NEOPLASIA
MOLECULAR LEVEL

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Mutations in cancer

- Nonlethal genetic damage lies at the heart of carcinogenesis
- A tumor is formed by the clonal expansion of a single precursor cell that has incurred genetic damage. The damage is passed onto daughter cells.
- Carcinogenesis results from the accumulation of complementary mutations in a stepwise fashion over time
- Loss-of-function mutations in genes that maintain genomic integrity appear to be a common early step on the road to malignancy, particularly in solid tumors.

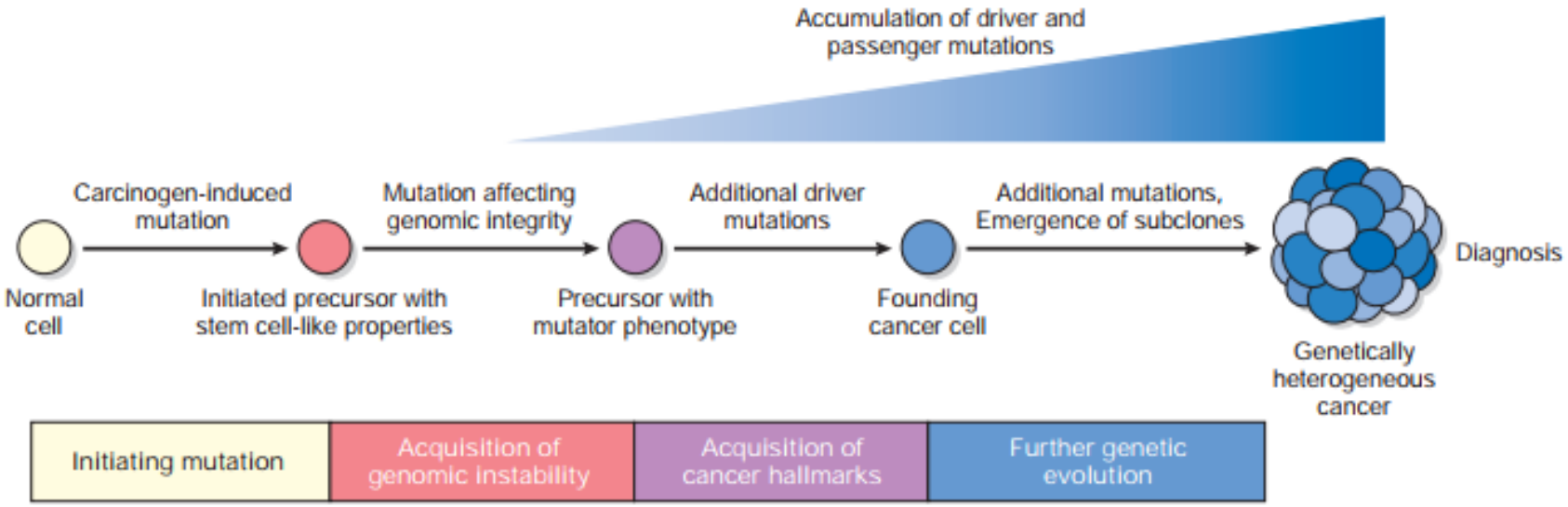


Figure 7-22 Development of a cancer through stepwise acquisition of complementary mutations. The order in which various driver mutations occur in initiated precursor cells is not known and may vary from tumor to tumor. See text for details.

Mutations in cancer

- When a tumor mass is detectable, it contains approximately one billion cells and has gone through 30 doublings.
- Subclones may demonstrate different mutations which were likely acquired after transformation during the outgrowth and spread of the tumor.

Mutations in cancer

- Four classes of normal regulatory genes are the principal genes involved in cancer causing mutations:
- Proto-oncogenes (growth promoting)
- Gain of function
- Tumor suppressor genes (growth inhibiting)
- Loss of function (usually biallelic)
- Genes that regulate programmed cell death (apoptosis)
- Genes involved in DNA repair

Mutations in cancer

- Epigenetic aberrations also contribute to the malignant properties of cancer cells.
- DNA methylation, which tends to silence gene expression
- Modifications of histones (the proteins that package DNA into chromatin)
- May either enhance or dampen gene expression.
- Together, these modifications dictate which genes are expressed, which in turn determines the lineage commitment and differentiation state of both normal and neoplastic cells.

Biological network

- Particular logic
- Complexity and connectivity are hallmarks
- Robust
- Ability to overcome perturbations, inhibitors, mutations
- Positive and negative feedback regulation
- Examples of positive feedback regulation
- TGF-α, heparin binding epithelial growth factor, VEGF transcription products as
- Examples of negative feedback regulation
- Growth factor induced endocytosis and receptor degradation, miRNA production of delayed early genes

Biological network

- Layered
- Hub dependent
- PI3K, AKT, mTOR as examples
- Ligands (or drugs) as inputs to receptor layer.
- Ligands activate surface receptors
- Thrombin, for example, interacts with a trimeric G-protein transmembrane receptor, leading to SRC activation, resulting in MYC transcription in the nucleus

Biological network

- Signal transduction follows, leading to transcription
- AND feedback regulation as delayed early genes inactivate and suppress transcription factors
- Outputs of differentiation, growth, migration, adhesion, apoptosis, as examples
- Cytokines react with transmembrane receptor dimers, leading to JAK activation, resulting in STAT transcription in the nucleus
- Growth factors interact with transmembrane receptor trimers, leading to activation of a variety of pathways (and cascades), resulting in transcription of MYC, JUN, FOS, ELK in the nucleus

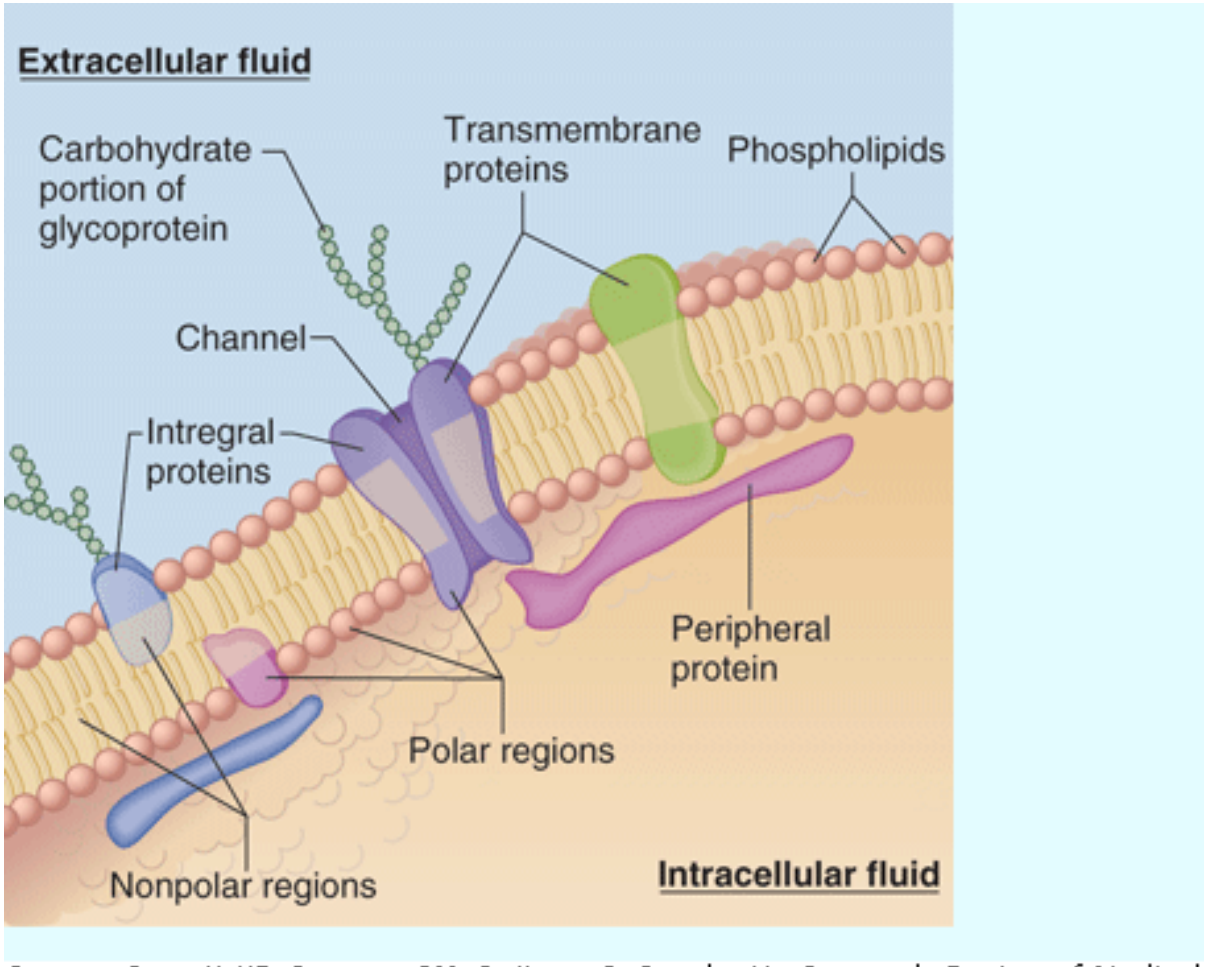
Oncogenesis

- A normal cell repairs DNA damage successfully. If there is a failure of DNA repair, apoptosis follows.
- Mutations in the repair mechanism as well as mutations in genes affecting cell growth (leading to loss or regulatory control) or apoptosis lead to clonal expansion and oncogenesis.
- It is a multi-step process.
- Germ line mutations are inherited.
- They involve a critical step in the multi-step cancer pathway. If the pathway is triggered, oncogenesis follows.

Oncogenesis

- Normally, oncogenesis results from somatic mutations.
- Mutations accumulate over time.
- When cell regulation or apoptotic control is lost, oncogenesis follows.
- Epigenetic changes involve methylation of cytosine C5 that occurs as part of a cytosine-phosphate-guanine dinucleotide (transcription) as well as histone deacetylation.
- Either change tightly binds DNA, rendering it transcriptionally inactive.

Cell membrane



Source: Barrett KE, Barman SM, Boitano S, Brooks H: *Ganong's Review of Medical Physiology, 23rd Edition*: http://www.accessmedicine.com

Reproduced with permission from Widmaier EP, Raff H, Strang K: Vander's Human Physiology: The Mechanisms of Body Function, 11th ed. McGraw-Hill, 2008.)

Fig. 2-2
Accessed 07/01/2010

Signal transduction

- G-protein coupled receptors are membrane based and are linked to a trimeric G-protein that controls the activity of a secondary messenger.
- Insulin, epithelial growth factor, TSH, ACTH, LH, FSH as examples.
- Cytokine receptors are membrane based and associated with cytosolic JAK kinases.
- Activate STAT transcription factors through phosphorylation.
- Growth hormone, prolactin, cytokines as examples.

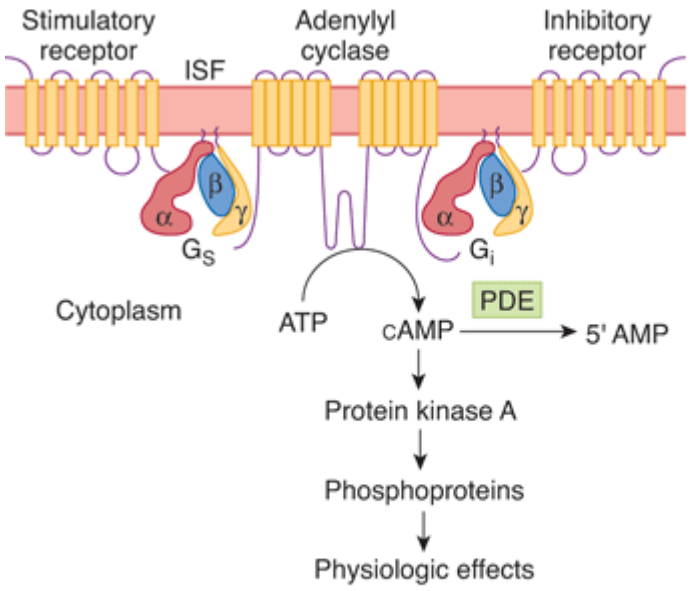
Signal transduction

- Receptor tyrosine kinases are cytosolic.
- Translocate to the nucleus and activate nuclear transcription factors through phosphorylation.
- For example, the GPC receptor in the membrane activates cAMP, phosphokinase A, and translocates to the nucleus where chromosome response element binding occurs.
- PLC activates PKC, leading to FOS transcription in the nucleus
- PI3K activates AKT (BAD, S6K)
- CRK activates ABL

Signal transduction

- NCK as well as VAV/RAC activation leads to PAK/JNNK/JNK cascade and ELK, JUN transcription in the nucleus
- SHC/GRB2/SOS (dephosphorylated by a p21-GDP complex that phosphorylates GAP) activation leads to RAF/MEK/MAPK cascade and ELK, FOS transcription in the nucleus as well as PI3K and AKT activation

cAMP system



Activation of adenylyl cyclase catalyzes the conversion of ATP to cAMP. Cyclic AMP activates protein kinase A, which phosphorylates proteins, producing physiologic effects. Stimulatory ligands bind to stimulatory receptors and activate adenylyl cyclase via G_s. Inhibitory ligands inhibit adenylyl cyclase via inhibitory receptors and G_i. ISF, interstitial fluid.

