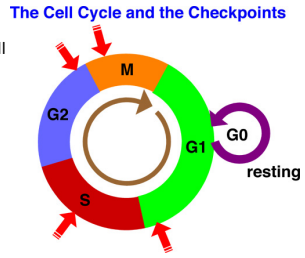
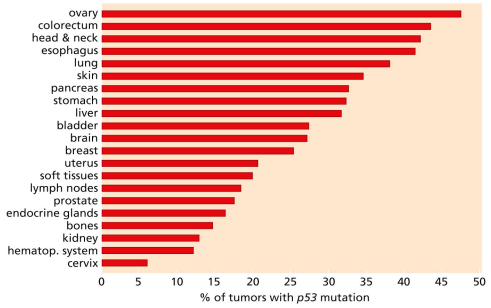
P53 and Apoptosis

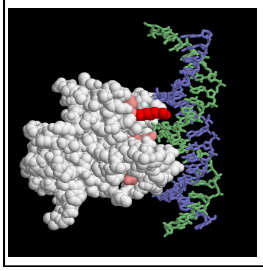
- P53 tumor suppressor TF responsible (with Rb) to control many of the cyclin proteins responsible for regulating checkpoint and activation of cell cycle
- DNA damage (single or double strand – ionizing radiation, viruses, chemcal issues …) activates p53 to stop cell cycle until repair
- Extended time or extend of damage will induce apoptosis
- Within one hour of activation – apoptosis will lead to disappearance of damaged cell



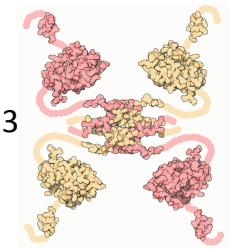
Frequency of mutant p53 alleles in tumor genomes



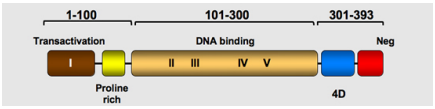
- Homotetramer – large flexible multi-domain transcription factor
- Low concentration until damaging signal occurs
- Tumor suppressor transcription factor
- Common mutation (Arg248 in red – fits into the minor groove forming a stabilizing interaction between protein (+) and DNA (- phosp)
- http://www.rcsb.org/pdb/101/motm.do?momID=31)



p53



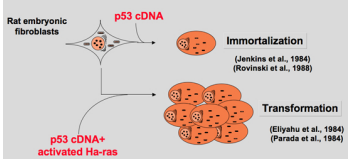
P53 Domains



- Human p53 protein (Hp53) can be divided into five domains, each corresponding to specific functions:
- I) The amino-terminus part 1-42 contains the acidic transactivation domain and the mdm2 protein binding site. It also contains the Highly Conserved Domain I (HCD I)
- II) Region 40-92 contains series repeated proline residues that are conserved in the majority of p53. it also contains a second transactivation domain.
- III) The central region (101-306) contains the DNA binding domain. It is the target of 90% of p53 mutations found in human cancers. It contains HCD II to V.
- IV) The oligomerization domain (307-355, 4D) consists of a beta-strand, followed by an alpha-helix necessary for dimerization, as p53 is composed of a dimer of two dimers. A nuclear export signal (NES) is localized in this oligomerization domain.
- V) The carboxy-terminus of p53 (356-393) contains 3 nuclear localization signals (NLS) and a non-specific DNA binding domain that binds to damaged DNA. This region is also involved in downregulation of DNA binding of the central domain.

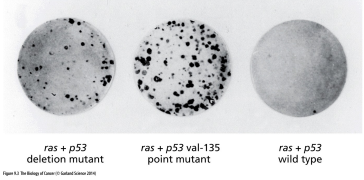
Questionable Origins

- First discovered as a protein bound to a viral (SV40) expressed protein (large T antigen) as a protein of 53-54 kilodaltons (p53)
  - p53 is a target but not product of SV40 transformation
- Several early studies showed p53 cooperated with H Ras.



P53 cDNA was cloned/ synthesized from tumor cells with mutant p53 instead of wild-type normal gene!

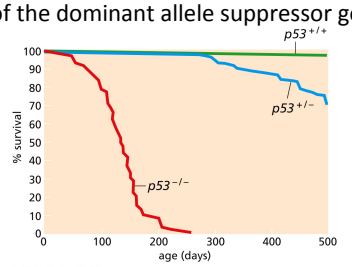
P53 as a tumor suppressor



- Use of point and deletions of wild-type p53 with Ras show true nature of protein function
  - Soft gel agar foci lost when normal p53 and mutant Ras expressed.

