Apoptosis and tumor suppressor proteins

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201 OM

Oncology and oncosurgery

What is apoptosis?

- **Apoptosis** is a regulated cellular **suicide mechanism** characterized by nuclear condensation, cell shrinkage, membrane blebbing, and DNA fragmentation.
- **Apoptosis**, or programmed cell death, is an evolutionary conserved genetic process of cellular suicide, which plays a crucial role in sculpting the developing organism and in "pruning" billions of **unwanted, unneeded, or damaged cells** every day during adult life

Importance of Apoptosis

- **1) Crucial for embryonic development**
- -Errors in Apoptosis can lead to Birth Defects
- **2) Important for maintaining homeostasis**
- - Cell death is balanced with mitosis to regulate cell number.
- **3) Improper regulation contributes to human disease**
- - Neurodegenerative diseases
- Parkinson's
- Alzheimer's
- -**Cancer**
- - Autoimmune diseases e.g. (diabetes type I)
- - Viral diseases

Morphology

- Cell shrinkage (condensation of cytoplasm)
- Breakdown of mitochondria; release of cytochrome C
- Nuclear condensation
- Nuclear fragmentation
- Cell membrane blebbing
- Fragmentation; apoptotic body formation: membrane-bound cellular fragments, which often lack nuclei
- Phagocytosis

How Apoptosis Differs from Necrosis?

- Apoptosis is **intrinsically controlled**, necrosis is not
- Apoptosis is more **rapid** (12-24 hours) than necrosis
- Apoptosis is induced by **endogenous or exogenous stimuli**, necrosis is always induced by exogenous harms
- Apoptosis is **limited to single or few cells** at a time, and occurs among healthy cell population, necrosis is usually **more extensive** & occurs in tissue exposed to injuries
- Cell cytoplasm **shrinks in apoptosis** and swells in necrosis.
- Nucleosomes of apoptotic cells are 180 bp fragments, contrary to the irregular ones in necrosis
- Apoptosis has **no inflammation**, while necrosis leads to liberation of pro-inflammatory mediators
- Apoptosis has **no systemic manifestations** contrary to most inflammations

Mechanism

- I. Four stages of apoptosis have been defined:
- i. Committment to death by extracellular or intracellular triggers/signals
- ii. Cell killing (execution) by activation of intracellular proteases (caspases)
- iii. Engulfment of cell corpse by other cells
- iv. Degradation of the cell corpse within the lysosomes of phagocytic cells

Death Factors

- Definition: cytokines that activate an apoptosis program by binding to their specific receptor.
- **Typical examples of death factors are:**
- Fasligand, FAS L
- TNF (tumor necrosis factor) and
- TRAIL (TNF-related apoptosis-inducing ligand).
- - Apoptosis can also be induced by cytotoxic T-lymphocytes using the enzyme granzyme.

III. Activation of Caspase cascade

- i. Various stimuli described above eventually activate the executioner (caspase) cascade
- ii. At least 14 different caspases exist in human cells
- iii. Caspase cascades are apparently required for complete execution

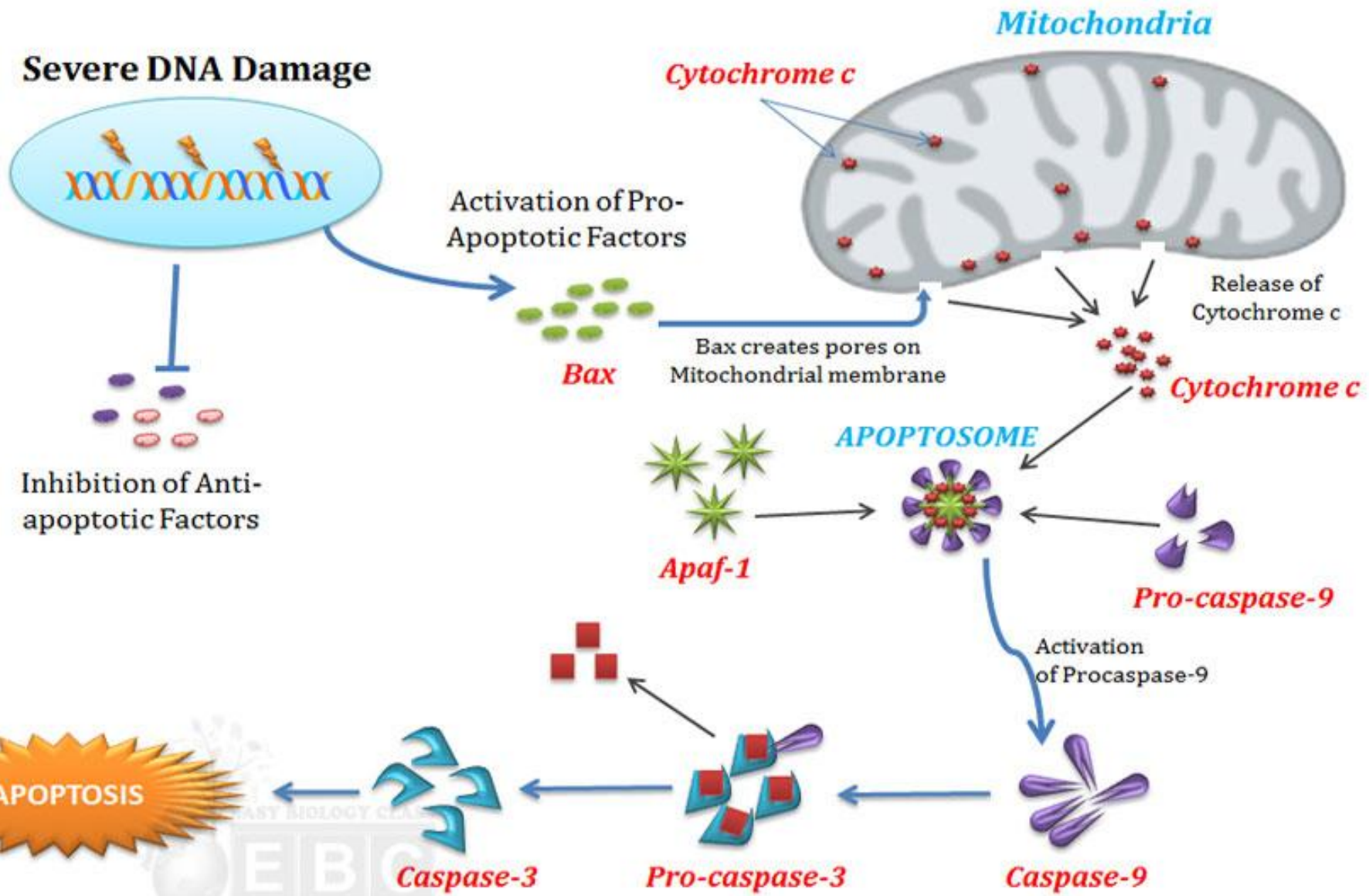
The intrinsic (mitochondrial) pathway of apoptosis.

- Death agonists cause changes in the inner mitochondrial membrane, resulting in the mitochondrial permeability transition (MPT) and release of cytochrome c and other pro-apoptotic proteins into the cytosol, which activate caspases.
- AIF= Apoptosis inhibitory factor;
- IAPs= Inhibitors of apoptosis proteins;
- Apaf-1= apoptosis protease activating factor

- Caspases are central initiators and executioners of apoptosis
- The term caspases is derived from cysteine-dependent aspartate-specific proteases
- The caspase cascade can be activated by:
- Granzyme B released by cytotoxic T lymphocytes which is known to activate caspase-3 and -7;
- death receptors (like FAS, TRAIL receptors and TNF receptor) which can activate caspase-8 and -10; and
- the apoptosome, regulated by cytochrome c and the Bcl-2 family, which activates caspase-9.