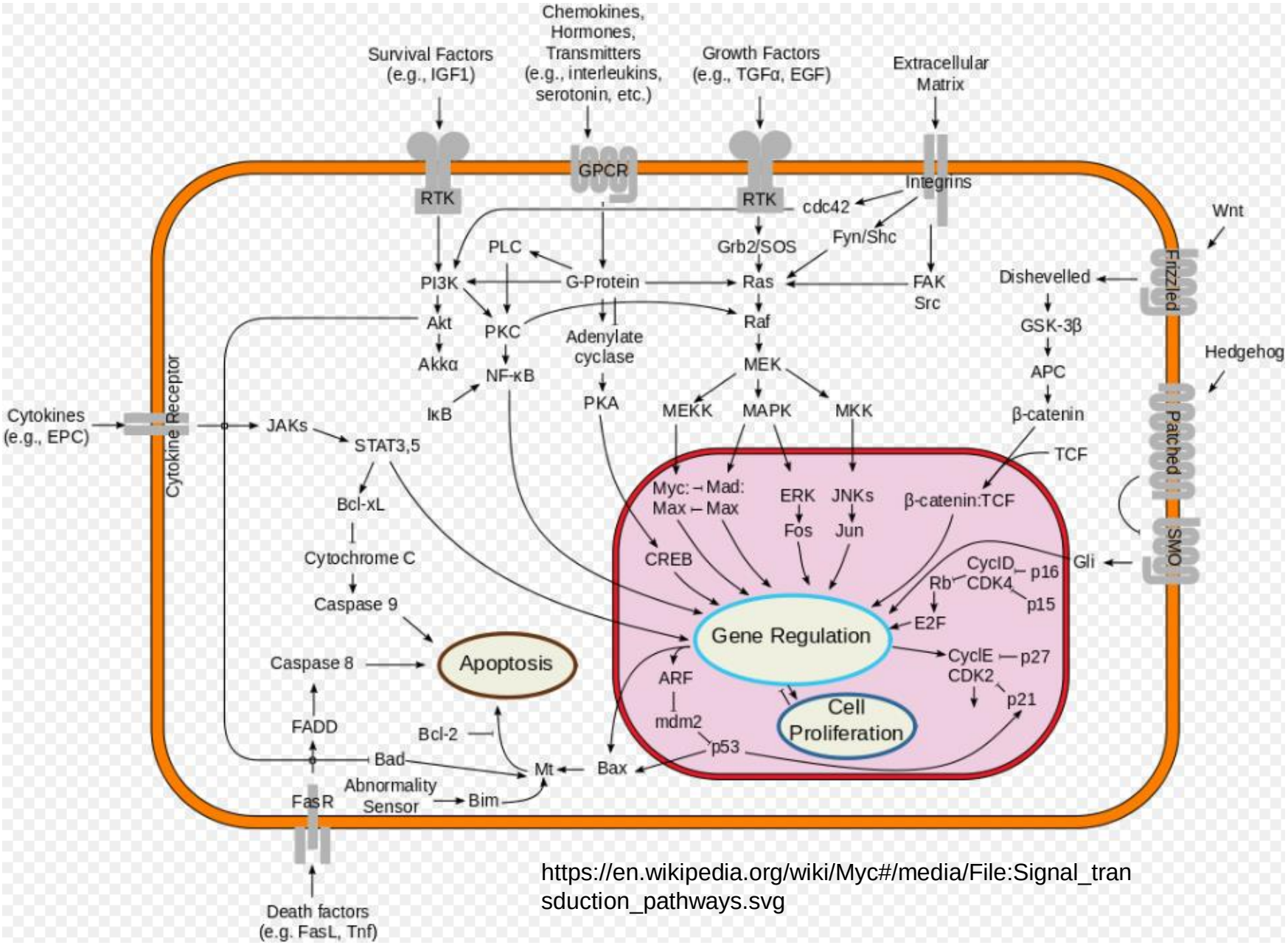
Fig. 2-28 Accessed 08/01/2010

Signal transduction

- TGF-β receptors are cytosolic but have serine-threonine kinase activity.
- Activate SMAD transcription factors in cytosol by phosphorylation.
- Activate differentiation signals p15, p16, p18, p19 (and block CYCLINs).
- TNF, for example, activates NF_K- B in cytosol.
- Dimerizes.
- Translocates to nucleus.

Signal transduction

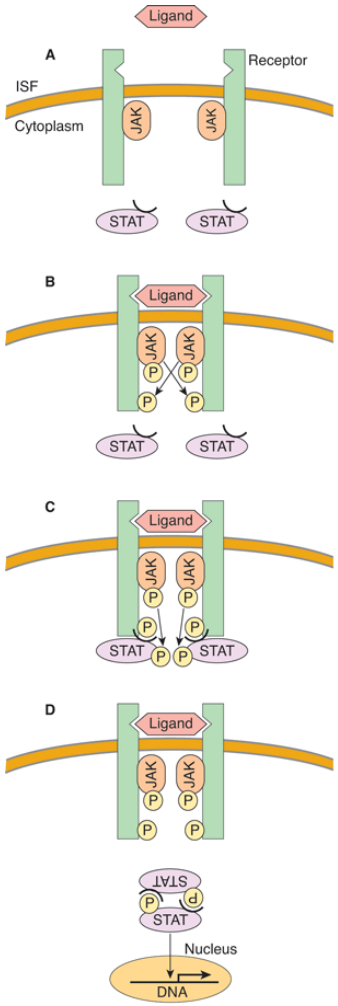
- Retinoic acid, vitamin D, steroids enter with heat shock protein chaperones and translocate to nucleus.
- Final common pathway for signaling is the chromosome binding protein.



https://en.wikipedia.org/wiki/Myc#/media/File:Signal_transduction_pathways.svg

Signal Transduction
Cytokine receptors

IFN-α induces pSTAT1 expression.
IL-2 induces pSTAT3, pSTAT5 expression.



JAK/STAT Pathway

A) Ligand binding leads to dimerization of receptor.
B) Activation and tyrosine phosphorylation of JAKs.
C) JAKs phosphorylate STATs.
D) STATs dimerize and move to nucleus, where they bind to response elements on DNA.

Myeloproliferative disorders.

(Modified from Takeda K, Kishimoto T, Akira S: STAT6: Its role in interleukin 4-mediated biological functions. J Mol Med 1997;75:317.)

Fig. 2-31 Accessed 08/01/2010

Growth hormone receptor signaling

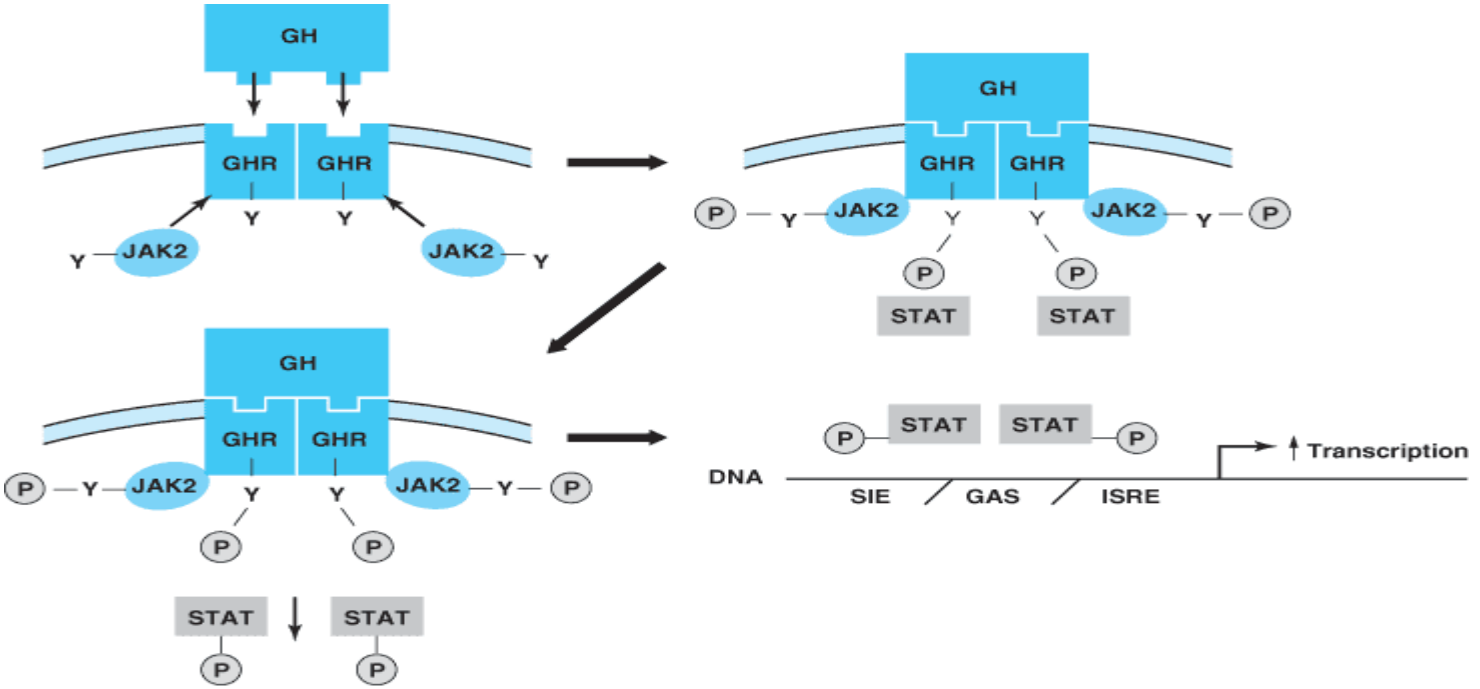


Fig. 2-9 Accessed 07/01/2010

Growth factor dependent pathway

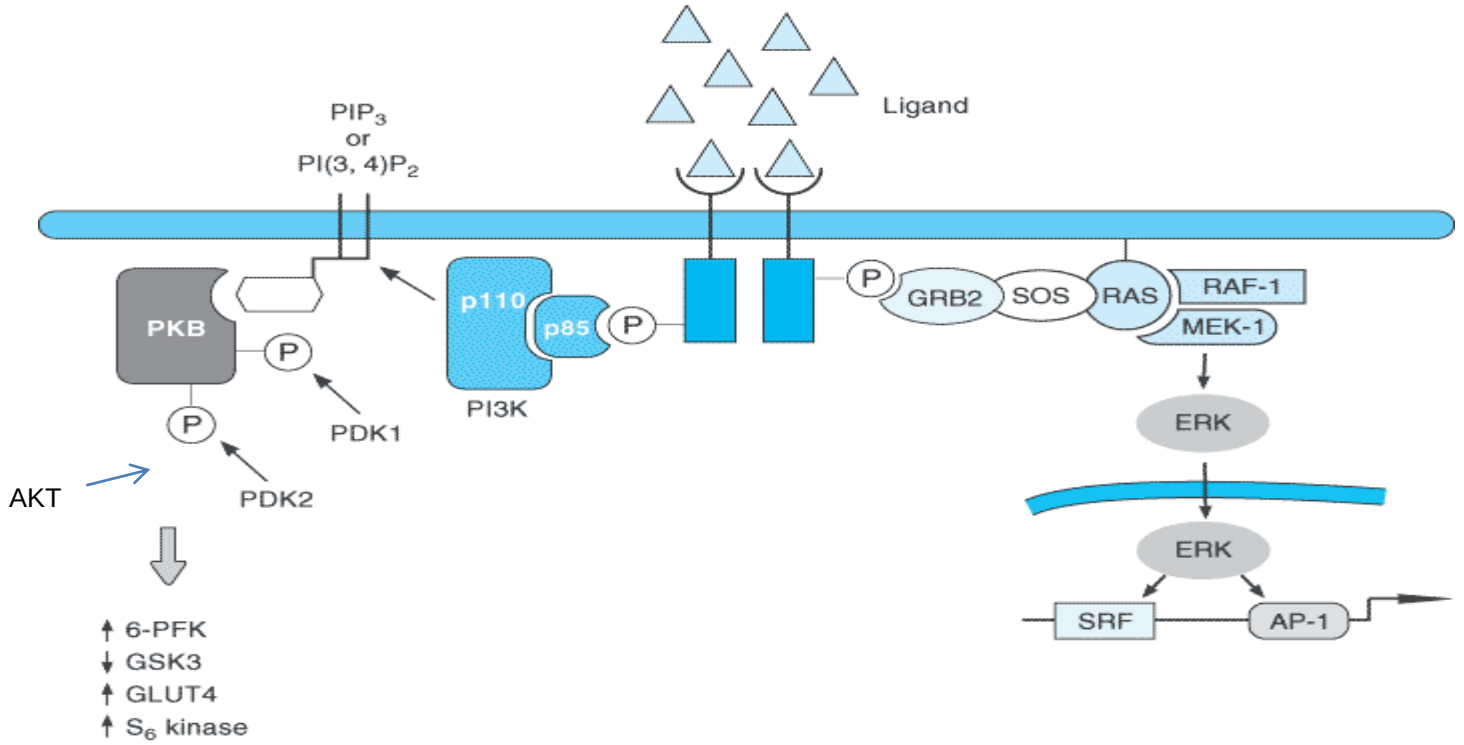


Fig. 2-8 Accessed 07/01/2010

Receptor endocytosis

- Receptor ubiquination in the endosome by the E3 ligase, CBL
- EGFR and HER2/neu differ:
- EGFR rapidly undergoes endocytosis and slowly recycles
- HER2 undergoes endocytosis slowly and rapidly recycles.
- In cancers over expressing HER2/neu
- Heterodimers containing both HER2/neu and EGFR are recycled back to the cell surface
- Avoid endocytosis and ubiquination
- Permit enhanced signaling

Cell signal pathways

- Activated EFGR receptor tyrosine kinases stimulate phosphorylation cascades:
- SHE to Growth factor receptor bound protein (GRB2) activates SOS, RAS, RAF, MEPK, ERK1 and ERK 2 (extracellular signal related kinase), affecting cell proliferation
- Phospholipase C (PLC) triggers PIP2 to IP3 and DAG). DAG triggers PKC, activates RAF.
- PKC activates FAK (focal adhesion kinase), activates paxillin, affecting cell migration.

Cell signal pathways

- IP_3 leads to iCa^{2+} influx to trigger eNOS and cPLAa (cytosolic phospholipases)
- Lead to nitrous oxide and prostaglandin generation.