Not two hit / LOH model

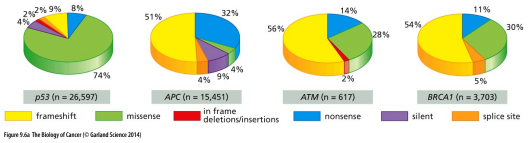
- First of the dominant allele suppressor genes

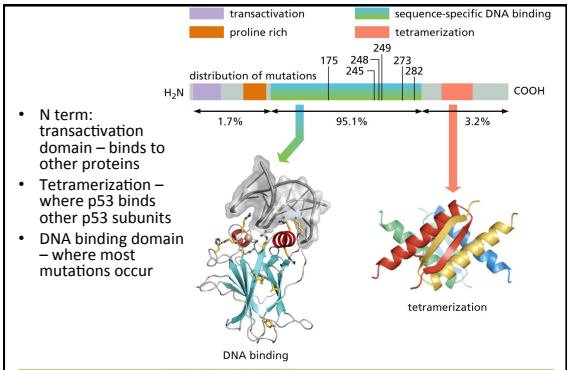


- ***LOSS of BOTH alleles causes death in months typically from lymphomas and sarcomas – indicates the gene is a TS***

Second clue to "novel" TS function

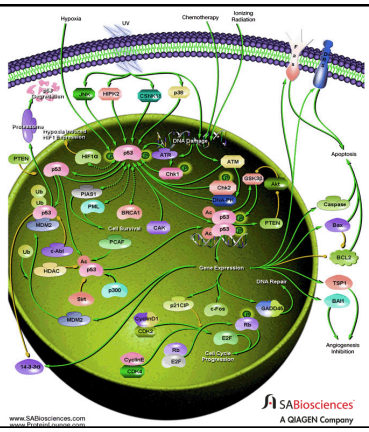
- Most mutations are point -missense mutations not truncation, knockout or nonsense codon mutations





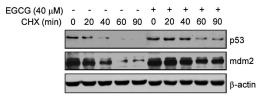
- N term: transactivation domain – binds to other proteins
- Tetramerization – where p53 binds other p53 subunits
- DNA binding domain – where most mutations occur

- Thus – mutations allow for protein interaction with wild-type and mutated p53 but poor binding to DNA – ineffective TF and dominant negative phenotype



p53 Expression – unusual case

- High level of p53 expression but half life is ~20 min.
  - Unusual to have high expression of unstable protein
  - Regulation takes place at transcription, mRNA stability or protein stability
  - Cycloheximide blocks tRNA-mRNA translocation and stops protein synthesis
  - Used to show level of p53 regulation



Jin et al. Oncology Reports 2013 pp1983

Epigallocatechin gallate (EGCC) promotes p53 accumulation in human lung cells

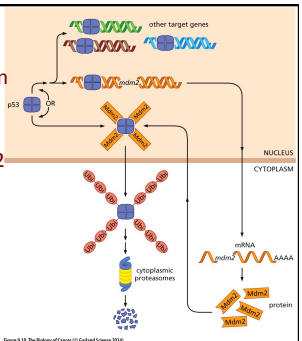
DNA -> mRNA -> protein -> aa

p53

Why make a protein only to destroy it?
Energetically costly –
To rapidly change level of protein to physiological response
Low steady state in normal conditions are easily increased by blocking degredation of protein!

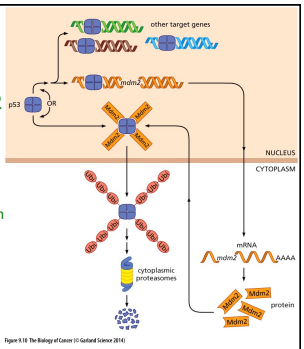
p53 activation

- "activation" is stabilization of protein from proteolysis –
- Two key regulators Mdm2 and p19ARF
- Mdm2 (found in mouse sarcoma cells) binds p53 and targets it for ubiquination