INTRINSIC PATHWAY OF APOPTOSIS

(Mitochondria Mediated Programmed Cell Death Pathway)



Activation of Caspase-3 by Caspase-9

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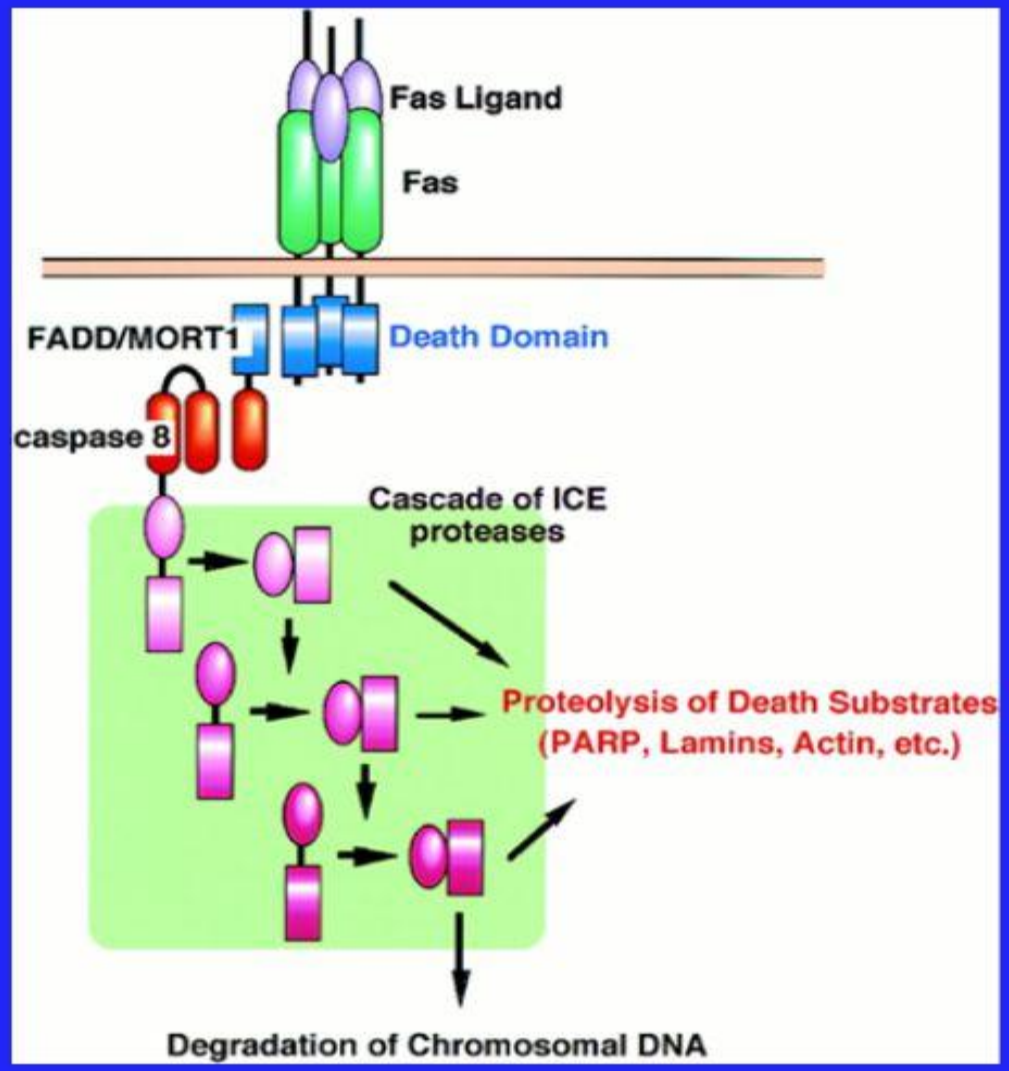
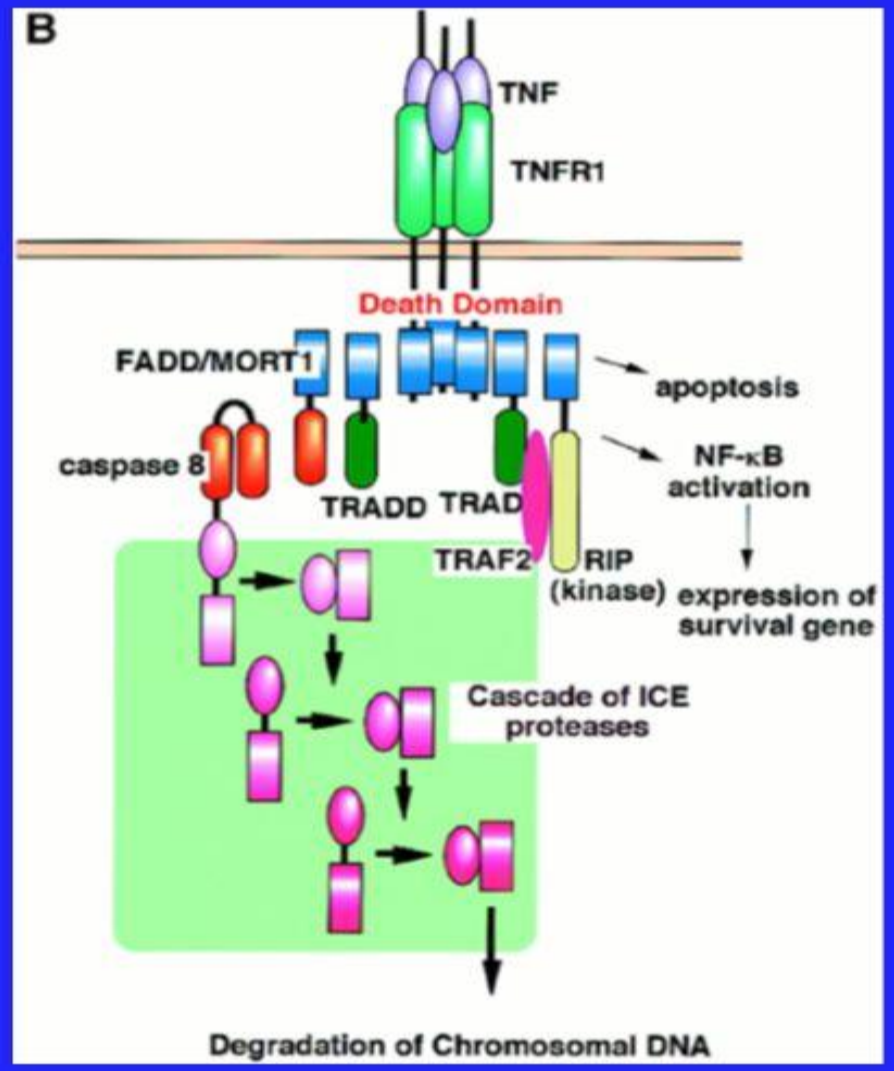
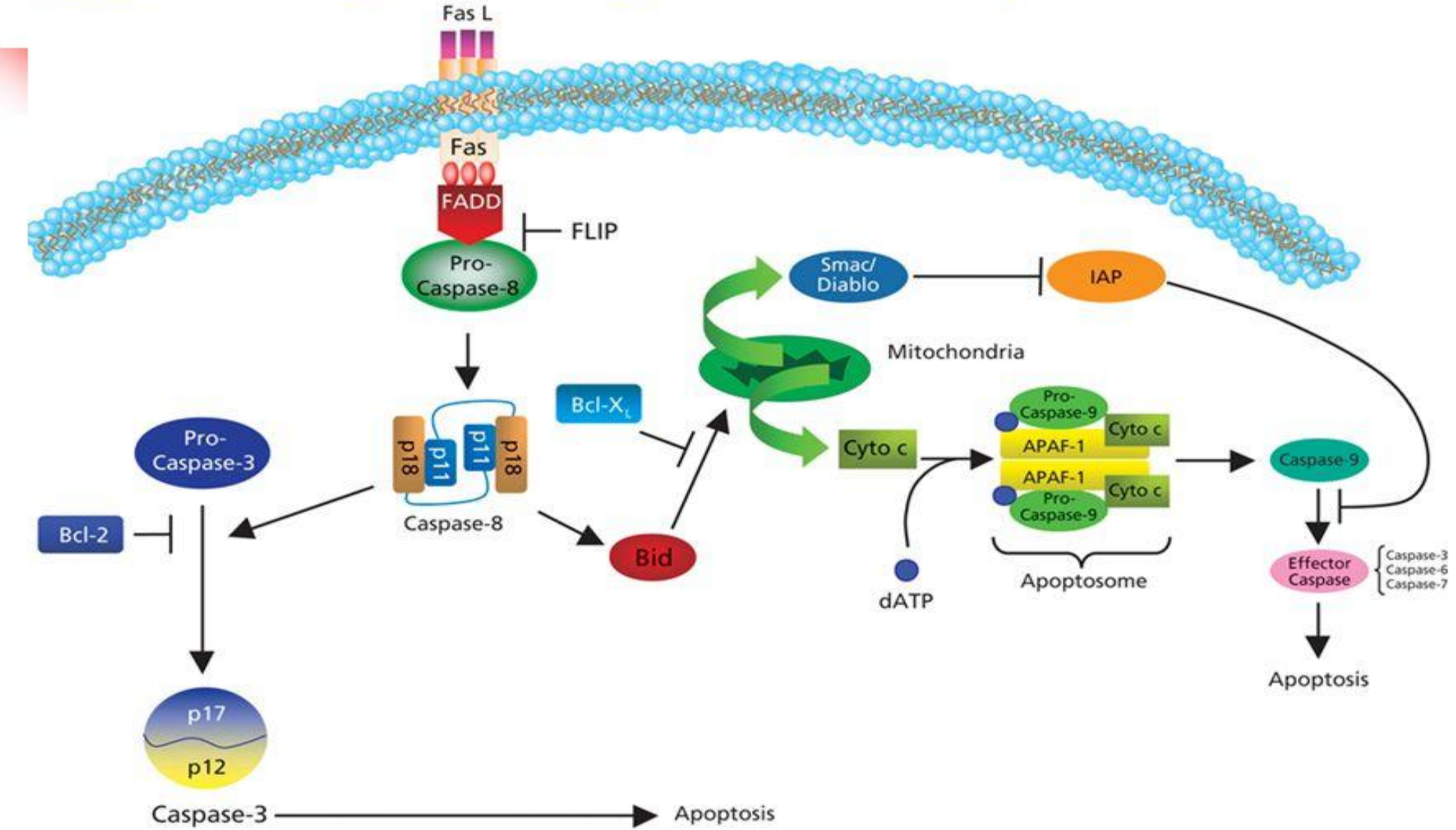
FAS/TNF Death Signaling Pathway