Phospholipase C

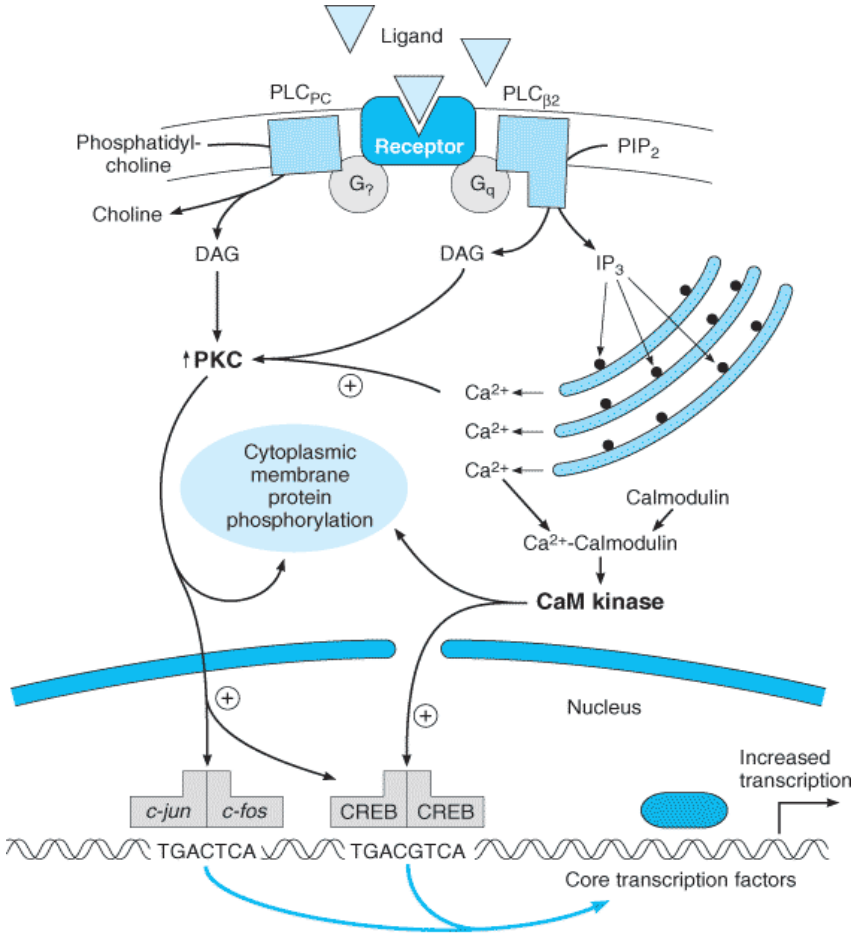


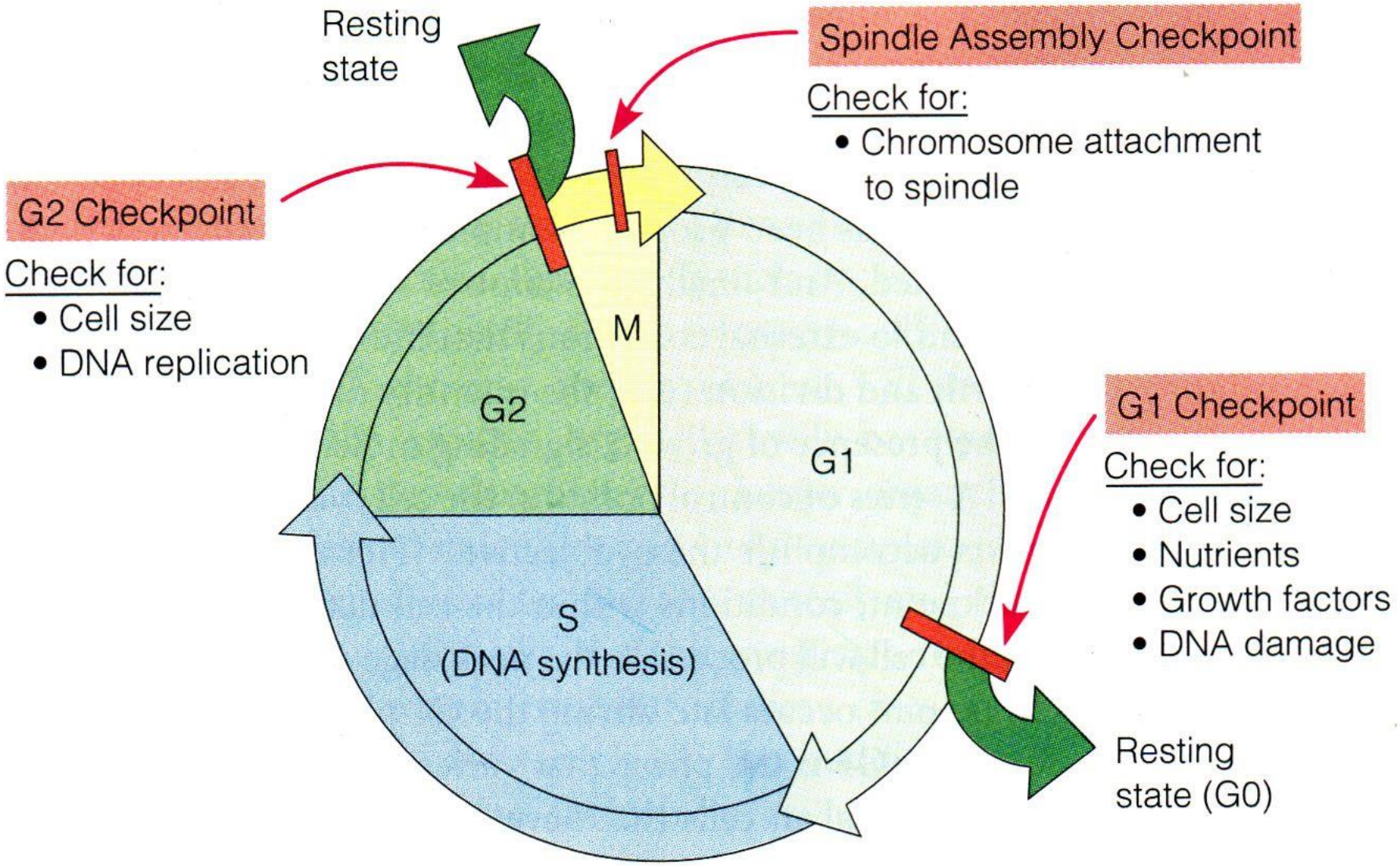
Fig. 2-5 Accessed 07/01/2010

Cell surface receptors

- Hedgehog (HH) receptors are tethered to membrane of signaling cell by a cholesterol anchor.
- Control processing of transcription factors by proteolysis.
- Hedgehog binding causes release from cytosolic complex.
- WNT receptors are palmitoylated ligands that bind to seven transmembrane proteins.
- Release an activated transcription factor of from a multiprotein cytosolic complex.

Cell surface receptors

- NOTCH receptor is a transmembrane protein (ligand) that binds to signaling cell.
- Cytosolic domain released by proteolysis and acts in concert with nuclear transcription factors.



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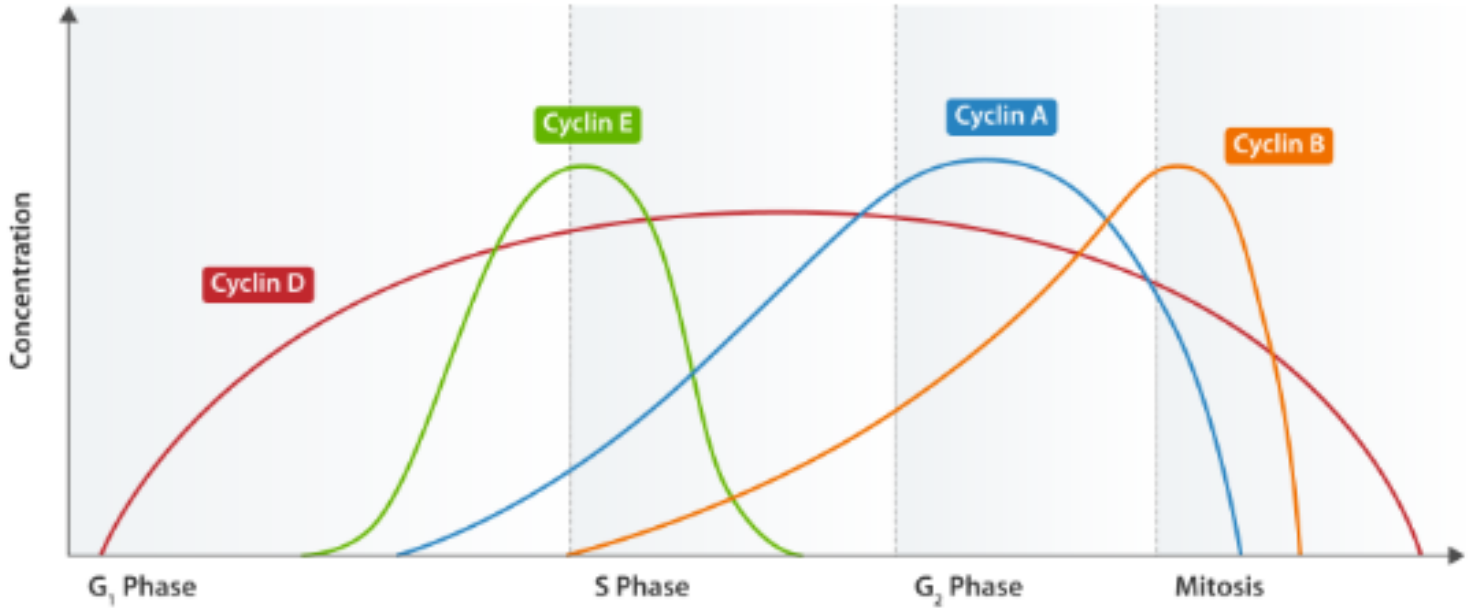

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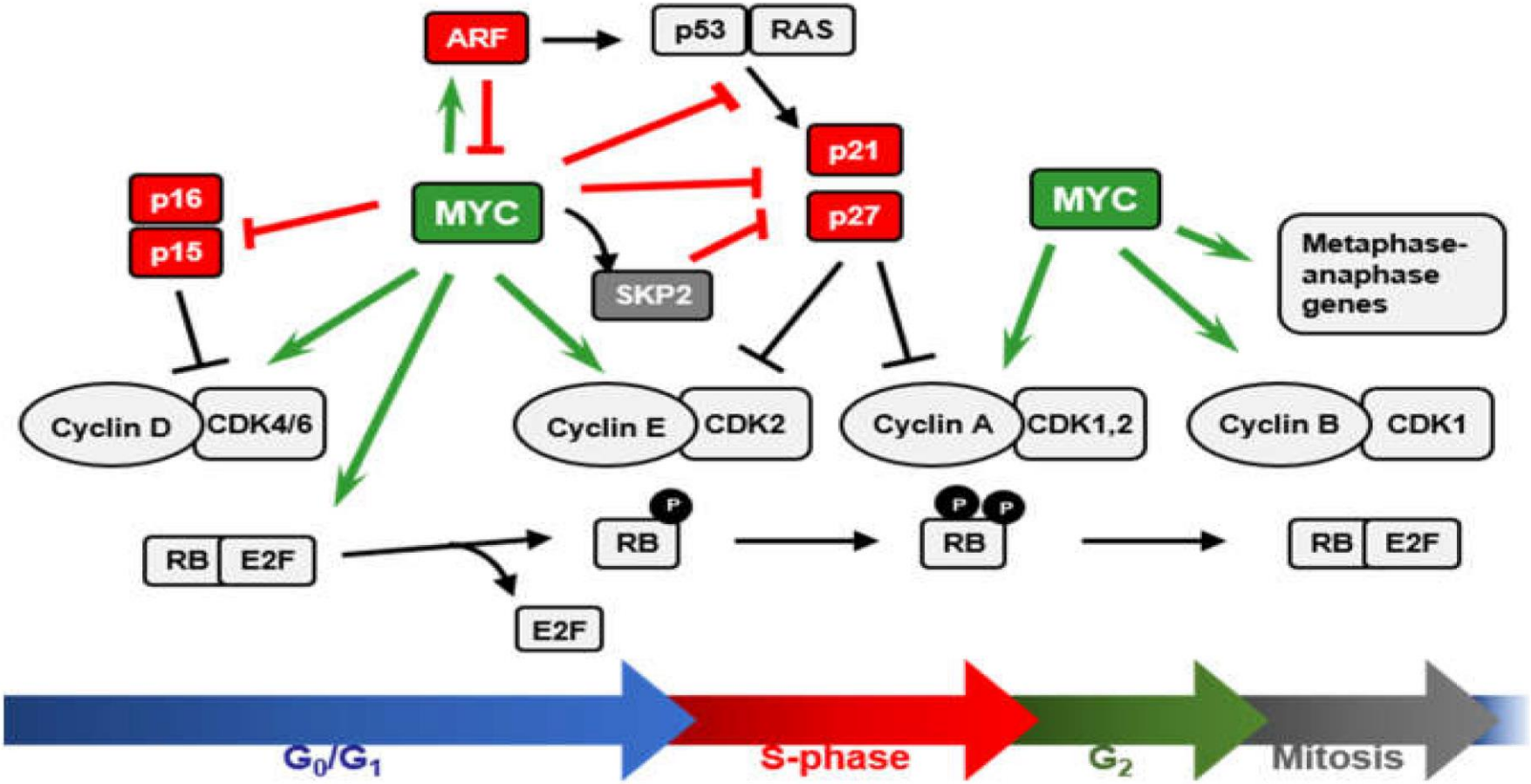


https://www.mdpi.com/genes/genes-10-00244/article_deploy/html/images/genes-10-00244-g002.png

Cell cycle regulation

- Following growth factor signal initiating transduction
- GSK3β activated
- Negative signal blocks β-catenin, thus blocks activation of CYCLINs
- RAS activated
- Positive signal activates CYCLIN D1
- Cell-cell-extracellular matrix contacts trigger GSK3β
- Cell-cell-extracellular matrix contacts trigger RhoA
- Indirect negative signal blocking $p21^{cip1}$ and $p27^{kip1}$, activating all CDKs

Cell cycle regulation

- G_0 resting phase of stable parenchymal cells
- G_1 synthesis of RNA, proteins, organelles, CYCLIN D1 and CDK4 as well as CYCLIN D2 and CDK6
- CYCLIN D, CYCLIN E, CYCLIN A, CYCLIN B appear sequentially
- CYCLIN D and CYCLIN E drive cell into S phase
- CYCLIN D1 accumulates following growth factor stimulation
- Maximal at mid-G_1 restriction point
- Mitogen stimulus no longer required and growth factors withdrawn
- TP53 independent process
- Cell committed to further development