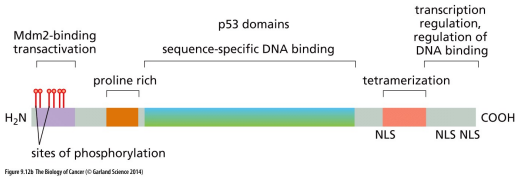
p53 activation

- One of the genes activated by p53 is Mdm2 (negative regulation)
  - Mdm2 binding decreases affinity of p53 for TF partners p300/CBP
  - Mdm2 binds p53 at transactivation domain and promotes ubiquitination
- Both Mdm2 and p53 are differentially phosphorylated regulating action



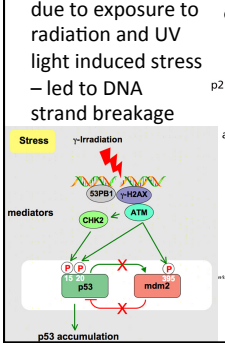
p53 Phosphorylation

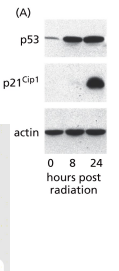
- DNA damage (single and double stranded breaks) activates ATM, Chk1 and Chk2 kinases – which phosphorylate p53 on N term – BLOCKING Mdm2 interaction – leads to higher cellular concentration

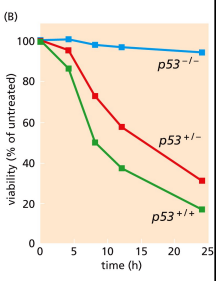


Response to UV/Radiation

- First observation of p53 activation was due to exposure to radiation and UV light induced stress – led to DNA strand breakage

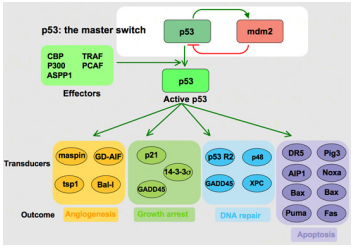




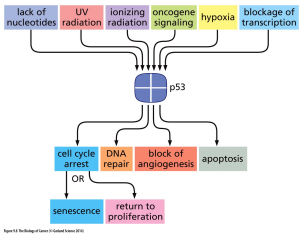


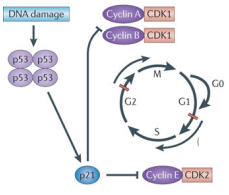
p53 Activation

- Basic activation is due to diminished Mdm2 driven degradation via ubiquitin/proteosome
- Some increase in translation (mRNA) will also occur with extended cellular insult
- Several other PTM and proteins also regulate p53



Cell Cycle Check Point and More





- p21 Cip1 (kinase) is upregulated by p53
- … p53 controls Apoptosis if damage is to great

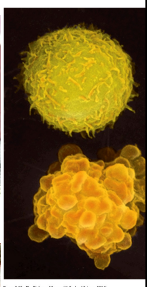
Class of genes	Name of gene	Function of gene product
p53 antagonist	*MDM2/HDM2*	induces p53 ubiquitylation
Growth arrest genes	*$p27^{Kip1}$*	inhibitor of CDKs, DNA polymerase
	Siah-1	aids β-catenin degradation
	14-3-3σ	sequesters cyclin B–CDC2 in cytoplasm
	Reprimo	G_2 arrest
DNA repair genes	*p53R2*	ribonucleotide reductase—biosynthesis of DNA precursors
	XPE/DDB2	global NER
	XPC	global NER
	XPG	global NER, TCR
	GADD45	global NER [?]
	DNA pol κ	error-prone DNA polymerase
Regulators of apoptosis	*BAX*	mitochondrial pore protein
	PUMA	BH3-only mitochondrial pore protein
	NOXA	BH3-only mitochondrial pore protein
	p53AIP1	dissipates mitochondrial membrane potential
	Killer/DR5	cell surface death receptor
	PIDD	death domain protein
	PERP	pro-apoptotic transmembrane protein
	APAF1	activator of caspase 9
	NF-κB	transcription factor, mediator of TNF signaling
	FAS/APO1	death receptor
	PIG3	mitochondrial oxidation/reduction control
	PTEN	reduces levels of the anti-apoptotic PIP3
	Bcl-2	repression of anti-apoptotic protein expression
	IGF-1R	repression of anti-apoptotic protein expression
	IGFBP-3	IGF-1-sequestering protein
Anti-angiogenic proteins	*TSP-1* (thrombospondin)	antagonist of angiogenesis

Abbreviations: NER, nucleotide excision repair; TCR, transcription-coupled repair.