Adapted from Nagata S. Cell 1997

Fas Signaling Pathway



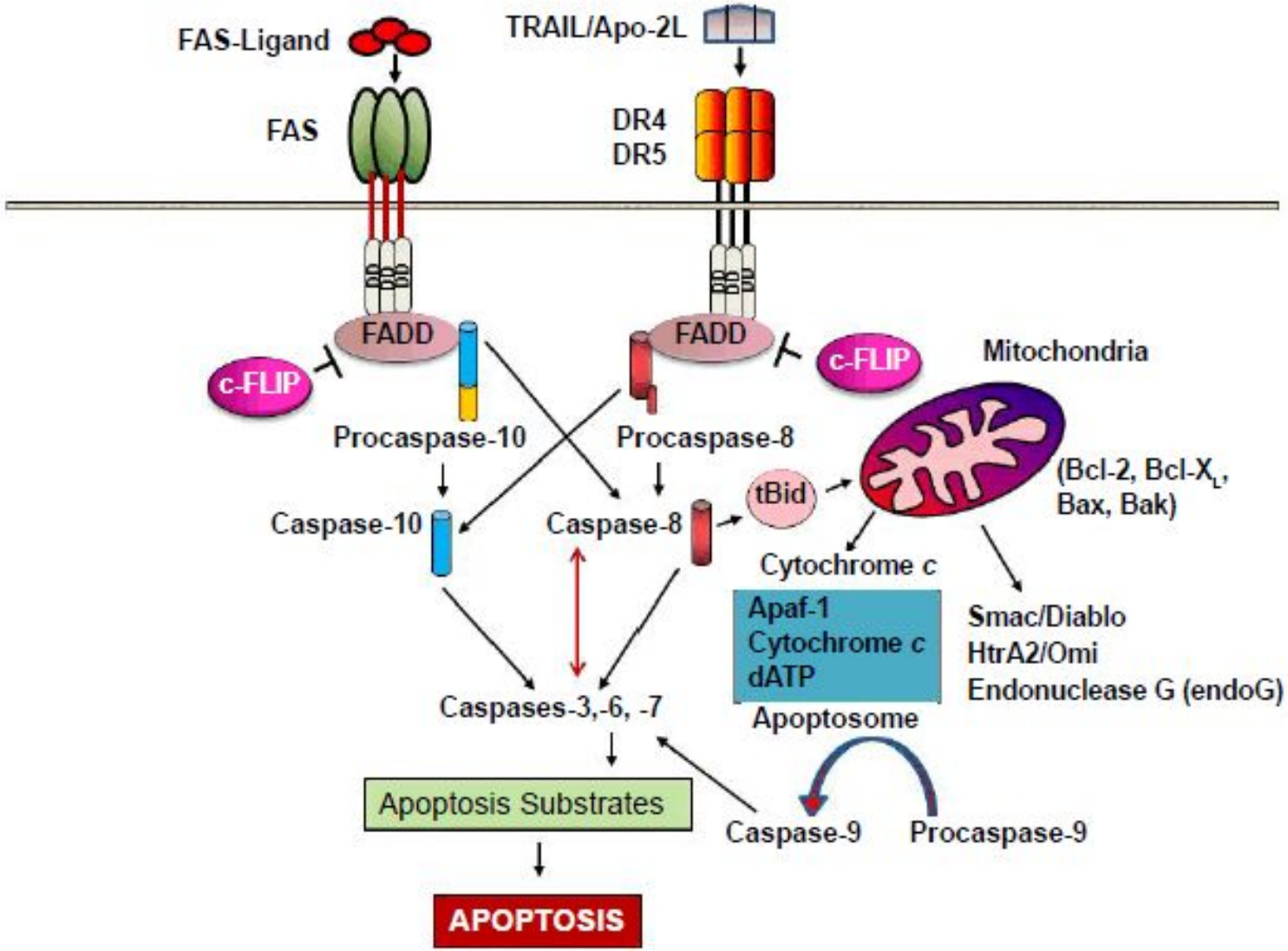
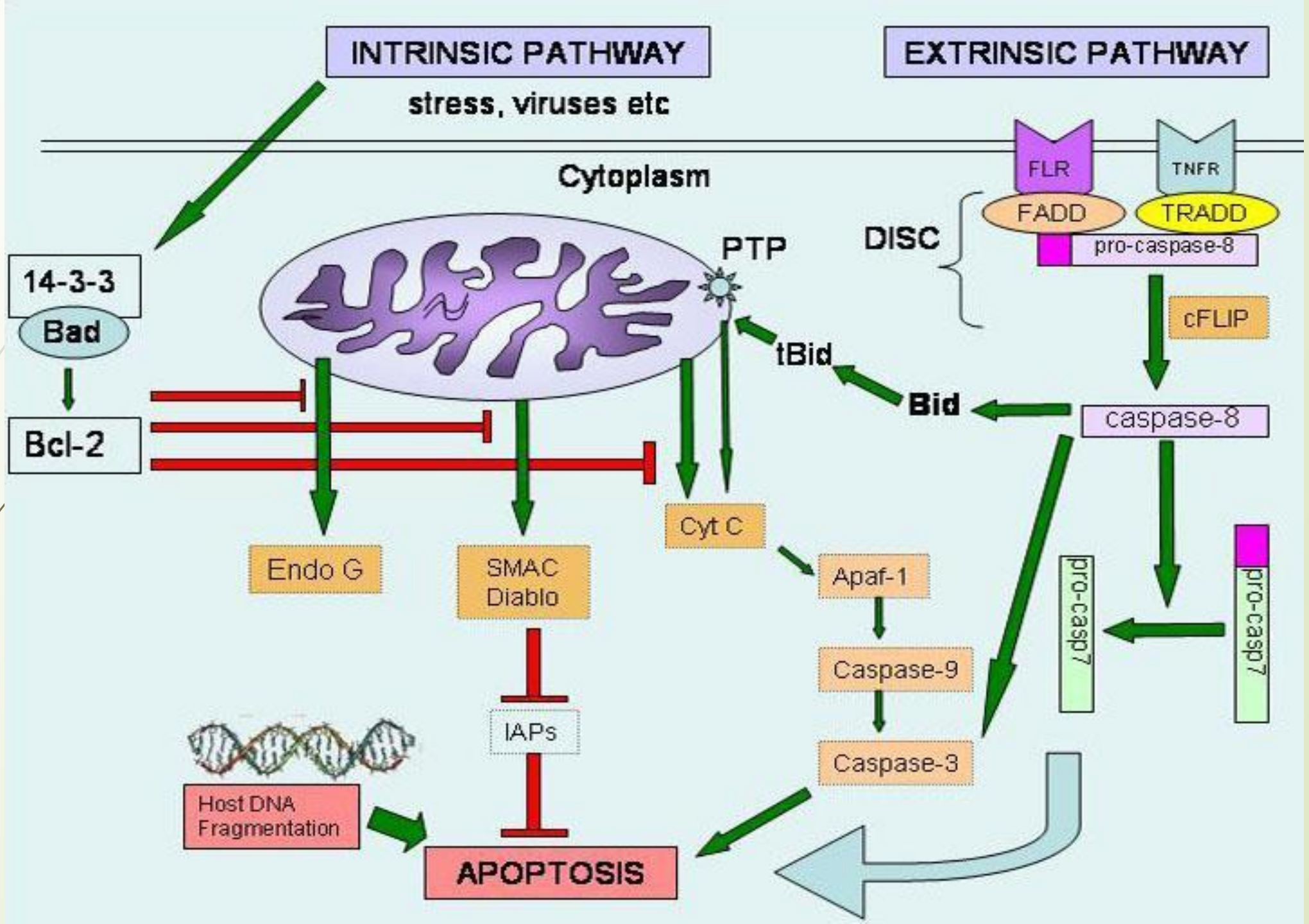
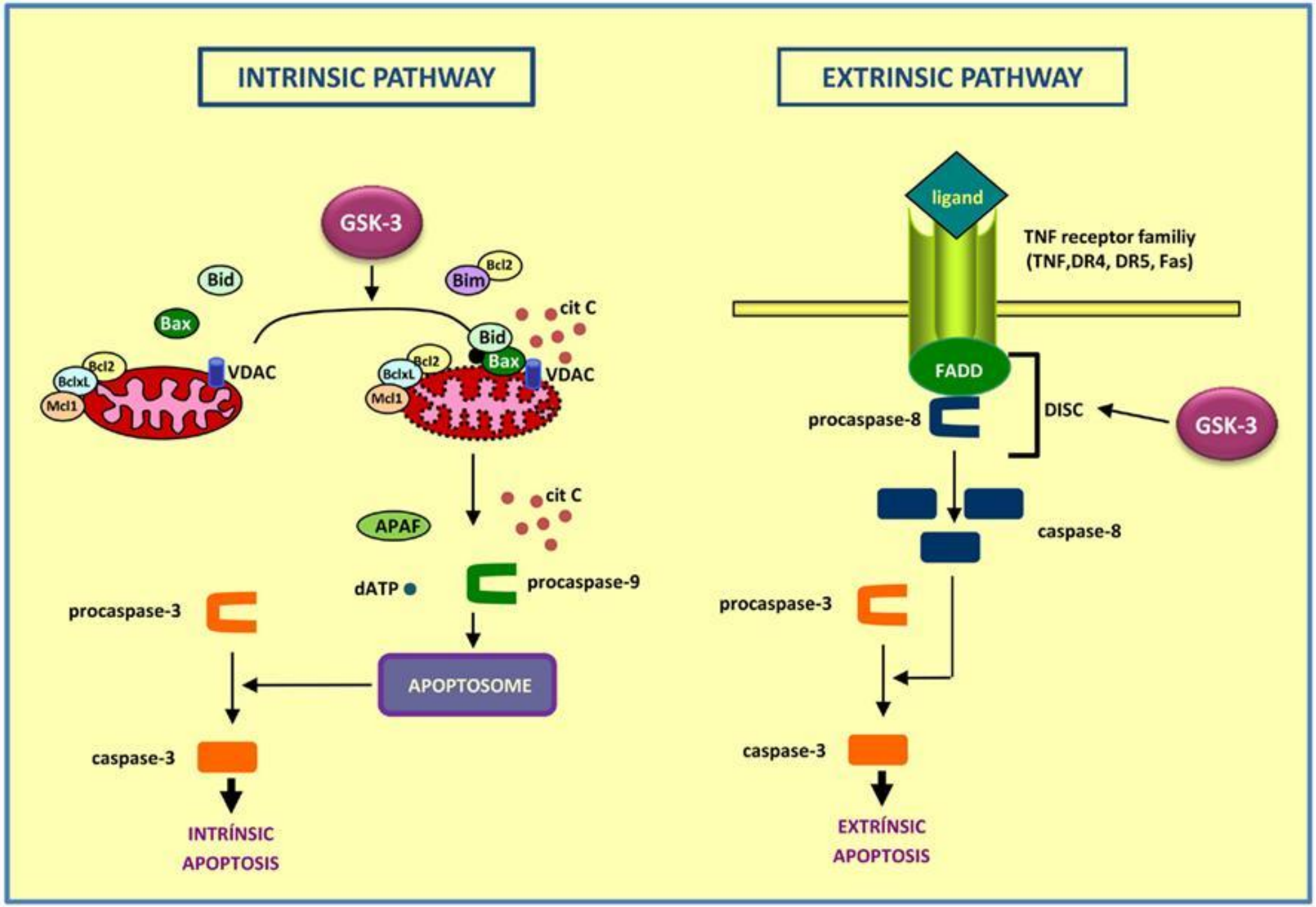