Cell cycle regulation

- CYCLIN D1-CDK4 complex, CYCLIN D2-CDK6 complex, CYCLIN E-CDK2 complex regulate G_1 to S transition (check point)
- Phosphorylate RB protein (E2F/DP1/RB complex)
- Phosphorylated RB does not bind to transcription factors.
- Transcription factors are not sequestered and genes critical for cell cycle progression are actively transcribed
- E2F removed
- Progress to S phase following pause at check point

Cell cycle regulation

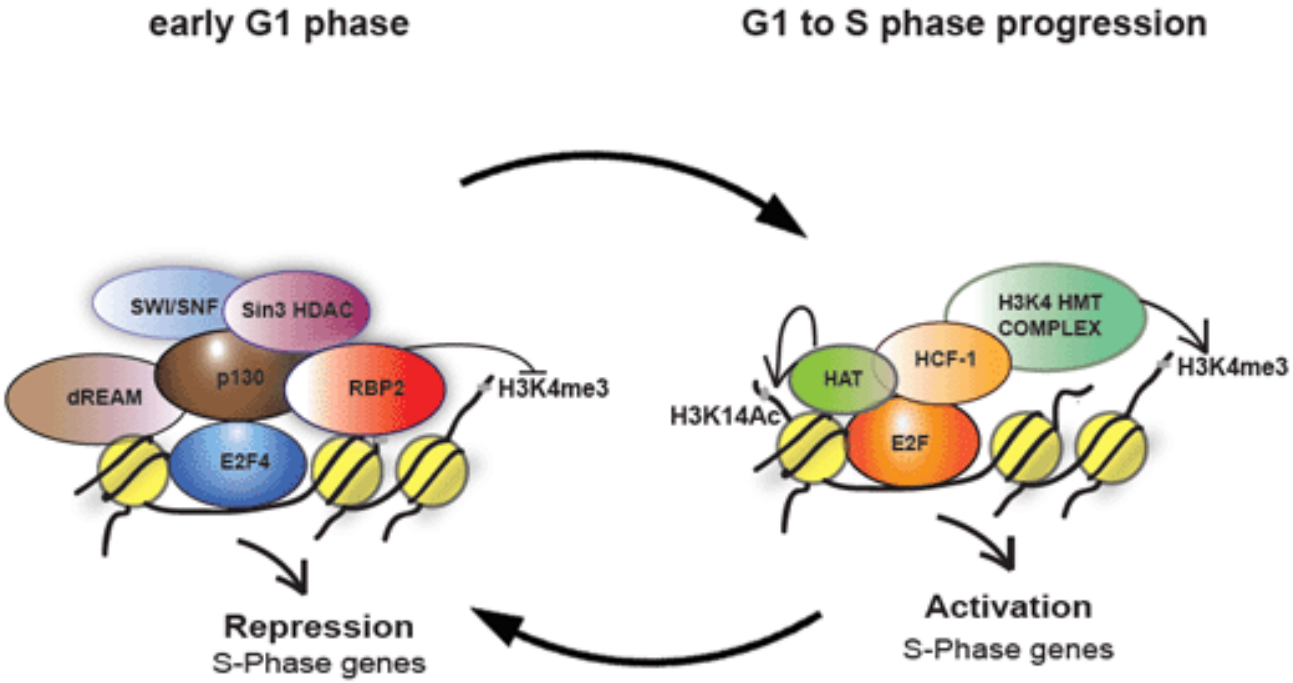
- Pause to check for DNA damage
- DNA damage stimulates ATM which activates p53 and indirectly activates $p21^{cip1}$ and $p27^{kip1}$.
- p53 (activates $p21^{cip1}$) inhibits CDK4 and blocks progression at G_1until DNA repair completed.
- SKIP, a transcription elongation factor, required for $p21^{cip1}$ function.

Cell cycle regulation

- p53 activates MDM2 to facilitate repair or apoptosis
- Upregulates GADD45 (DNA repair).
- Interacts with PTEN and BAX
- BAX inhibits BCL2 anti-apoptosis gene
- p14ARF blocks p53
- Servo feedback on p53.
- Polyadenosine ribose polymerase also active in base excision repair.
- E2F transactivates CYCLIN E and CYCLIN A nucleotide synthesis

Cell cycle regulation

- S synthesis of DNA, RNA, protein
- CYCLIN A-CDK2 and CYCLIN A-CDK1 active in S phase
- CDK1 is an inactive kinase, produced constitutively at S phase.



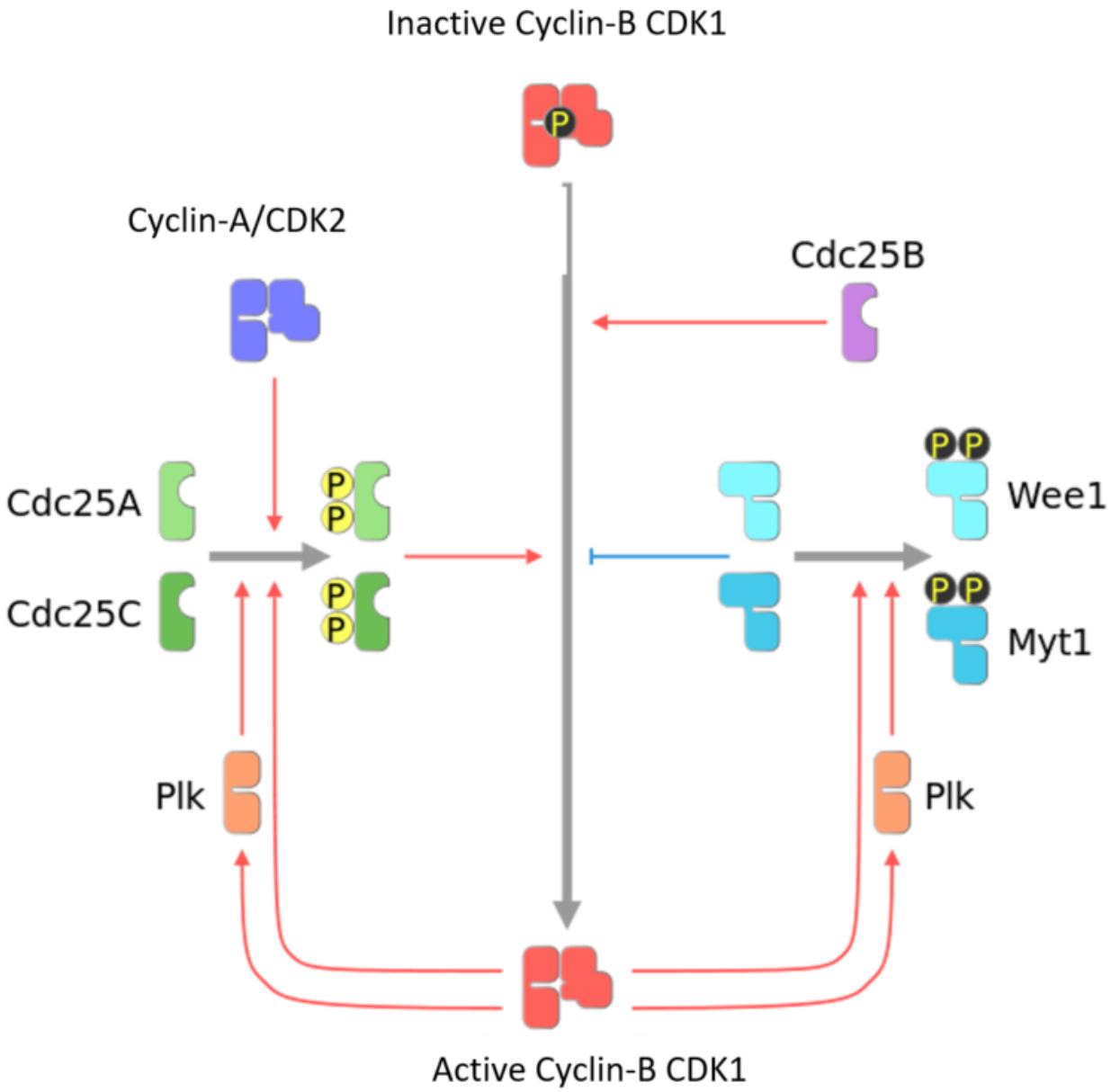
http://www.cdfd.org.in/images/cellcycle/lccr_fig1.gif

Cell cycle regulation

- G_2 Final growth before mitosis
- CYCLIN B synthesized at beginning of G_2.
- CYCLIN B-CDK1 active complex controls movement from G_2 to M phase.
- Check point to check for damaged or unduplicated DNA.
- M mitosis
- CYCLIN B removed in M phase.

Cell cycle regulation

- CYCLIN dependent kinase inhibitors are p21, p27, p57
- Inhibit all CDKs
- INK4 inhibitors are CDKN2B (p16), CDKN2A (p15), CDKN2C (p18), CDKN2D (p19)
- Act on CYCLIN D-CDK4 and CYCLIN-CDK 6
- Reinforce G_1-S and G_2-M checkpoints
- WEE 1 inactivates CDK2.



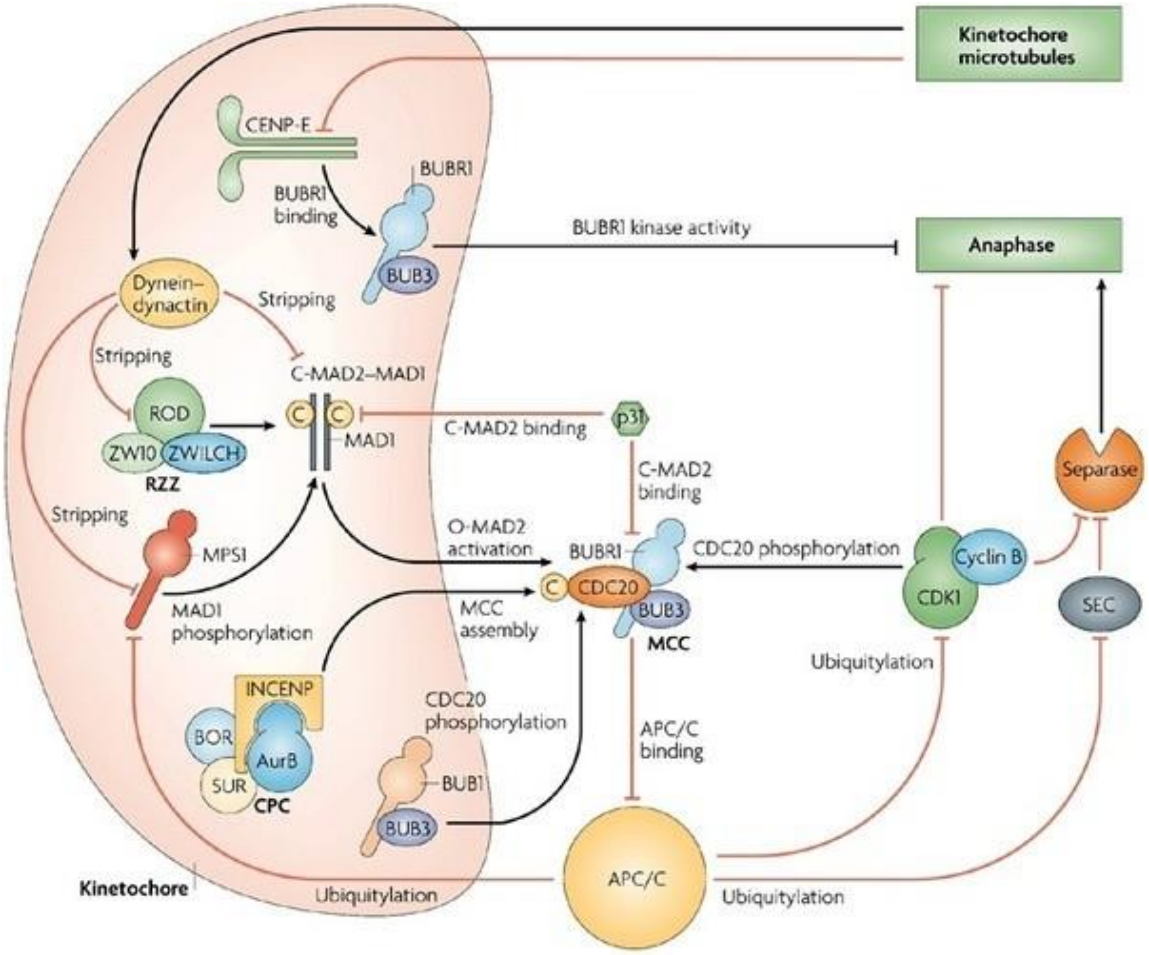
https://en.wikipedia.org/wiki/G2_phase#/media/File:G2-M_feedback_loops.png

Spindle activation complex

- At metaphase, all the sister chromatids are attached to each other via cohesin complexes.
- Before cohesin can be cleaved, all the chromosomes need to be properly attached to the mitotic spindle via their kinetochores.

Spindle activation complex

- Cleavage of cohesin after release of the spindle activating complex (SAC) is accompanied by pulling forces from the spindle poles, resulting in chromosome segregation
- Active Cdc20 associated with the APC/C (anaphase promoting complex/cyclosome) degrades securin (SEC), which is a separase inhibitor, which when active cleaves cohesin.