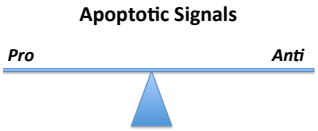
Apoptosis – Eat Me

Programmed Cell Death

- Phosphatidyl Serine and annexin flip from inner-plasma membrane to extracellular leaflet
- Signals phagocytosis by macrophages
- Cells begin complicated series of proteolysis, DNA degradation and membrane/organelle elimination

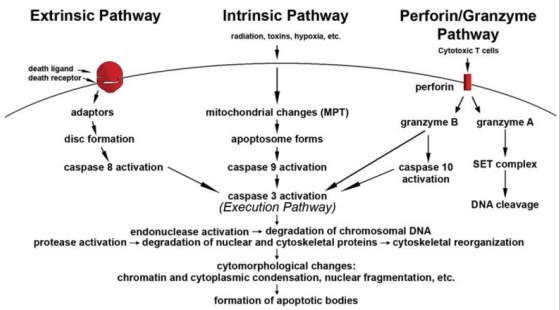


Repair or Die



- p53 commits cells to increase apoptosis
- Cancer cells find ways to mute p53 signaling allowing damaged cells to continue to grow and collect additional mutations
- Fate of cell is a matter of balance of pro and anti apoptotic signals

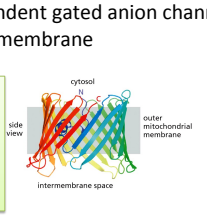
	Apoptosis	Necrosis
Provoking stimuli		
	programmed tissue remodeling	metabolic stresses
	maintenance of cell pool size	absence of nutrients
	genomic damage	changes in pH, temperature
	metabolic derangement	hypoxia, anoxia
	hypoxia	
	imbalances in signaling pathways	
Morphological changes		
Affected cells	individual cells	groups of cells
Cell volume	decreased	increased
Chromatin	condensed	fragmented
Lysosomes	unaffected	abnormal
Mitochondria	morphologically normal initially	morphologically aberrant
Inflammatory response	none	marked
Cell fate	apoptotic bodies consumed by neighboring cells	lysis
Molecular changes		
Gene activity	required for program	not needed
Chromosomal DNA	cleaved at specific sites	random cleavage
Intracellular calcium	increased	unaffected
Ion pumps	continue to function	lost

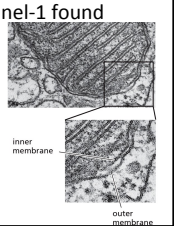


Apoptosis- Intrinsic and Extrinsic pathways

- Intrinsic pathways – driven by pro-apoptotic (death) signals opening ion channels in mitochondria – release cytochrome C to activate caspase proteolytic pathway
  - Bcl-2 blocks apoptosis by keeping VDAC1 closed
  - Voltage-dependent gated anion channel-1 found in inner-mito membrane

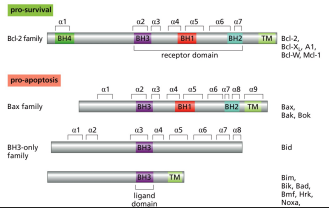
Regulators Bcl and others bind to inner oligo peptide and selected strands to regulate opening and closing



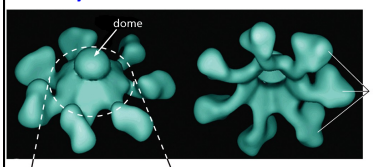


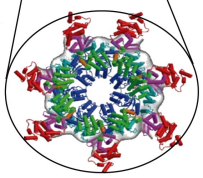
p53 regulated proteins

- Bcl-2 – close VDAK1
- Bax, Bad, Bak and Bid Open channel – some are activated by phosphorylation (Akt/PKB)
- Pro-apoptotic proteins cluster at mito membrane inducing fragmentation of organelle