Figure 1: Apoptosis signaling pathways and roles of c-FLIP in preventing apoptosis. Interaction of TRAIL with its receptors DR4 and DR5 or binding of Fas ligand to Fas receptor initiates the death receptor (extrinsic) and subsequently mitochondrial apoptosis signaling pathways through FADD-dependent autocatalytic activation of caspases-8 and -10 and Bid cleavage to truncated Bid. c-FLIP isoforms suppress caspase-8 and -10 activation, therefore preventing the downstream apoptosis cascade.


INTRINSIC PATHWAY
EXTRINSIC PATHWAY
stress, viruses etc
Cytoplasm
FLR
TNFR
FADD
TRADD
DISC
pro-caspase-8
cFLIP
PTP
14-3-3
Bad
Bcl-2
tBid
Bid
caspase-8
Cyt C
Endo G
SMAC
Diablo
Apaf-1
pro-casp7
pro-casp7
Caspase-9
IAPs
Caspase-3
Host DNA
Fragmentation
APOPTOSIS


INTRINSIC PATHWAY
EXTRINSIC PATHWAY
GSK-3
Bid
Bim
Bcl2
Bax
cit C
Bid
Bax
Bcl2
BclxL
Mcl1
VDAC
VDAC
APAF
cit C
dATP
procaspase-9
procaspase-3
APOPTOSOME
caspase-3
INTRÍNSIC
APOPTOSIS
ligand
TNF receptor familiy
(TNF,DR4, DR5, Fas)
FADD
DISC
GSK-3
procaspase-8
caspase-8
procaspase-3
caspase-3
EXTRÍNSIC
APOPTOSIS

Resistance to Fas signalling in cancer

Figure 1. Fas apoptotic pathway and mechanisms of resistance to Fas signaling in cancer. The activated Fas receptor recruits the adapter molecule FADD by homotypic interaction through their death domains. Fas-associated death domain (FADD) recruits the initiator procaspase 8 by the death effector domain to form the death-inducing signaling complex (DISC). Procaspase 8 is cleaved in the DISC to give rise to active caspase 8, which dissociates from the DISC to proceed with the activation of the caspase cascade. In type 1 cells, sufficient caspase-8 is generated to directly activate caspase-3. However, in type 2 cells, the signal needs to be amplified through the mitochondria. Caspase-8 induces the cleavage and activation of B cell leukemia 2 homology domain 3 interacting domain death agonist (Bid) and its translocation to the mitochondria, which in turn causes the release of pro-apoptotic molecules from the intermembrane space of mitochondria, like cytochrome c (Cyto c). It clusters with apoptotic peptidase activating factor 1 (Apaf-1) and Procaspase-9 to form the apoptosome, producing active caspase-9, which in turn converges with the extrinsic pathway in the activation of caspase-3, the cleavage of death substrates in the cell, and ultimately in the death of the cell. Often, cancer cells protect themselves from Fas-mediated apoptosis, which is achieved through different mechanisms leading to resistance acquisition (mechanisms 1 to 6 in figure, further explained in boxes).