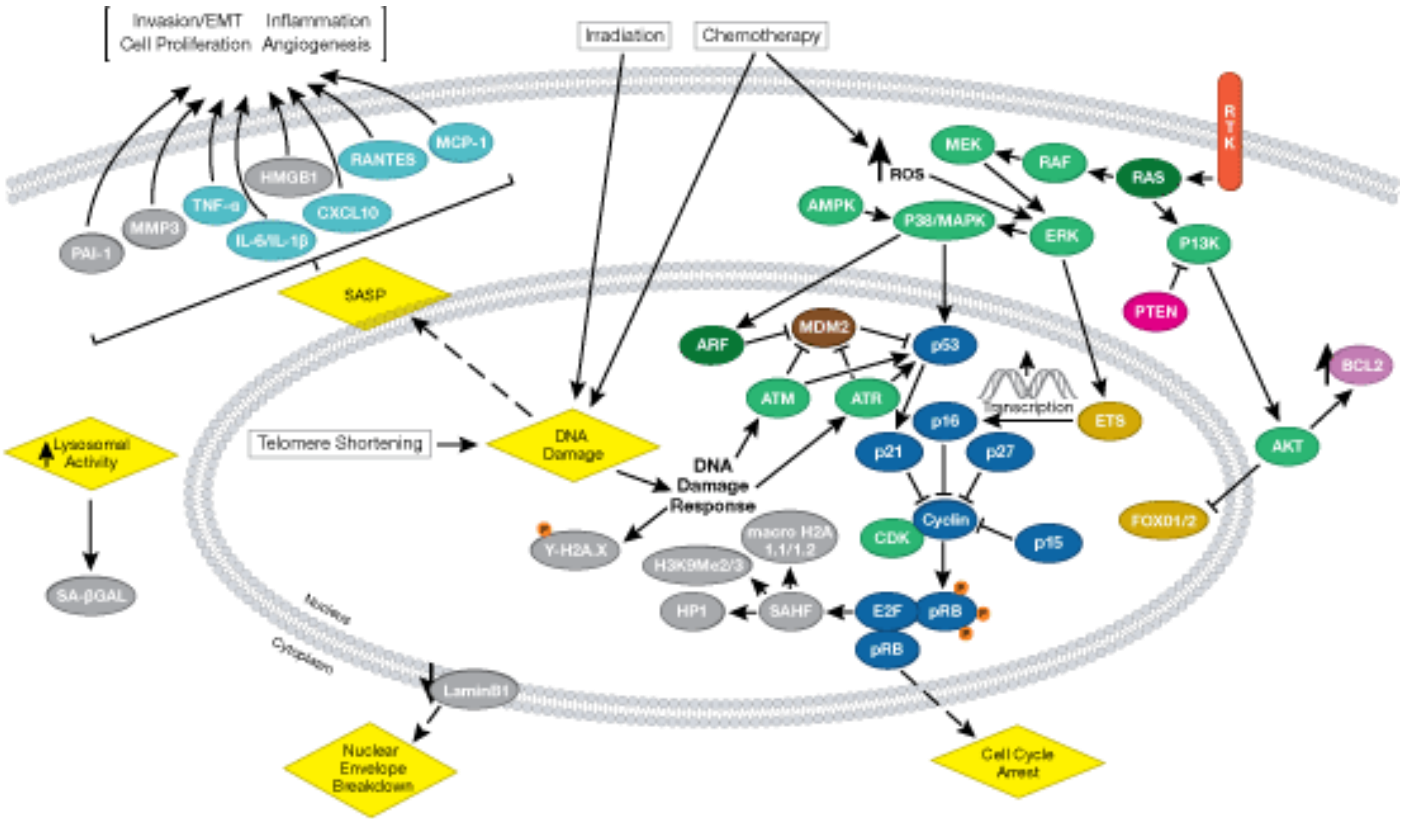
Nature Reviews | Molecular Cell Biology

Senesence

- All cancers contain cells that are immortal and have limitless replicative potential.
- Most normal human cells have the capacity to divide 60 to 70 times.
- After this, the cells become senescent, permanently leaving the cell cycle and never dividing again.

Senescence

- The senescent state is associated with upregulation of tumor suppressors such as p53 and INK4a/p16 in response to the accumulation of DNA damage over time.
- Maintain RB in a hypo-phosphorylated state that favors cell cycle arrest at the RB-dependent G_1/S cell cycle checkpoint.
- While cells that are resistant to senescence have increased replicative capacity, they are still not immortal
- They eventually enter into a phase referred to as mitotic crisis and die.



http://media.cellsignal.com/www/images/categories/pathways/cell-cycle/cell-cycle-senescence.png

Telomeres

- Telomeres are special DNA sequences at the ends of chromosomes that bind several types of protective protein complexes.
- Most somatic cells reproduce slowly and do not express telomerase
- Telomerase is highly active in cells that divide rapidly, such as cells that line the lungs and gastrointestinal tract, cells in bone marrow, and cells of the developing fetus.
- Telomerase maintains telomere length as it acts as a reverse transcriptase that adds simple sequence repeats to chromosome ends by copying a template sequence within the RNA component of the enzyme.

Telomerase

- The telomerase enzyme consists of two major components that work together.
- The component produced from the TERT gene (5p15.33) is known as hTERT.
- The other component is produced from a gene called TERC (3q6.22) and is known as hTR.
- The hTR component provides a template for creating the repeated sequence of DNA that telomerase adds to the ends of chromosomes.
- The hTERT component then adds the new DNA segment to chromosome ends.

Telomerase

- Catalyzes the RNA-dependent extension of 3'-chromosomal termini with the 6-nucleotide telomeric repeat unit, 5'-TTAGGG-3'.
- The catalytic cycle involves primer binding, primer extension and release of product once the template boundary has been reached or nascent product translocation followed by further extension.
- More active on substrates containing 2 or 3 telomeric repeats.
- Modulates WNT signaling.

Telomerase

- With each cell division the telomeres of somatic cells shorten.
- When the telomeric DNA is eroded, the exposed chromosome ends are “sensed” as double-stranded DNA breaks.

Telomerase

- If the affected cells have functional p53, the cell arrests its growth and may undergo apoptosis.
- If p53 is dysfunctional, the nonhomologous end joining pathway is activated and may join the “naked” ends of two chromosomes.
- This results in dicentric chromosomes that are pulled apart at anaphase, resulting in new double-stranded DNA breaks.
- The snowballing genomic damage caused by repeated “bridge-fusion breakage” cycles eventually produces mitotic crisis and cell death.

Telomerase

- If cells in crisis reactivate telomerase, the cells can restore their telomeres and survive; but are at high risk for malignant transformation.
- Another mechanism for cancer cells to maintain their telomeres probably depends on DNA recombination.
- Telomere shortening in prostate cancer is associated with increase in abnormalities at 8q21.21(C-MYC).
- Idiopathic pulmonary fibrosis is associated with mutations in the TERC gene.

Telomerase

- Cancers also arise from long term stem cells.
- Tissue stem cells and germ cells express telomerase
- Are resistant to mitotic crisis
- Avoid the genetic and epigenetic alterations that trigger senescence.
- Long-lived stem cells also possess the capacity for self-renewal.
- Each time a stem cell divides at least one of the two daughter cells remains a stem cell.

Autophagy

- Autophagy is an evolutionarily conserved process, with both cytoprotective and programmed cell death mechanisms.
- Beclin 1
- Essential autophagic protein,
- A BH3-domain-only protein that binds to BCL-2 anti-apoptotic family members.
- The dissociation of beclin 1 from its BCL-2 inhibitors is essential for its autophagic activity.

Autophagy

- Death associated protein kinase (DAPK) phosphorylates beclin 1 and facilitates its dissociation from BCL-XL
- Leads to autophagy rather than cell death
- Tumor cells can recycle and reuse tumor DNA from cells that have died off through autophagy

Horizontal gene transfer

- Tumor cells can recycle and reuse tumor DNA from cells that have died off through apoptosis
- This process may allow the tumor cells to accumulate genetic alterations that promote malignant transformation or resistance to therapy.
- Tumor DNA is also transferred from tumor cells to endothelial cells in vivo.
- Functional gene transfer between tumor cells and endothelium may be a novel mechanism by which tumors manipulate their microenvironment to support their growth.

Apoptosis

- Programmed cell death
- Extrinsic pathway
- Initiated when CD95/Fas binds to its ligand, CD95L/FasL, leading to trimerization of the receptor and its cytoplasmic death domains
- Attract the intracellular adaptor protein FADD.
- Recruits pro-caspase 8 to form the death-inducing signaling complex (DISC).
- Pro-caspase 8 is activated by cleavage into smaller subunits, generating caspase 8.
- Caspase 8 then activates downstream executioner caspase 3.

Apoptosis

- Both the regulators in apoptosis pathway such as caspase-8 and caspase-9 and the key factors in autophagy such as Beclin 1 and ATG7 can regulate the TRAIL-induced (TNF) apoptosis and autophagy
- Some molecular switchers such as RIP1, regulate the balance between TRAIL-induced apoptosis and autophagy by dynamic expression and modification
- They share the same regulators even pathways to control the complicated process
- Additionally, caspase 8 can cleave and activate the BH3-only protein BID, activating the intrinsic pathway as well.

Apoptosis

- TRAIL (TNF Receptor ligand) triggers both apoptosis signaling pathways, the death receptor (extrinsic) and the mitochondrial (intrinsic) pathways.
- TRAIL triggers the extrinsic apoptosis pathway upon receptor binding of the TRAIL trimer
- Recruits FADD
- In “type I” cells, the procaspases 8 and 10 form homodimers. This induces a conformational change that exposes their proteolytical active sites, resulting in auto activation.
- When sufficient caspase-8 is produced, it stimulates effector caspase-3 to induce apoptosis

Apoptosis

- “Type II” cells generate less-active caspase-8 at the DISC. These cells induce apoptosis requiring further signal amplification by the intrinsic/mitochondrial pathway.
- An intracellular complex is activated
- Then caspase-8-mediated cleavage of Bid to truncated Bid (tBid) activates the mitochondrial pathway, activating caspase 9, eventually leading to mitochondrial outer membrane permeabilization.
- Release of Smac augments apoptosis by antagonizing the inhibitor of apoptosis (IAP) proteins

Apoptosis

- Immunogenic cell death results from early expression of chaperones (e.g., calreticulin, heat shock proteins)
- OR late release of HMG-β which acts on TL34
- Enhance antigen presentation
- Inhibitor apoptosis proteins (BIR domain) promote degradation of capiases.
- Mitochondrial.
- Usually impaired in malignancy

Apoptosis

- Intrinsic pathway
- 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: BCL2, BCL-XL, MCL1

Apoptosis

- A third set of proteins (so-called BH3-only proteins), including BAD, BID, and PUMA, sense death-inducing stimuli and promote apoptosis by neutralizing the actions of anti-apoptotic proteins like BCL2 and MCL1.
- When the sum total of all BH3 proteins expressed “overwhelms” the anti-apoptotic BCL2/BCL-XL/MCL1 barrier, BAX and BAK are activated and form pores in the mitochondrial membrane.

Apoptosis

- Cytochrome c leaks into the cytosol, where it binds to APAF1, activating caspase 9.
- Caspase 9 then activates downstream caspase 3 that cleaves DNA and other substrates to cause cell death.
- Caspases cleave cytostructural proteins
- Caspases are held in check in healthy cells by members of the inhibitors of apoptosis protein (IAP) family.

Apoptosis

- In the DISC, the main regulator protein is cellular FLICE-like inhibitory protein (cFLIP) and caspase-8,
- cFLIP contains a death domain, which allows them to interact with proteins of the TRAIL DISC, thereby blocking the transmission of the proapoptotic signal and preventing caspase-8 activation
- cFLIP closely resembles caspase-8 but lacks the protease activity required for apoptosis induction

Apoptosis

- The cFLIP-S isoform can inhibit caspase-8 activation in a dominant-negative manner by competing with it for binding to FADD.
- cFLIP-L can also completely prevent DR-induced apoptosis when it is expressed at high levels.
- Several studies have demonstrated that cancer cells exploit over expression of cFLIP to evade TRAIL-induced
- Over expression of cFLIP is a frequent event in human cancers and has been correlated with resistance to the induction of apoptosis, including TRAIL-mediated cell death

Apoptosis

- Hypermethylation is responsible for low or even absent caspase-8 expression
- Results in resistance or decreased sensitivity to TRAIL-induced apoptosis .
- Caspase-8 function can be suppressed in a dominant-negative manner
- The tyrosine kinase Src phosphorylates caspase-8 at Thr-308, which impairs the enzymatic function of caspase-8
- These regulation factors can influence the activity of caspase-8 that causes the change of TRAIL-induced apoptosis.

Apoptosis

- TRAIL can also induce cell survival signaling such as proinflammatory pathways (through NF-kB, Akt, MAPK, and JNK activation).
- TRAIL can promote a variety of cell survival cascades leading, for example, to proliferation, migration, invasion, and even metastasis, especially in cancers in which the cell death signaling part of the signaling network is impaired
- Elevated C-reactive protein associated with TRAIL down-regulation.

