exemplified by BCL2 and BCL-XL.**
- **A third set of proteins (so-called BH3-only proteins) including BAD, BID, and PUMA, regulate the balance between the pro- and anti-apoptotic members of the**

- **The BH3-only proteins promote apoptosis by neutralizing the actions of anti-apoptotic proteins like BCL2 and BCL-XL.**
- **When the sum total of all BH3 proteins expressed "overwhelms" the anti-apoptotic BCL2/BCLXl protein barrier, BAX and BAK are activated and form pores in the mitochondrial membrane.**
- **Cytochrome *c* leaks into the cytosol where it binds to APAF-1, activating caspase 9.**

- **Like caspase 8 of the extrinsic pathway, caspase 9 can cleave and activate the executioner caspases.**
- **Because of the pro-apoptotic effect of BH3 only proteins, efforts are underway to develop of BH3 mimetic drugs.**

- **<u>Malignent cells can escape apoptosis through different ways:</u>**
- **1-Reduced levels of CD95 may render the tumor cells less susceptible to apoptosis by Fas ligand (FasL).**
- **2-Some tumors have high levels of FLIP, a protein that can bind death-inducing signaling complex and prevent activation of caspase 8.**

- **3-Reduced egress of cytochrome *c* from mitochondrion as a result of up-regulation of BCL2.**
- **4- Reduced levels of pro-apoptotic BAX resulting from loss of p53.**
- **5-Loss of APAF-1.**
- **6-Up-regulation of inhibitors of apoptosis.**

- **Of all these genes the role of BCL2 in protecting tumor cells from apoptosis is best established.**
- **85% of B-cell lymphomas of the follicular type carry a characteristic t(14;18) (q32;q21) translocation.**
- **14q32, the site where immunoglobulin heavy-chain genes are found, is also involved in the pathogenesis of Burkitt lymphoma.**
- **Juxtaposition of this transcriptionally active locus with *BCL2* (located at 18q21) causes overexpression of the BCL2 protein.**

- **This in turn increases the BCL2/BCL-XL buffer protecting lymphocytes from apoptosis and allowing them to survive for long periods.**
- **Steady accumulation of B lymphocytes resulting in lymphadenopathy and marrow infiltration.**
- **BCL2-overexpressing lymphomas arise in large part from reduced cell death rather than explosive cell proliferation they tend to be indolent (slow growing) compared with many other lymphomas.**

# Limitless Replicative Potential

- **Most normal human cells have a capacity of 60-70 doublings.**
- **After this, the cells lose the capacity to divide and enter senescence.**
- **This phenomenon is due to progressive shortening of *telomeres* at the ends of chromosomes.**

- **Tumor cells, unlike normal cells, are capable of limitless replication.**
- **Markedly shortened telomeres are recognized by the DNA repair machinery as double-stranded DNA breaks leading to cell cycle arrest and senescence mediated by *TP53* and *RB.***

- **In cells in which *TP53* or *RB* mutations are disabled by mutations, the nonhomologous end-joining pathway is activated in a last effort to save the cell joining the shortened ends of two chromosomes**

- **Short telomeres are recognized by the DNA repair machinery leading to cell cycle arrest mediated by *p53* and *RB.***
- **Cells in which the checkpoints are disabled by *p53* or *RB* mutations the nonhomologous end-joining pathway is activated as a last effort to save the cell joining the shortened ends of two chromosomes.**

- **This inappropriately activated repair system results in dicentric chromosomes that are pulled apart at anaphase, resulting in new double-stranded DNA breaks.**
- **The resulting genomic instability from the repeated bridge-fusion-breakage cycles eventually produces mitotic catastrophe, characterized by massive cell death.**