DcR3: Decoy receptor 3; FLIP: FADD-like IL-1β-converting enzyme-like apoptotic protein-inhibitory protein; IAPs: Inhibitors of apoptosis proteins; mFAS: Membrane Fas; miRNA: microRNA; sFAS: Soluble Fas; sFASL: Soluble Fas ligand.

Figure 2. Approaches targeting the Fas/FasL signaling for cancer therapy. In order to sensitize tumor cells to Fas-mediated apoptosis, various approaches have been developed to target the Fas/FasL system at each of the levels than can be affected. The approaches are summarized in three major groups (1 to 3), see text for details. Group 3 is subdivided into six subgroups of approaches, according to the level of action.

DISC: Death-inducing signaling complex; FADD: Fas-associated death domain; FLIP: FADD-like IL-1β-converting enzyme-like apoptotic protein-inhibitory protein; HDIs: Histone deacetylases inhibitors; IAPs: Inhibitors of apoptosis; mFAS: Membrane Fas; miRNAs: microRNAs; MMPIs: MMP inhibitors; sFAS: Soluble Fas; sFASL: Soluble Fas ligand.

- **Ингибиторы апоптоза** (антиапоптические факторы). К наиболее серьезным ингибиторам апоптоза относятся ростовые факторы. Другие: нейтральные аминокислоты, цинк, эстрогены, андрогены, некоторые белки.
- Пример: Белки семейства 1AP — подавляют активность каспаз 3 и 9, один из этих белков (Survin) обнаружен в опухолевых клетках. С ним связывают резистентность опухолевых клеток к химиотерапии.
- **Активаторы апоптоза**(проапоптические факторы). Это проапоптические гены и их продукция: гены семейства BCL-2 (BAX и BID); гены Rb и P53 (запускают апоптоз, если клетка задержана механизмом checkpoint).
- Патогенез многих заболеваний, в том числе и опухолевых, связан со снижением способности клеток подвергаться апоптозу. Отсюда накопление поврежденных клеток и формирование опухоли.

Bcl-2

- Bcl2 was the first apoptosis-related gene that was recognized to play a role in **tumorigenesis**, and indeed, Bcl-2 is overexpressed in a variety of cancers, contributing to cancer cell survival through direct inhibition of apoptosis.
- BCL-2 is a **human proto-oncogene** located on chromosome 18.
- Its product is an **integral membrane protein (called Bcl-2)** located in the membranes of the endoplasmic reticulum (ER), nuclear envelope, and in the outer membrane of the mitochondria.
- The gene was discovered as the translocated locus in a **B-cell leukemia** (hence the name). This translocation is also found in some **B-cell lymphomas.**

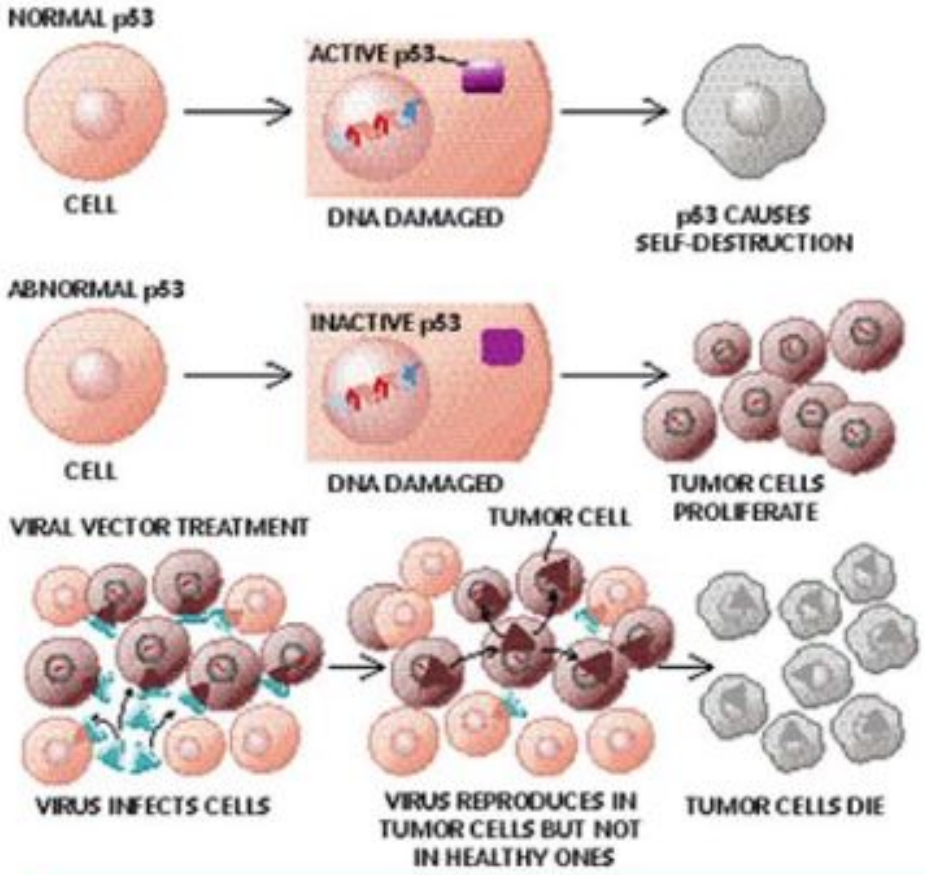
Белки супрессоры

- 1. Обнаружение повреждения в структуре ДНК. Этот факт - стимул для активации генов-супрессоров.
- 2. Гены-супрессоры продуцируют белки Rb и p53.
- 3.Белки Rb и p53 запускают апоптоз поврежденной клетки. Это - индукторы апоптоза. Белок p53 индуцирует апоптоз в момент G_1/S. Белок Rb индуцирует апоптоз в момент G_2/M.

- **Биологическая роль генов-супрессоров:** они не пропускают в митоз клетку с поврежденной ДНК. Дефект гена-супрессора ведет к размножению поврежденной клетки. Пролиферация поврежденной клетки - основа опухолевого роста.
- **Наследование генов-супрессоров.** В каждой клетке есть по два аллеля любых генов. Значит, в каждой клетке есть два гена-супрессора. Дефект одного гена-супрессора повышает риск пропуска в митоз поврежденной клетки. Дефект обоих генов-супрессоров всегда приводит к пропуску в митоз поврежденной клетки и опухолевому росту.
- Пример: наследственная ретинобластома - опухоль сетчатки глаза - диагностируется в раннем детском возрасте (зрачок отсвечивает красным). Этиология - наследственный дефект гена-супрессора Rb и как следствие - постоянный пропуск в митоз клеток с поврежденной ДНК.

Loss of Cell Cycle Controls in Cancer Cells

- Cancer cells
  - Do not respond normally to the body's control mechanisms
  - Form tumors



P53 is a tumor suppressor protein that in humans is encoded by the ***TP53* gene**

***Ras* proteins** also play a role in cell growth and division. Overactive Ras signaling can ultimately lead to cancer

(**ra**t **s**arcoma)