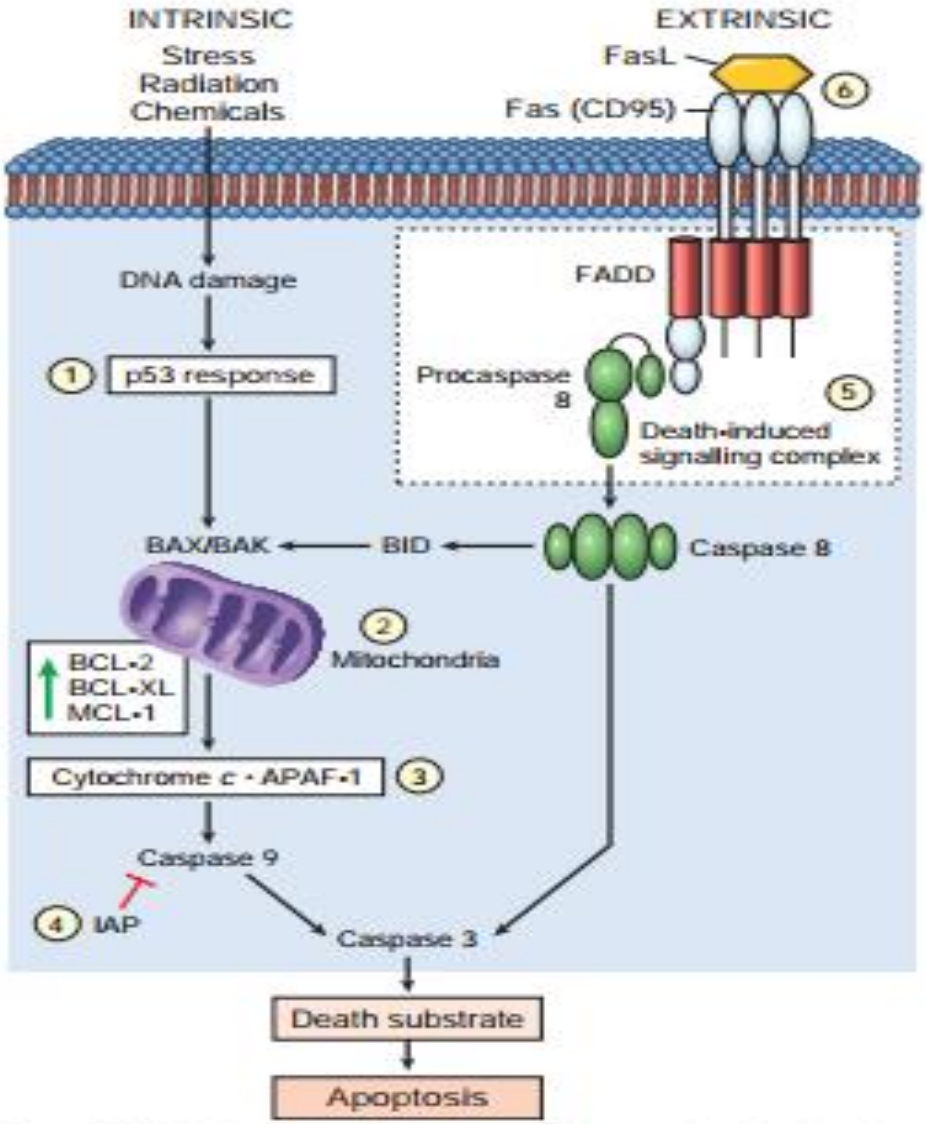


Figure 7-33 Intrinsic and extrinsic pathways of apoptosis and mechanisms used by tumor cells to evade cell death. (1) Loss of p53, leading to reduced function of pro-apoptotic factors such as BAX. (2) Reduced egress of cytochrome c from mitochondria as a result of upregulation of anti-apoptotic factors such as BCL2, BCL-XL, and MCL-1. (3) Loss of apoptotic peptidase activating factor 1 (APAF1). (4) Upregulation of inhibitors of apoptosis (IAP). (5) Reduced CD95 level. (6) Inactivation of death-induced signaling complex. FADD, Fas-associated via death domain.

Other cellular responses

- FOXP3+ tumor cells associated with poor prognosis
- Infiltrate of FOXP3+ T_{reg} cells (immunosuppressive) associated with good prognosis.
- Proliferating macrophages associated with poor prognosis
- Lymphocytic infiltrates about a tumor are not a prognostic indicator
- Regional recurrence has worse prognosis than does local recurrence.
- Elevated tumor markers associated with worse prognosis than are normal levels of tumor markers.

Overview of mutations in cancer

- On average, cancer genomes contain 4–5 driver mutations when combining coding and non-coding genomic elements.
- Non-coding driver point mutations account for 99% of single nuclear variants (passenger mutations)
- With the exception of the TERT promoter, individual enhancers and promoters are only infrequent targets of driver mutations.

Overview of mutations in cancer

- The molecular functional impact of passenger gene mutations has a multimodal distribution.
- There are a reduced fraction of high-impact passengers gene mutations with an increase in the total mutation frequency.
- This may be attributed to the underlying mutational signatures or may signify the presence of weak negative selection among a subset of passengers genes.

Overview of mutations in cancer

- Depletion of passenger loss of function genes are found in key gene categories, including DNA repair and cell-cycle, potentially suggesting the presence of weak negative selection
- There is differential mutational burdening for early and late sub-clonal mutations at the pan-cancer level.

Overview of mutations in cancer

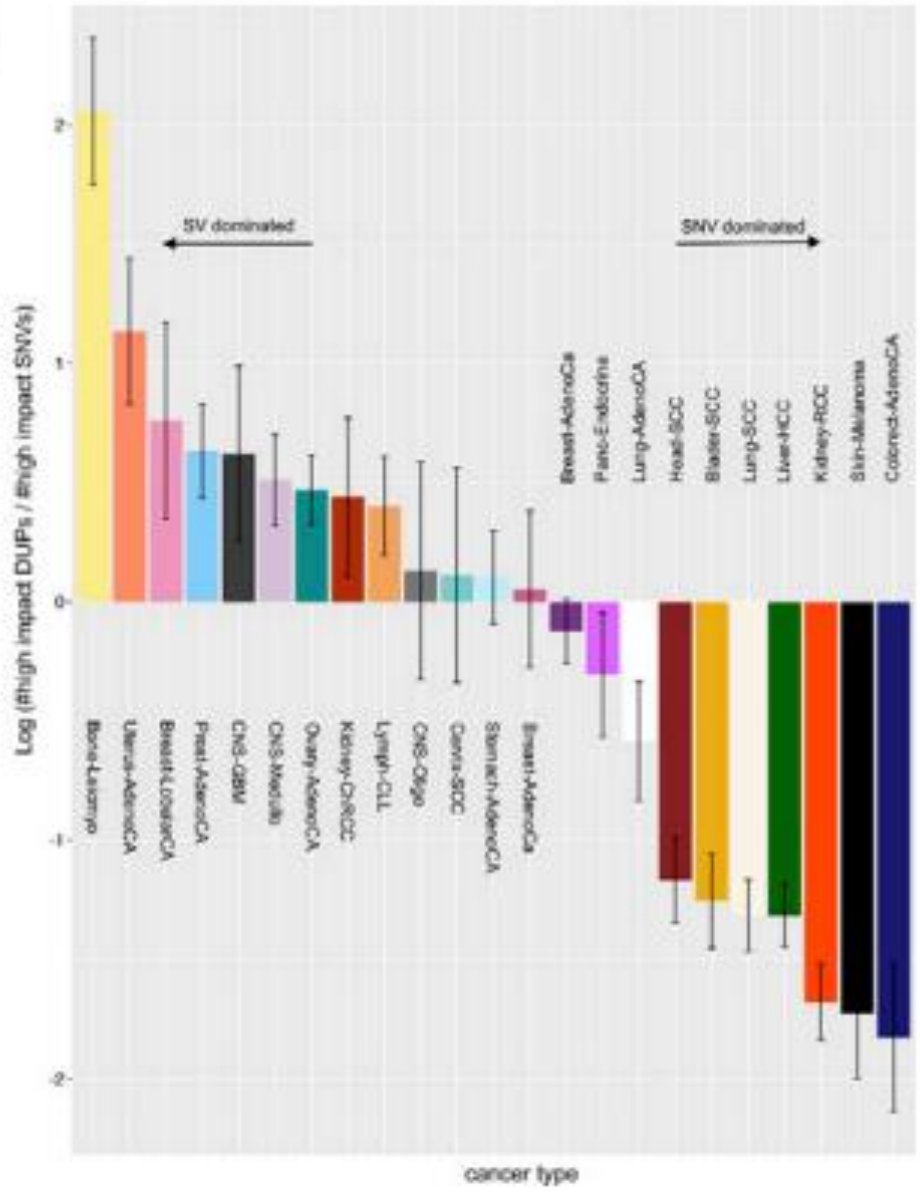
- There is an opposing enrichment and depletion of passenger mutations among tumor suppressors and oncogenes, respectively.
- This suggests that the subset of passenger mutations in tumor suppressors may confer weak driver activity
- Those in oncogenes may impair oncogenic activity to the detriment of tumor fitness.

Overview of mutations in cancer

- Across tumor types, structural variants and point mutations have different relative contributions to tumorigenesis.
- Driver structural variants are more prevalent in
- Breast adenocarcinomas
- Ovary adenocarcinomas
- Large deletions are known to act as the predominant drivers in ovarian cancer

Overview of mutations in cancer

- Driver point mutations have a larger contribution in
- Colorectal adenocarcinomas
- Mature B cell lymphomas
- Single nucleopeptide variants (SNP, SNV) often exclusively drive clear cell kidney cancer



Log plot of the ratio of high impact duplication events to high impact SNVs. Similar to deletions, cohorts such as bone-leiomyoma, adenocarcinoma of the uterus, and lobular adenocarcinoma of the breast harbor high impact duplications compared to high impact SNVs.

In contrast, colorectal, melanoma and renal cell cancers are dominated by high impact SNVs compared to large duplications

Kumar, S, et al., “Passenger Mutations in More Than 2,500 Cancer Genomes: Overall Molecular Functional Impact and Consequences 2020, Cell 180, 915–927 https://doi.org/10.1016/j.cell.2020.01.032

Overview of mutations in cancer

- Any driver mutations that affect tumor suppressor genes are two-hit inactivation events.
- 77% of cancers with p53 mutations had both alleles mutated
- 96% of which combined a somatic point mutation that affected one allele with somatic deletion of the other allele.
- 17% of patients had rare germline protein-truncating variants in cancer-predisposition genes, DNA-damage response genes, and somatic driver genes.

Overview of mutations in cancer

- Biallelic inactivation due to somatic alteration on top of a germline protein truncating variant was observed in 4.5% of patients overall
- 81% of these affecting known cancer-predisposition genes (such as BRCA1, BRCA2 and ATM).

Overview of mutations in cancer

- Some somatic mutational processes generate multiple mutations in a single catastrophic event, typically clustered in genomic space, leading to substantial reconfiguration of the genome.
- Three such processes have previously been described:

Term	Greek Root	Definition	Characteristics			
			Chromosomes	Breakpoints	Distribution	Dosage alt
chromothripsis	*chromo* for chromosome and *thripsis* for shattering into pieces	phenomenon by which hundreds of rearrangements originate through random shattering and reshuffling of clustered chromosome regions within a single catastrophic event. The predominant method of re-assembly of the shattered pieces is c-NHEJ	typically 1 (cancer) but 1–4 observed in congenital cases	≥5, up to 65 observed in congenital cases and hundreds in cancer	clustered	typically unbalanced (cancer) and balanced (congenital)
chromoplexy	*chromo* for chromosome and *pleko* for to twist or enfold	phenomenon where derivative chromosomes are generated by the chimeric joining of DNA segments from two or more chromosomes. Chromosome re-assembly is predicted to occur by c-NHEJ or alt-EJ repair	≥2	≥5 to a couple dozen	interspersed	typically balanced but deletions can also be present at junctions
chromoanasynthesis	*chromo* for chromosome and *anasynthesis* for reconstitution	phenomenon by which multiple combinations of SVs are generated through errors in DNA replication, namely FoSTes and MMBIR	typically 1	≥5 to a couple dozen	clustered	unbalanced (gains and losses)

https://doi.org/10.1016/j.ajhg.2019.02.024

Overview of mutations in cancer

- (1) Chromoplexy results in shuffled chains of rearrangements
- Repair of co-occurring double-stranded DNA breaks
- Typically on different chromosomes
- 17.8% of all cancers
- Chromoplexy is prominent in prostate adenocarcinoma, lymphoid malignancies, and thyroid adenocarcinoma.

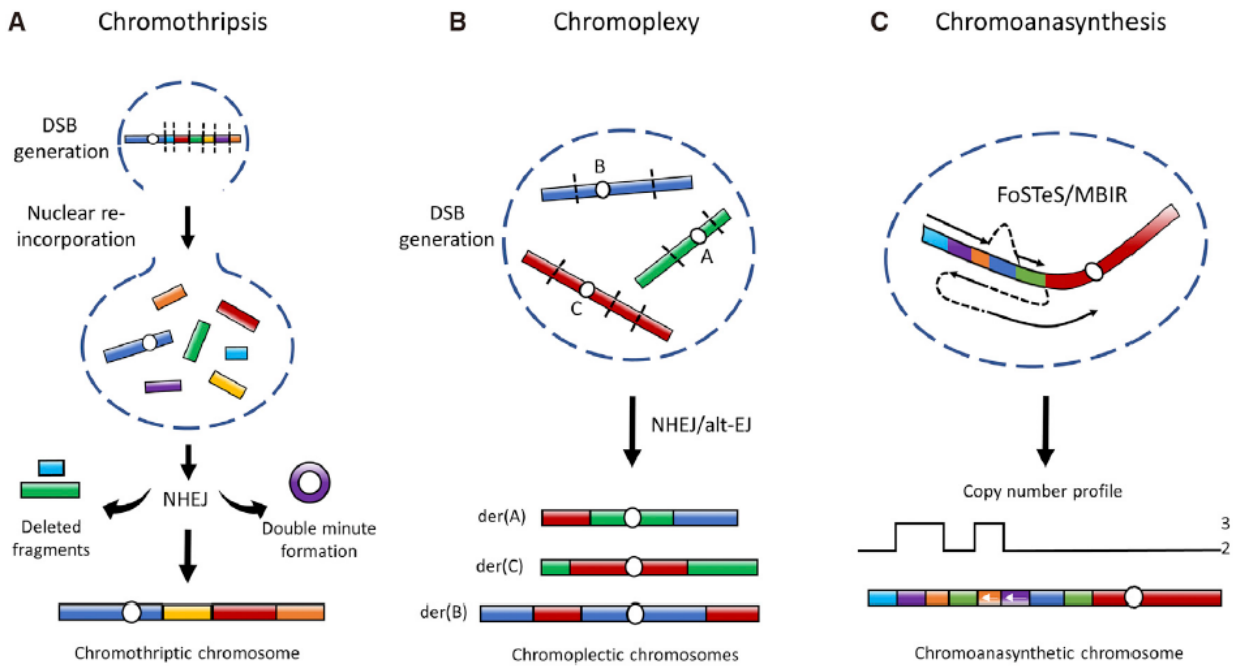


Figure 1. Characteristics of Chromothripsis, Chromoplexy, and Chromoanasynthesis-Derived SVs

(A) A chromosome in a micronucleus can undergo massive DNA damage and result in multiple double-strand breaks (DSBs, depicted with dashed black lines). When the micronucleus is re-incorporated into the nucleus during mitosis, the DSBs undergo repair through NHEJ, where chromosome segments are randomly stitched back together, lost, or become double minutes. Functionally relevant segments could become double minutes and undergo amplification, as has been observed in *MYC* and other oncogene-containing segments in various cancer cases.[19,29,30]

(B) In chromoplexy, different DSBs can be repaired with or without DNA loss at the breakpoints and be arranged into various derivative configurations, as shown here by the rearrangements of example chromosomes A, B, and C.