Cytochrome C starts things badly



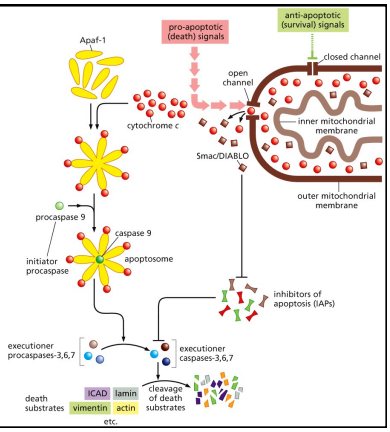


- Apoptosome – Apoptotic protease activating factor 1 (Apaf-1) protein: Central hub of apoptotic complex. WD40 (like βγ subunit of G-proteins) forms complex with procaspase 9 in presence of cytochome C and activates by hydrolysis to caspase 9

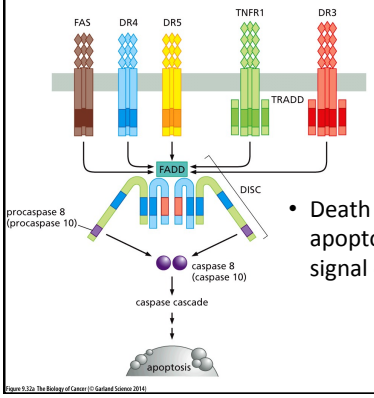
Cytochrome C starts things badly

- Caspase (**C**ysteine **Asp**artyl Prote**ases**) start proteylytic cascade
- Smac/DIABLO inactivates anti-apoptotic IAP (inhibitors of apoptosis) which ubiquinate caspases blocking their action by removal
- Cascade ends in release of ***death substrates*** – activate other proteases responsible for digestion of cell, DNA fragmentation, and cytoskeletal proteins

Intrinsic Pathway

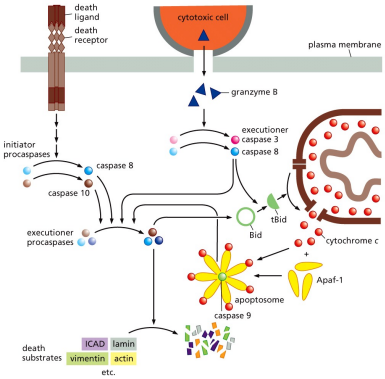


Extrinsic Apoptosis

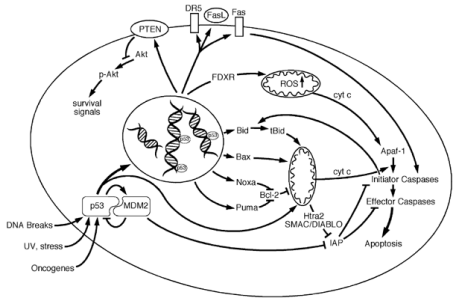


- Death receptors – pro-apoptotic signaling signal caspases

Convergence of Intrinsic and Extrinsic



p53 and apoptosis



- Most p53 control of apoptosis comes from ability to regulate Bcl-2 family proteins
- Each (Bax, Bid…) have p53 promoter element
- Most p53 signaling is intrinsic by inducing Apaf-1
- Fas/CD95 are targeted by p53
- PKB/PI3K signaling (PTEN) also targets for p53
- High p53 induces ROS – inducing greater damage

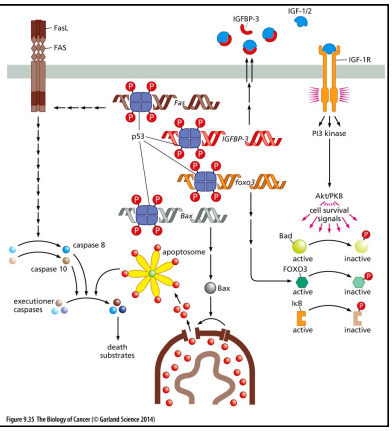


Figure 9.35 The Biology of Cancer (© Garland Science 2014)

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, 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	~½ of human tumors
PTEN inactivation	hyperactivity of Akt/PKB kinase	glioblastoma, prostate carcinoma, endometrial carcinoma

Table 9.5 (part 1 of 2) The Biology of Cancer (© Garland Science 2014)

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

Alteration	Mechanism of anti-apoptotic action	Types of tumors
IGF-1/2 overexpression	activates PI3K	many types
IGFBP repression	loss of anti-apoptotic IGF-1/2 antagonist	many types
Casein kinase II overexpression	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
USP9X overexpression	deubiquitylates Mcl-1	lymphomas
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.

Table 9.5 (part 2 of 2) The Biology of Cancer (© Garland Science 2014)