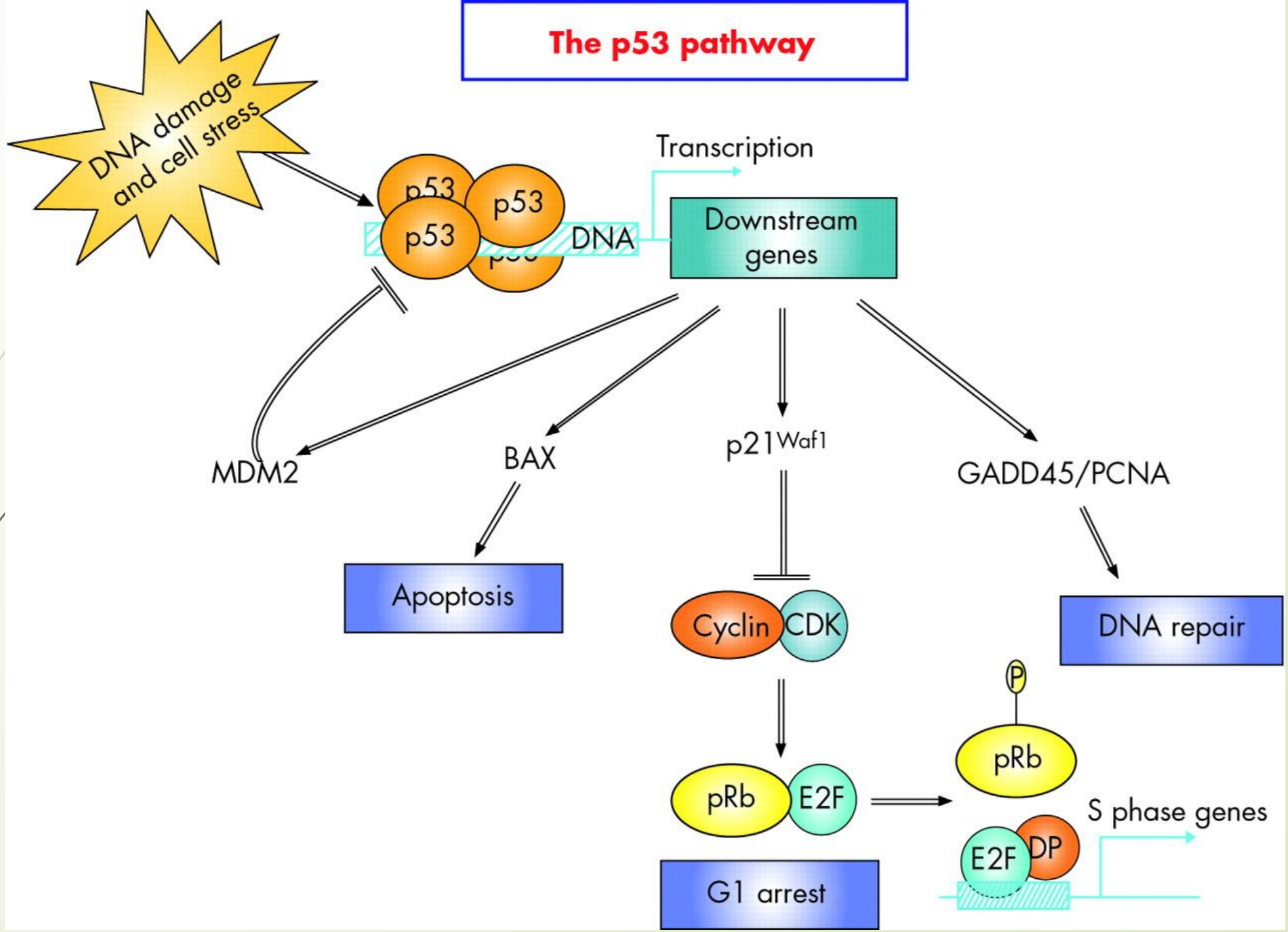
BRCA1 and BRCA 2 are tumor-suppressor genes expressed in the cells of breast and other tissue, where it helps repair damaged DNA, or destroy cells if DNA cannot be repaired. If BRCA itself is damaged, damaged DNA is not repaired properly and this increases risks for cancers

P53 protein

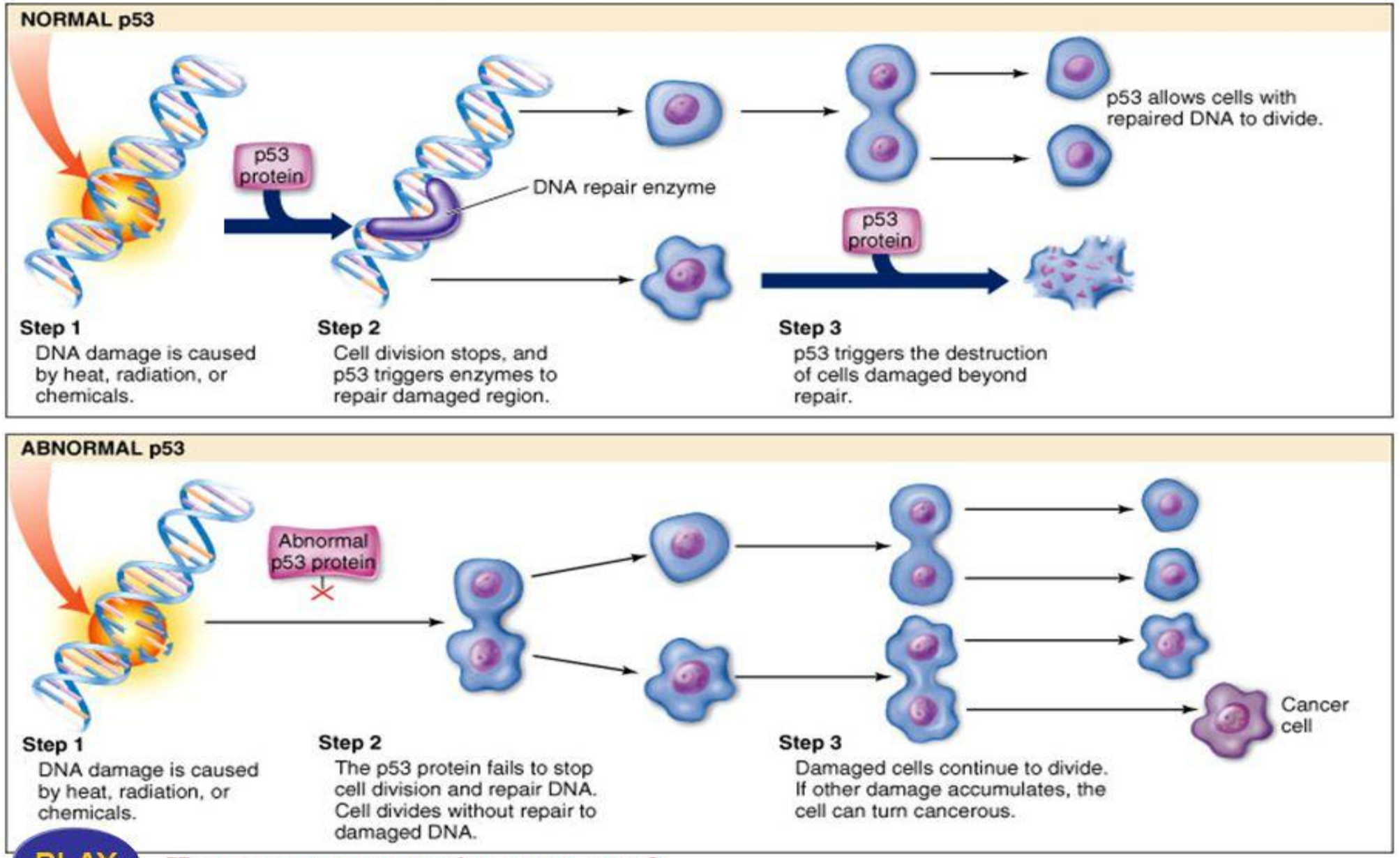
- Acts as a tumor suppressor gene
- 2 Main Functions:
  - halts growth and division in cell cycle under aberrant conditions
  - induces apoptosis
- Loss of p53 function leading cause in 30-50% of various types of cancers

- **Сигнальный путь № 1** (связан с повреждением ДНК):
- 1. Повреждение ДНК
- 2. Активация гена p53 и продукция соответствующего белка
- 3. Активация проапоптических генов семейства BCL-2 (BAX и BID)
- 4. Образование белков этих генов
- 5. Активация каспазы 9
- 6. Активация каспазы 3
- 7. Активация других каспаз и протеаз
- 8. Апоптоз

- The p53 gene like the Rb gene, is a tumor suppressor gene, i.e., its activity stops the formation of tumors. If a person inherits only one functional copy of the p53 gene from their parents, they are predisposed to cancer and usually develop several independent tumors in a variety of tissues in early adulthood. This condition is rare, and is known as Li-Fraumeni syndrome. However, mutations in p53 are found in most tumor types, and so contribute to the complex network of molecular events leading to tumor formation.
- The p53 gene has been mapped to chromosome 17. In the cell, p53 protein binds DNA, which in turn stimulates another gene to produce a protein called p21 that interacts with a cell division-stimulating protein (cdk2). When p21 is complexed with cdk2 the cell cannot pass through to the next stage of cell division. Mutant p53 can no longer bind DNA in an effective way, and as a consequence the p21 protein is not made available to act as the 'stop signal' for cell division. Thus cells divide uncontrollably, and form tumors.