(C) In chromoanasynthesis, a normal chromosome can undergo DNA segment re-synthesis (dashed lines to show template switches and solid arrows to show replication) mediated by replication processes such as FoSTeS and MMBIR. These mechanisms lead to templated insertions that exhibit higher copy-number and may be arranged in different orientations (depicted in purple and orange with white arrows signifying inverted sequence orientation). Notice the chromoanasynthesis chromosome has a copy-number profile exhibiting intercalating duplication-normal-duplication (dup-nml-dup) copy-number states, as seen in previous studies.[21]

https://doi.org/10.1016/j.ajhg.2019.02.024.

Overview of mutations in cancer

- (2) Kataegis
- A focal hypermutation process that leads to locally clustered nucleotide substitutions, biased towards a single DNA strand
- Kataegis events are found in 60.5% of all cancers
- Abundant in lung squamous cell carcinoma, bladder cancer, acral melanoma and sarcomas

Overview of mutations in cancer

- Typically, comprises C > N mutations in a TpC context, which are probably caused
- by APOBEC3B activity
- Also T > N error-prone polymerase signature
- And showed cytidine deamination in an alternative GpC or CpC context.
- Sarcomas

Overview of mutations in cancer

- (3) Chromothripsis
- Frequently an early event in tumor evolution
- Tens to hundreds of DNA breaks occur simultaneously, clustered on one or a few chromosomes, with near-random stitching together of the resulting fragments
- 22.3% of cancers
- Most frequently among osteosarcoma, glioblastoma, lung squamous cell carcinoma, melanoma and breast adenocarcinoma

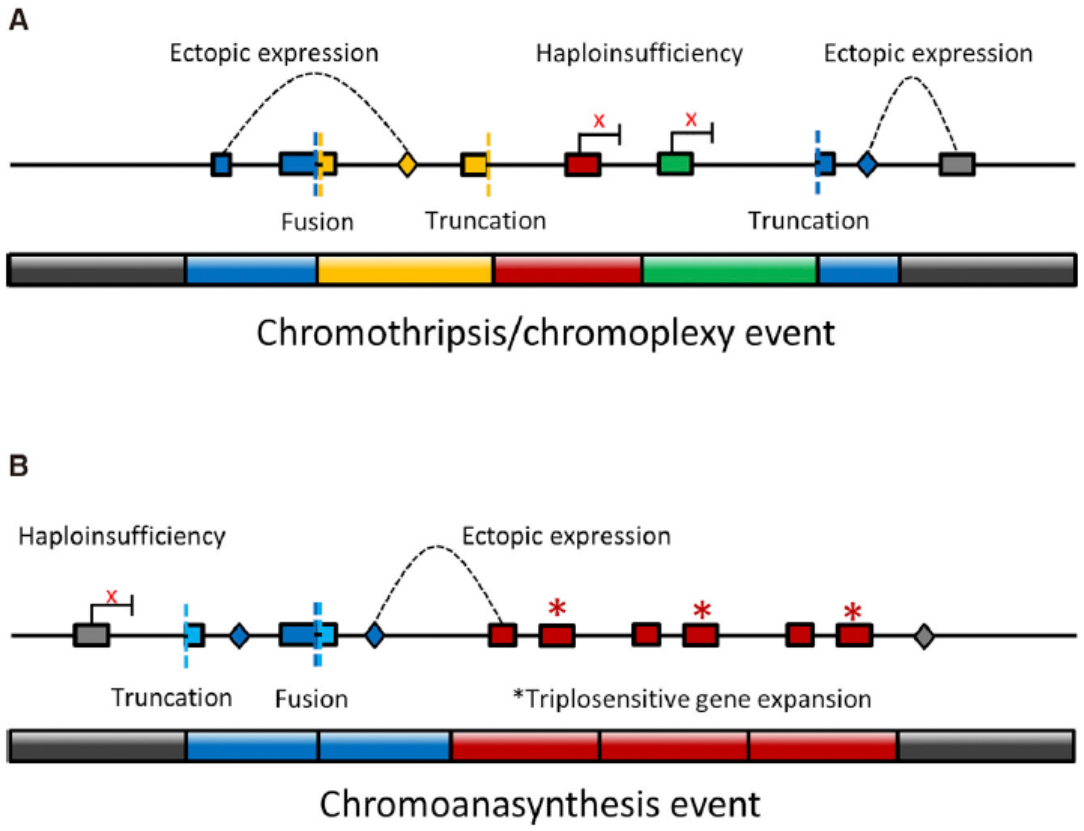


Figure 3. Functional Consequences of Chromoanagenesis

Rearranged chromosome fragments are colored in blue, yellow, red, and green. Grey fragments represent the remainder of the chromosome, to pter (left gray fragments) and qter (right gray fragment).

(A) Chromothripsis/chromoplexy events can lead to gene truncation (colored boxes and colored dashed lines), fusions (adjacent colored boxes and colored dashed lines), gene haploinsufficiency due to removal of regulatory elements (enhancers marked as colored diamonds and haploinsufficient gene transcription marked with an x), or ectopic expression caused by position effects (enhancers marked as colored diamonds and dashed lines indicate the genes on which they are exerting their effects).

(B) Similar to chromothripsis/chromoplexy, chromoanasynthesis can lead to gene truncation, gene fusion, gene haploinsufficiency due to removal of regulatory elements or ectopic expression. In addition, expansion and transcription of triplosensitive genes could be observed in chromoanasynthesis.

https://doi.org/10.1016/j.ajhg.2019.02.024

Overview of mutations in cancer

- In liposarcoma, for example, chromothripsis events often involved multiple chromosomes, with universal MDM2 amplification and co-amplification of TERT in 20% of cases.
- In glioblastoma the events tended to affect a smaller region on a single chromosome that was distant from the telomere
- Resulting in focal amplification of EGFR and MDM2 and loss of CDKN2A.

Overview of mutations in cancer

- Acral melanomas frequently exhibited CCND1 amplification
- Squamous cell carcinomas, SOX2 amplifications
- In chromophobe renal cell carcinoma, chromothripsis nearly always affects chromosome 5
- Breakpoints immediately adjacent to TERT, increasing TERT expression

Table 7-5 Selected Oncogenes, Their Mode of Activation, and Associated Human Tumors

Category	Proto-Oncogene	Mode of Activation in Tumor	Associated Human Tumor
Growth Factors			
PDGF-β chain	*PDGFB*	Overexpression	Astrocytoma
Fibroblast growth factors	*HST1* *FGF3*	Overexpression Amplification	Osteosarcoma Stomach cancer Bladder cancer Breast cancer Melanoma
TGF-α	*TGFA*	Overexpression	Astrocytomas
HGF	*HGF*	Overexpression	Hepatocellular carcinomas Thyroid cancer
Growth Factor Receptors			
EGF-receptor family	*ERBB1 (EGFR)* *ERBB2 (HER)*	Mutation Amplification	Adenocarcinoma of lung Breast carcinoma
FMS-like tyrosine kinase 3	*FLT3*	Point mutation	Leukemia
Receptor for neurotrophic factors	*RET*	Point mutation	Multiple endocrine neoplasia 2A and B, familial medullary thyroid carcinomas
PDGF receptor	*PDGFRB*	Overexpression, translocation	Gliomas, leukemias
Receptor for KIT ligand	*KIT*	Point mutation	Gastrointestinal stromal tumors, seminomas, leukemias
ALK receptor	*ALK*	Translocation, fusion gene formation Point mutation	Adenocarcinoma of lung, certain lymphomas Neuroblastoma
Proteins Involved in Signal Transduction			
GTP-binding (G) proteins	*KRAS* *HRAS* *NRAS* *GNAQ* *GNAS*	Point mutation Point mutation Point mutation Point mutation Point mutation	Colon, lung, and pancreatic tumors Bladder and kidney tumors Melanomas, hematologic malignancies Uveal melanoma Pituitary adenoma, other endocrine tumors
Nonreceptor tyrosine kinase	*ABL*	Translocation Point mutation	Chronic myelogenous leukemia Acute lymphoblastic leukemia
RAS signal transduction	*BRAF*	Point mutation, Translocation	Melanomas, leukemias, colon carcinoma, others
Notch signal transduction	*NOTCH1*	Point mutation, Translocation Gene rearrangement	Leukemias, lymphomas, breast carcinoma
JAK/STAT signal transduction	*JAK2*	Translocation	Myeloproliferative disorders Acute lymphoblastic leukemia
Nuclear Regulatory Proteins			
Transcriptional activators	*MYC* *NMYC*	Translocation Amplification	Burkitt lymphoma Neuroblastoma
Cell Cycle Regulators			
Cyclins	*CCND1* (Cyclin D1)	Translocation Amplification	Mantle cell lymphoma, multiple myeloma Breast and esophageal cancers
Cyclin-dependent kinase	*CDK4*	Amplification or point mutation	Glioblastoma, melanoma, sarcoma

Pan-cancer analysis of whole genomes The ICGC/TCGA Pan-Cancer Analysis of Whole Genomes Consortium Nature (2019) https://doi.org/10.1038/s41586-020-1969-6

Scientific area	Key findings
Driver mutations	
Discovery of non-coding drivers	• Estimated ~10-fold more coding than non-coding driver point mutations. • Variation in point mutation density in non-coding regions influenced more by mutational processes than selection.
Drivers by pathways and networks	• Both coding and non-coding alterations contribute to cancer pathways. • Some pathways, such as RNA splicing, are primarily driven by non-coding mutations.
Evolution and heterogeneity	
Timing of cancer evolution	• Each tumour type has a distinct pattern of early and late-occurring driver events. • Earliest somatic mutations may occur decades prior to diagnosis, providing opportunities for early diagnosis. • Intra-tumour heterogeneity is widespread and tumour subclones contain drivers that are under positive selection.
Structural variants	
Patterns of structural variation	• Replication-based mechanisms of genome rearrangement frequent in many cancers, often causing driver structural variants. • 16 signatures of SV, including break-and-ligate patterns and copy-and-insert patterns, varying by size range, replication timing, tumour type and patient.
Functional consequence of structural variation	• 52 regions with recurrent structural breakpoints and 90 recurrently fused pairs of loci show evidence of positive selection. • Oncogenic fusions are shaped by juxtaposition of proto-oncogenes with tissue-specific regulatory elements.
Patterns of retrotransposition	• Many flavours of somatic retrotransposition in many cancers: LINE element mobilisation; transductions, pseudogenes, Alu elements. • Retrotranspositions can induce genomic instability, including large deletions and breakage-fusion-bridge cycles amplifying cancer genes.
Chromothripsis	• Chromothripsis pervasive across cancers, with frequency >50% in several tumour types. • Replicative processes and templated insertions contribute to rearrangement.
Mutational signatures	
Signatures of point mutations	• >70 distinct mutational signatures, encompassing SNVs, doublet subs and indels. • Multiple signatures from unknown processes of DNA damage, repair and replication.
Mutation distribution across genome	• Uneven distribution of somatic mutations and structural variants across the genome explained by epigenetic state of tissue, cell of origin and topological associated domains. • Can be used to identify a tumour's type and presumed tissue/cell of origin.
Transcriptional consequences of somatic mutation	
RNA effects of somatic mutation	• Genomic basis for RNA alterations across ~1200 tumours, including quantitative trait loci, allele specific expression and alternative splicing. • Link between mutational signatures and expression; classification of gene fusions; identification of genes recurrently altered at RNA level.
Others	
Tumour subtypes from genome sequencing	• Genomic distribution of somatic mutations, mutational signatures and driver mutations accurately distinguish major tumour types of primaries and metastases.
Mitochondrial DNA mutations	• Somatic mitochondrial truncating mutations frequent in certain cancer types, associated with activation of critical signaling pathways.
Telomere biology and sequences	• Activating *TERT* promoter mutations are the single most frequent non-coding driver. • In *ATRX*/*DAXX*-mutant tumours, aberrant telomere variant repeat distribution is common.

Single nucleopeptide polymorphism or variant (SNP, SNV)

- rSNPs at promoter regions; alter gene regulation
- 5’-UTR SNPs at non-coding exons; alter translation
- 3’-UTR SNPs at non-coding exons; alter mRNA stability
- nsSNPs at coding exons; alter protein sequence
- sSNPs at coding exons; alter mRNA
- X-SNPs at coding exons; alter protein sequence
- Splice-site SNPs at AG dinucleotide in intron before exons
- OR at GT dinucleotide in intron after exons; alter mRNA splicing.

Translocations

- During DNA duplication, DNA sequences may be translocated between chromosomes.
- Some translocation events can create fusion proteins.
- Burkitt's lymphoma
- t(8;14) rearrangement leads to the placement of the c-MYC gene under control of the promoter for the heavy chain gene. MYC over expression.
- and B-cell acute lymphoblastic leukemias

Translocations

- 85% of B-cell lymphomas of the follicular type carry a characteristic (14;18)(q32;q21) translocation.
- 14q32, the site where immunoglobulin heavy chain (IgH) genes are found, is also involved in the pathogenesis of Burkitt lymphoma.
- Juxtaposition of this transcriptionally active locus with BCL2 (located at 18q21) leads to over-expression of the BCL2 protein.
- This in turn protects lymphocytes from apoptosis and contributes to the survival of transformed B cells.

Translocations

- Chronic myelogenous leukemia
- t(9;22) rearrangement places the c-ABL gene near the cluster break point (BCR)
- Non-homologous end-joining then leads to a reciprocal translocation that creates an oncogenic BCR-ABL fusion gene on the derivative chromosome 22 (the so-called Philadelphia chromosome).
- The BCR-ABL fusion gene encodes a chimeric BCR-ABL protein with constitutive tyrosine kinase activity.

Translocations

- B-cell acute lymphoblastic leukemias (B-ALL) possess a cytogenetically identical translocation similar to that in CML.
- However, the structure of the resulting BCR-ABL fusion genes and proteins they encode usually differ slightly in these two tumors.