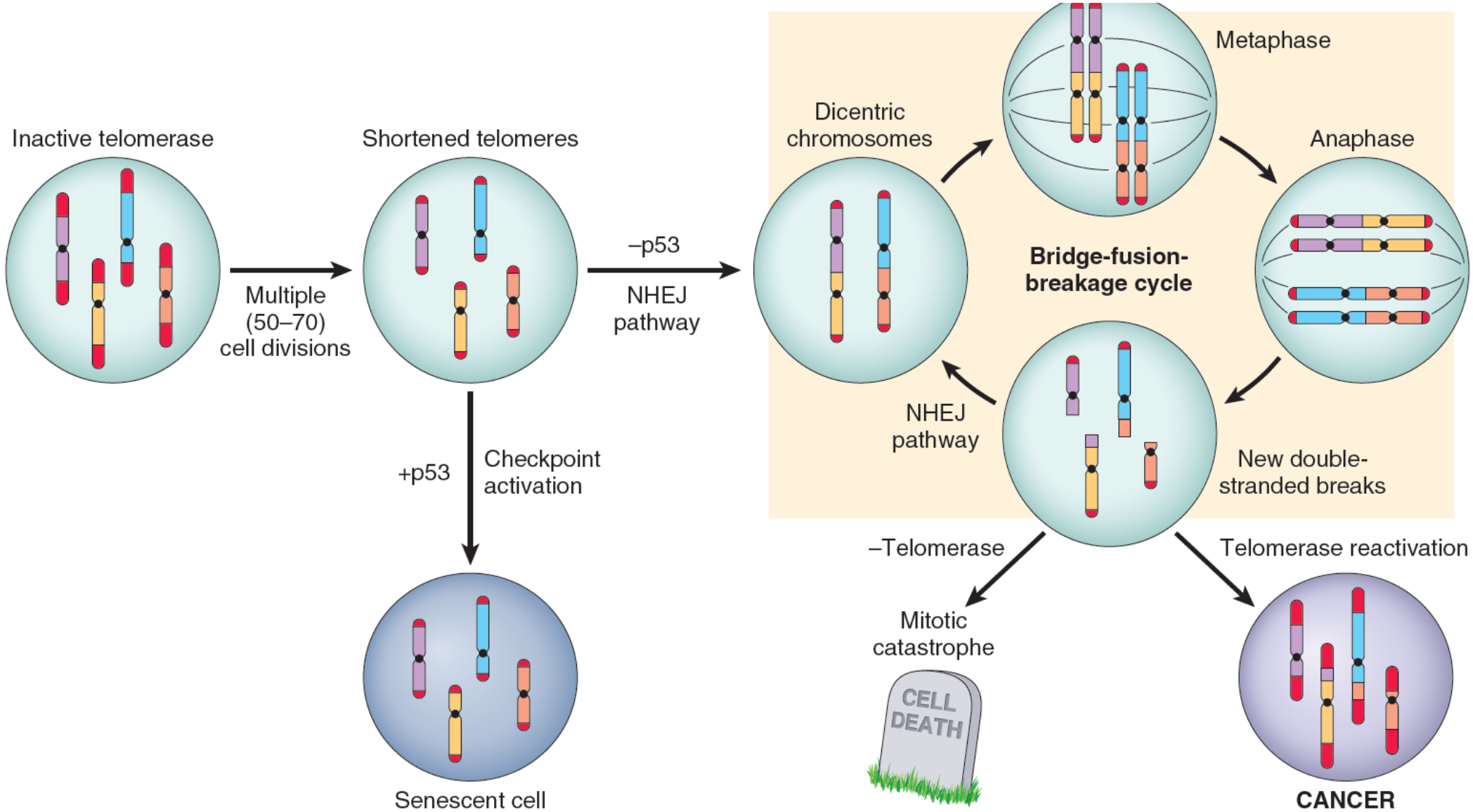



**Fig. 6.26** Escape of cells from replicative senescence and mitotic catastrophe caused by telomere shortening.

- **Such an inappropriately activated repair system results in dicentric chromosomes that are pulled apart at anaphase resulting in new double-stranded DNA breaks.**
- **The resulting genomic instability from the repeated bridge–fusion–breakage cycles eventually produces mitotic catastrophe characterized by massive apoptosis.**

- **It follows that for tumors to grow indefinitely, as they often do, loss of growth restraints is not enough.**
- **Tumor cells must also develop ways to avoid both cellular senescence and mitotic catastrophe .**

- **If during crisis a cell manages to reactivate telomerase the bridge-fusion-breakage cycles cease and the cell is able to avoid death.**
- **During this period of genomic instability that precedes telomerase activation, numerous mutations could accumulate.**
- **Passage through a period of genomic instability probably explains the complex karyotypes frequently seen in human carcinomas.**

- **Telomerase, active in normal stem cells, is normally absent from, or at very low levels in, most somatic cells.**
- **telomere maintenance is seen in virtually all types of cancers.**
- **In 85-95% of cancers due to up-regulation of the enzyme telomerase.**

- **A few tumors use other mechanisms, termed alternative lengthening of telomeres, which probably depend on DNA recombination.**

- **In the progression from colonic adenoma to colonic adenocarcinoma, early lesions had a high degree of genomic instability with low telomerase expression**
- **Malignant lesions had complex karyotypes with high levels of telomerase activity consistent with a model of telomere-driven tumorigenesis in human cancer.**