The p53 pathway
DNA damage and cell stress
p53
p53
p53
DNA
Transcription
Downstream genes
MDM2
BAX
p21Waf1
GADD45/PCNA
Apoptosis
Cyclin
CDK
DNA repair
P
pRb
pRb
E2F
S phase genes
E2F
DP
G1 arrest

Cancer, cell division, and p53 protein



PLAY How tumor suppression genes work

В инициации программы апоптоза важная роль принадлежит гену-супрессору wt53.

Белок этого гена – p53 локализован в ядре клетки и является регулятором транскрипции других генов – ген белка p21 и других, которые могут задерживать клетку в G-фазе клеточного цикла.

В норме ген wt53 в клетке молчит. При повреждениях ДНК происходит активация этого гена – много белка его. Он блокирует клеточный цикл в фазах G1 и G2 до репликации ДНК и митоза, делая возможной репарацию ДНК, и этим предотвращает появление клеток с эпимутациями и мутациями. Если репарация не произошла, то индуцируется апоптоз для защиты организма от присутствия дефектных по геному, т.е. предраковых клеток, способных превращаться в раковые клетки.

В половине случаев раковых клеток разного типа ген-супрессор wt53 имеет мутации. Это ведет к превращению предраковой клетки в раковую клетку и возникновению из нее рака. То есть раковая клетка в отличие от любой другой клетки не подвергается апоптозу, если в ней дефекты в ее генах-супрессорах.

Генетические изменения в раковых клетках, ведущие к подавлению обоих путей индукции апоптоза

В них закономерно обнаруживаются:

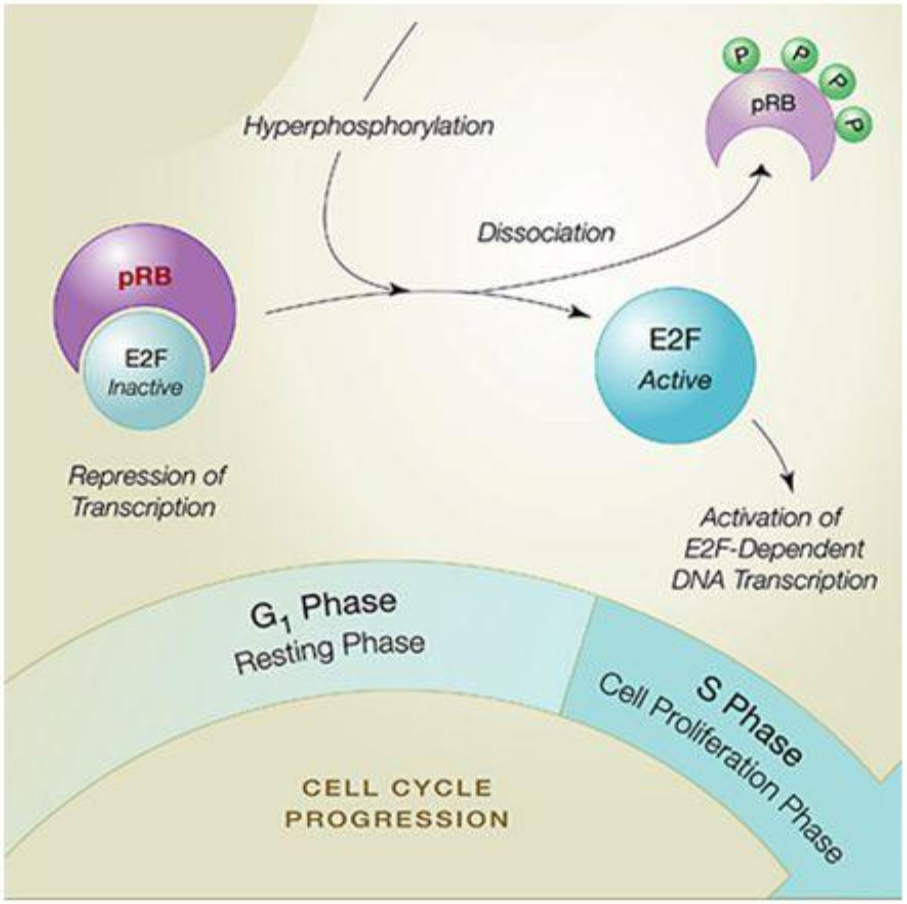
- потеря экспрессии на поверхности раковой клетки рецептора смерти Fas; при наличии бы рецептора Fas взаимодействие его с FAS-L или с моноклональными антителами приводило бы раковую клетку к феноптозу;

- нарушения проведения апоптогенного сигнала к митохондриям. Например, при мутации в «страже» генома гене wt53 и мутации или эпимутации в гене-супрессоре PTEN;

- ингибирование пор во внутренней мембране митохондрии для цитохрома с и AIF, вследствие экспрессии «генов жизни» через их белки – Bcl-2. Эти белки не дают открыть поры во внутренней мембране митохондрии;

- блокирование активации эффекторных каспаз. Например, при потере экспрессии белка Apaf-1 в результате метилирования его гена и др.

Rb, the retinoblastoma protein regulates the cell cycle



Cell cycle = OFF

Rb binds to E2F: no transcription, no entry into S phase

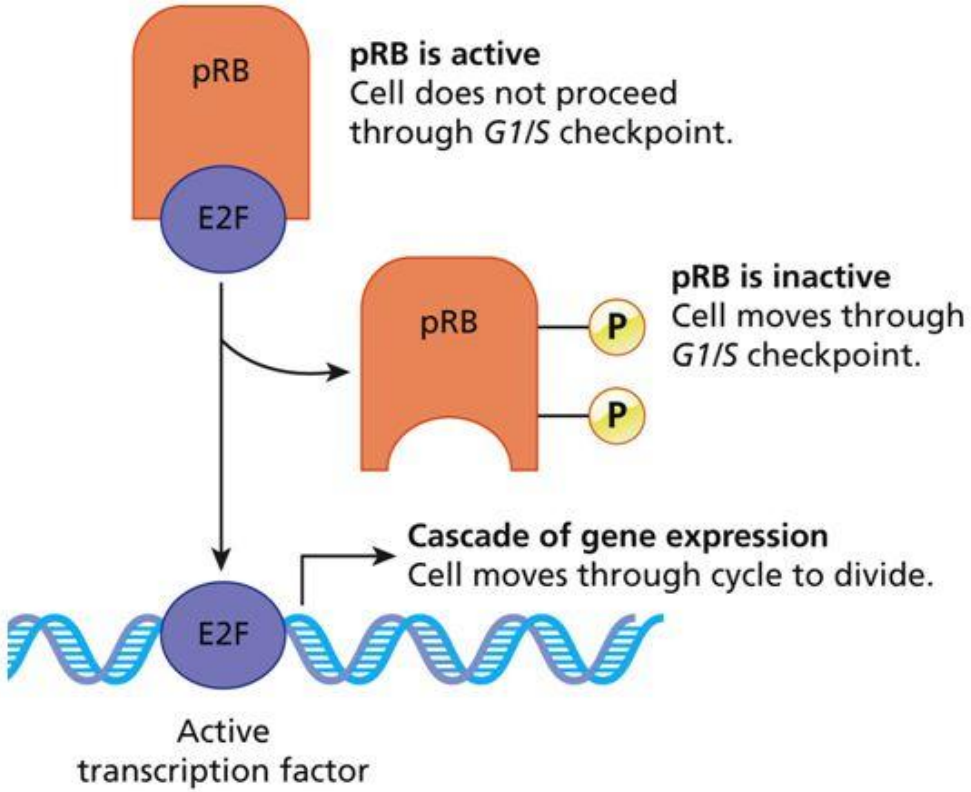
Cell cycle = ON

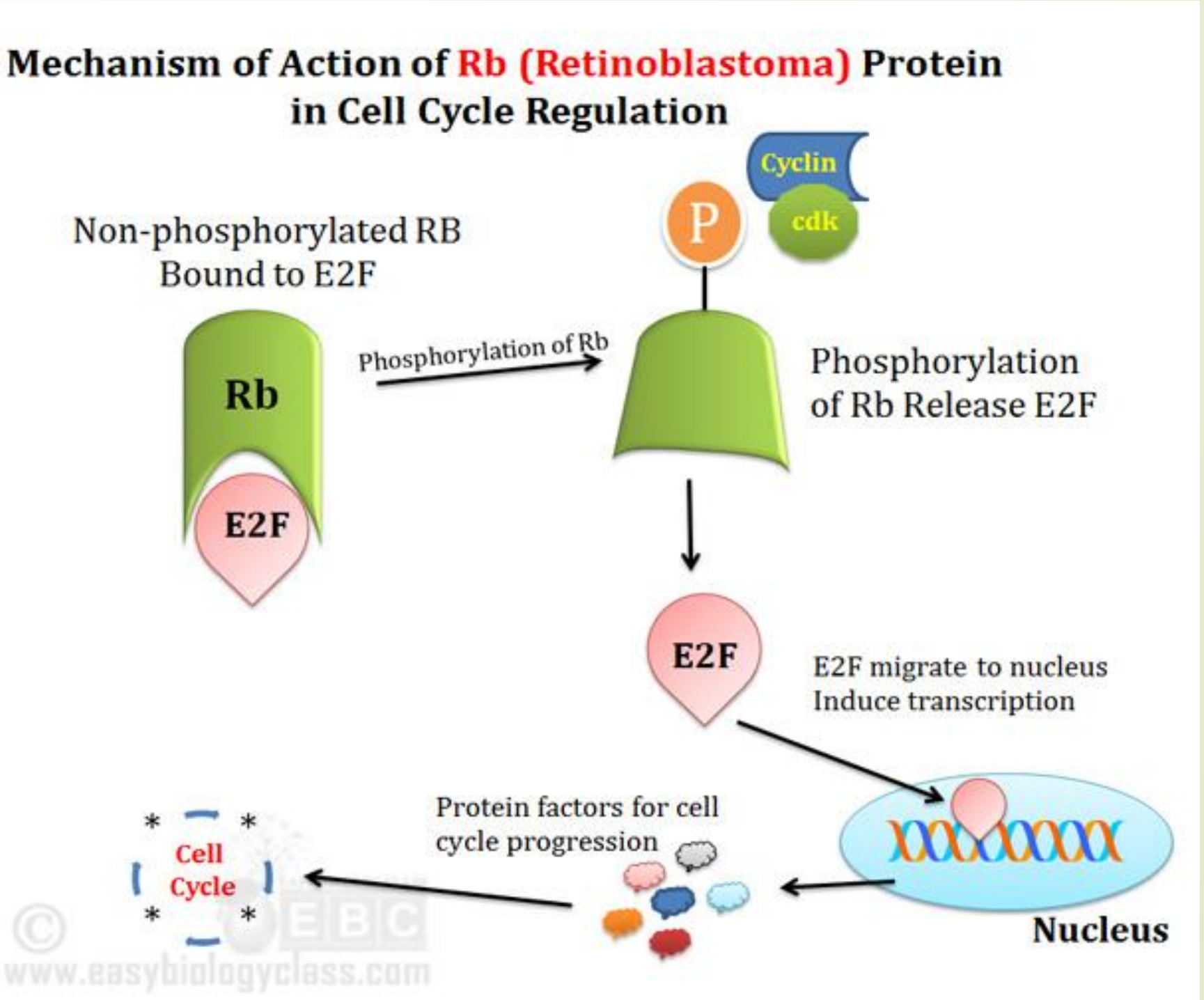
Rb does not bind to E2F: transcription and entry into S phase

w/o 2 copies of Rb: no cell cycle arrest

Tumor-Suppressor Gene

- RB1 is another tumor-suppressor gene found in many cells
- Produces a protein called pRB that blocks cell cycle
- RB1 failure is linked to retinoblastoma and other cancers




Mechanism of Action of Rb (Retinoblastoma) Protein in Cell Cycle Regulation
Non-phosphorylated RB Bound to E2F
Rb
E2F
Phosphorylation of Rb
Cyclin
cdk
P
Phosphorylation of Rb Release E2F
E2F
E2F migrate to nucleus Induce transcription
Nucleus
Protein factors for cell cycle progression
Cell Cycle
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Familial Cancer Syndrome	Tumor Suppressor Gene	Function	Chromosomal Location	Tumor Types Observed
Li-Fraumeni Syndrome	P53	cell cycle regulation, apoptosis	17p13.1	brain tumors, sarcomas, leukemia, breast cancer
Familial Retinoblastoma	RB1	cell cycle regulation	13q14.1-q14.2	retinoblastoma, osteogenic sarcoma
Wilms Tumor	WT1	transcriptional regulation	11p13	pediatric kidney cancer, most common form of childhood solid tumor
Neurofibromatosis Type 1	NF1, protein = neurofibromin 1	catalysis of RAS inactivation	17q11.2	neurofibromas, sarcomas, gliomas
Neurofibromatosis Type 2 GeneReviews	NF2, protein = merlin or neurofibromin 2	linkage of cell membrane to actin cytoskeleton	22q12.2	Schwann cell tumors, astrocytomas, meningiomas, ependymonas
Familial Adenomatous Polyposis	APC	signaling through adhesion molecules to nucleus	5q21-q22	colon cancer
Tuberous sclerosis 1 GeneReviews	TSC1, protein = hamartin	forms complex with TSC2 protein, inhibits signaling to downstream effectors of mTOR	9q34	seizures, mental retardation, facial angiofibromas
Tuberous sclerosis 2 GeneReviews	TSC2, protein = tuberin	see TSC1 above	16p13.3	benign growths (hamartomas) in many tissues, astrocytomas, rhabdomyosarcomas

Pancreatic Carcinoma 4, Familial juvenile polyposis syndrome GeneReviews	DPC4, also known as SMAD4	regulation of TGF-β/BMP signal transduction	18q21.1	pancreatic carcinoma, colon cancer
Deleted in Colorectal Carcinoma	DCC	transmembrane receptor involved in axonal guidance via netrins	18q21.3	colorectal cancer
Familial Breast Cancer GeneReviews	BRCA1	functions in transcription, DNA binding, transcription coupled DNA repair, homologous recombination, chromosomal stability, ubiquitination of proteins, and centrosome replication	17q21	breast and ovarian cancer
Familial Breast Cancer GeneReviews	BRCA2: same as the FANCD1 locus	transcriptional regulation of genes involved in DNA repair and homologous recombination	13q12.3	breast and ovarian cancer
Cowden syndrome GeneReviews	PTEN: **p**hosphatase and **ten**sin homolog	phosphoinositide 3-phosphatase, protein tyrosine phosphatase	10q23.3	gliomas, breast cancer, thyroid cancer, head & neck squamous carcinoma

- https://www.youtube.com/watch?v=8kbAQq_Pp8gb - Intrinsic Pathway
- https://www.youtube.com/watch?v=Aqf-n3pHv1I – Induction of apoptosis
- https://www.youtube.com/watch?v=1_s7KS2rit4 – Role of Mitochondria on apoptosis
- https://www.youtube.com/watch?v=RIk9ZzInzuA – Extrinsic Pathway/ TNF
- https://www.youtube.com/watch?v=f8CpWI-Tqf8 – E/Fas ligand

Literature:

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- https://www.cambridge.org/core/books/molecular-oncology/induction-of-apoptosis/0E2E934B7A64CCF86992F04BF081D8C2#fndtn-information
- https://www.cellsignal.com/contents/science-cst-pathways-apoptosis/regulation-of-apoptosis-interactive-pathway/pathways-apoptosis-regulation
- https://www.nature.com/articles/7290060

- https://www.ncbi.nlm.nih.gov/books/NBK22268/ p53
- https://themedicalbiochemistrypage.org/tumor-suppressors.php

? http://humbio.ru/humbio/cytology/00118f2b.htm p53

Cancers:

- http://cancerlink.ru/6346-2/молекулярная-биология-рака-почек/vhl-tumor-suppressor-protein/