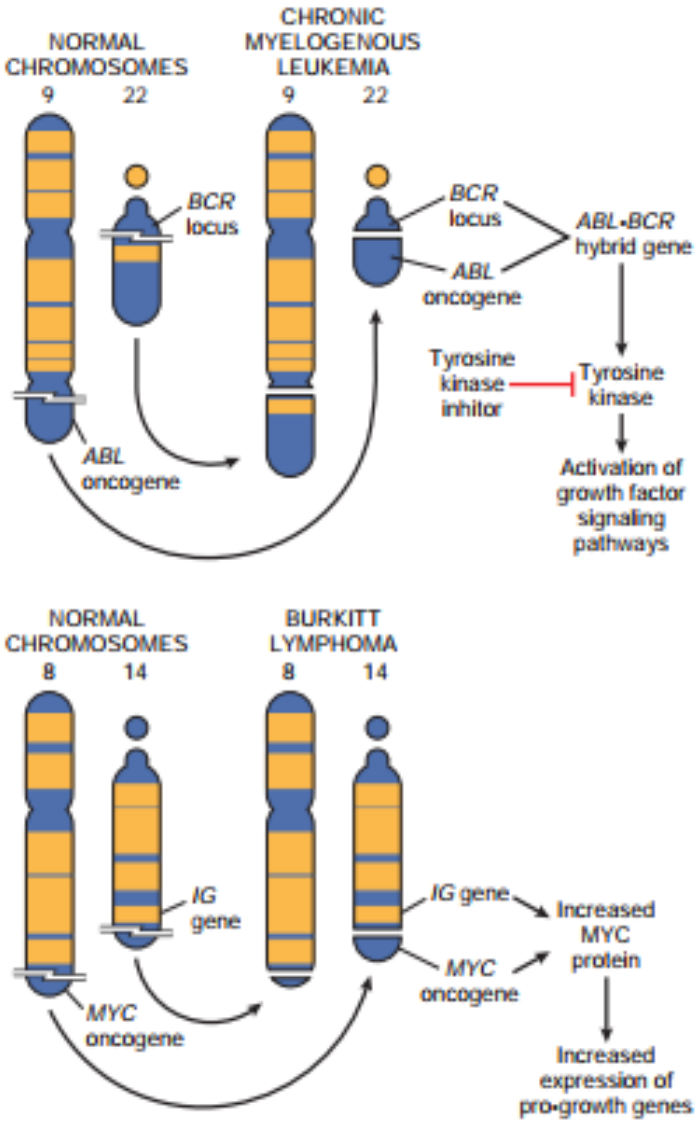


Figure 7-26 The chromosomal translocation and associated oncogenes in Burkitt lymphoma and chronic myelogenous leukemia.

Translocations

- Acute promyelocytic leukemia (APML)
- t(15;17) produces a PML-RARA fusion gene.
- The fusion gene encodes a chimeric protein consisting of part of a protein called PML and part of the retinoic acid receptor-α (RARα).
- Normal RARα binds to DNA and activates transcription in the presence of retinoids.
- Among the RARα responsive genes are a number that are needed for the differentiation of myeloid progenitors into neutrophils.

Translocations

- At physiologic levels retinoids do not bind to PML-RARα to any significant degree.
- Thus, the fusion gene retains the capacity to bind DNA
- But instead of activating transcription, it inhibits transcription through recruitment of transcriptional repressors.
- This interferes with the expression of genes that are needed for differentiation, leading to a “pile-up” of proliferating myeloid progenitors that replace normal bone marrow elements.

Translocations

- T-cell acute lymphoblastic leukemias
- Aberrations involving the 14q11 (TCRA/D) and 7q34(TCRB) regions can be detected in 35% of patients.
- They juxtapose enhancer elements of the TCR genes with transcription factors involved in T-cell differentiation (SCL/TAL1 at 1p32), with deregulation of hemopoiesis.
- In addition, high expression levels of TAL1 are observed in about 40% of T-ALL.

Table 1.

Rearrangements involving T-cell receptor genes.

Translocation	Involved gene	Fusion gene function	Frequency
t(7;10)(q34;q24) and t(10;14)(q24;q11)	*TLX1 (HOX11)*	Transcription factor	7% children 31% adults
t(5;14)(q35;q32)	*TLX3 (HOX11L2)*	Transcription factor	20% children 13% adults
inv(7)(p15q34), t(7;7)	*HOXA* genes	Transcription factor	5%
t(1;14)(p32;q11) and t(1;7)(p32;q34)	*TAL1*	Transcription factor	3%
t(7;9)(q34;q32)	*TAL2*	Transcription factor	<1%
t(7;19)(q34;p13)	*LYL1*	Transcription factor	<1%
t(14;21)(q11.2;q22)	*BHLHB1*	Transcription factor	<1%
t(11;14)(p15;q11)	*LMO1*	Protein-protein interaction	2%
t(11;14)(p13;q11) and t(7;11)(q35;p13)	*LMO2*	Protein-protein interaction	3%
t(1;7)(p34;q34)	*LCK*	Signal transduction	<1%
t (7;9)(q34;q34.3)	*NOTCH1*	Fate determination, differentiation	<1%
t(7;12)(q34;p13) and t(12;14)(p13;q11)	*CCND2*	Cell cycle activator	<1%

Chiaretti, S, Foa, R, “T-cell acute lymphoblastic leukemia,” Haematologica. 2009; 94(2): 160–162. doi: 10.3324/haematol.2008.004150 Accessed 05/05/2020

Table 2.

Lesions involving known oncogenes.

Translocation	Involved gene	Fusion gene function	Frequency
1p32 deletion	*SIL-TAL1*	Transcription factor	9-30%
t(10;11)(p13;q14)	*CALM-AF10*	Transcription factor	10%
t(9;9)(q34;q34)	*NUP214-ABL1*	Signal transduction	~5%
t(9;14)(q34;q32)	*EML1-ABL1*	Signal transduction	<1%
t(9;22)(q34;q11)	*BCR-ABL1*	Signal transduction	<1%
t(9;12)(p24;p13)	*ETV6-JAK2*	Signal transduction	<1%
Mutations			
Notch1	*Notch1*	Fate determination, differentiation	50%
JAK1	*JAK1*	Signal transduction	18%

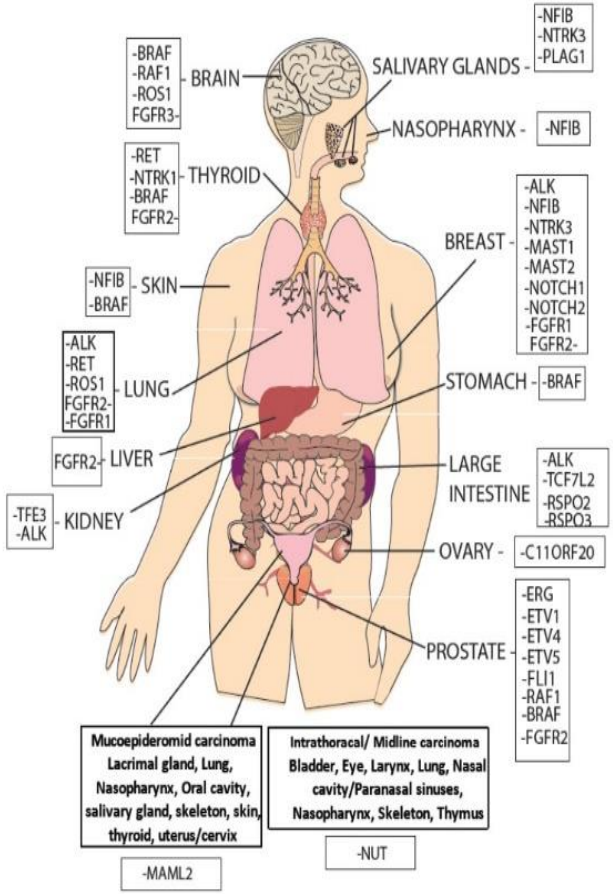
Chiaretti, S, Foa, R, “T-cell acute lymphoblastic leukemia,” Haematologica. 2009; 94(2): 160–162. doi: 10.3324/haematol.2008.004150 Accessed 05/05/2020

Table 7-8 Selected Examples of Oncogenes Created by Translocations

Malignancy	Translocation	Affected Genes*
Chronic myelogenous leukemia (CML)	(9;22)(q34;q11)	*ABL* 9q34 *BCR* 22q11
Acute myeloid leukemia (AML)	(8;21)(q22;q22) (15;17)(q22;q21)	*AML* 8q22 *ETO* 21q22 *PML* 15q22 *RARA* 17q21
Burkitt lymphoma	(8;14)(q24;q32)	*MYC* 8q24 *IGH* 14q32
Mantle cell lymphoma	(11;14)(q13;q32)	*CCND1* 11q13 *IGH* 14q32
Follicular lymphoma	(14;18)(q32;q21)	*IGH* 14q32 *BCL2* 18q21
Ewing sarcoma	(11;22)(q24;q12)	*FLI1* 11q24 *EWSR1* 22q12
Prostatic adenocarcinoma	(7:21)(p22;q22) (17:21)(p21;q22)	*TMPRSS2* (21q22.3) *ETV1* (7p21.2) *ETV4* (17q21)

*Genes in bold type are involved in multiple rearrangements.

Gene Fusions in Epithelial Tumors



Genomic Landscape

The genomic landscape of these R/R tumors reflects those of the corresponding tumors at diagnosis

Pathogenic or likely pathogenic germline variants in 8.7% of patients (including variants in *TP53*, *NF1*, *PTPN11*, *MUTYH*, *BRCA2*)

78.3% of tumors bore somatic alterations in a known cancer gene

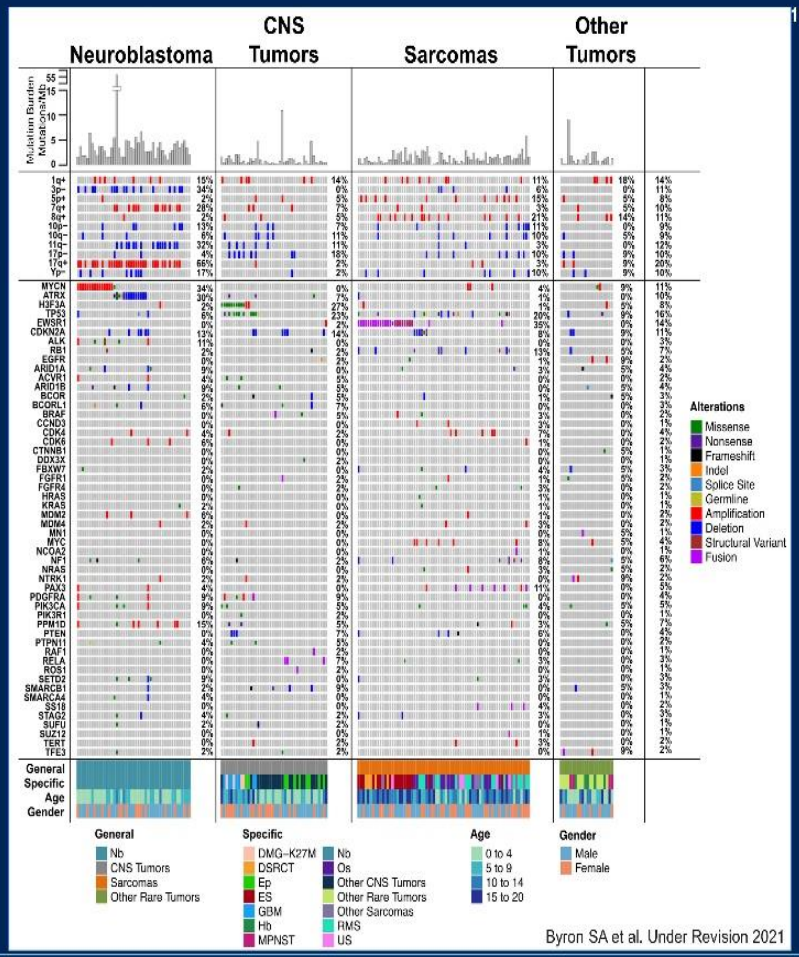
Pathognomonic fusions in 25% of tumors

- *EWSR1-FLI1/ERG* fusions in Ewing sarcoma
- *EWSR1-WT1* fusions in DSRCT
- *PAX-FOXO1* fusions in rhabdomyosarcoma
- *KIAA1549-BRAF* fusions in JPA
- *QKI-RAF1* fusion in pilomyxoid astrocytoma
- *EEF1G-ROS1* fusion in infantile astrocytoma

Hotspot mutations in 16.3% of tumors

- MAPK Pathway (*BRAF*, *HRAS*/*KRAS*/*NRAS*, *PTPN11*)
- PI3K Pathway (*PIK3CA*, *PIK3R1*, *FBXW7*)
- Receptor Tyrosine Kinases (*ALK*, *EGFR*, *FGFR4*, *PDGFRA*)

***TP53*, *CDNK2A*, *RB1*, *NF1*, and *PIK3CA* alterations occur across tumor types**



Tumor suppressor gene or oncogene

- Tumor suppressor gene
- Loss of function
- Germline mutation

- Activation of proto-oncogene may lead to oncogenesis
- Gain of function
- Somatic line mutations only

Important tumor suppressor genes

Gene	Chromosome	Mechanism (Loss of function)
RB1	**13q14**	**When dephosphorylated, blocks transition from G1 to S (to G2, to M) by binding to and inhibiting transcription of E2F; downstream affector of ABL**
BRCA-1 **BRCA-2**	**17q21** **13q12**	**Blocks replication of damaged DNA** **Blocks replication of damaged DNA**
p53	**17p13**	**Holds at G1/S checkpoint (bound to p21/CDK) ; at G2 (with 14-3-3s) if DNA damaged; induce apoptosis (BAX, PUMA, NOXA)**
APC1	**5q21**	**Binds β-catenins for ubiquination (in cytoskeleton, inhibits wtn)**
p16	**9p21.3**	**CDKN2A**

Important tumor suppressor genes

Gene	Chromosome	Mechanism
NF-1	17q11	Neurofibromin stimulates GTPase activity (p21) (bound to RAS on membrane, inactivating tyrosine kinase activity)
NF-2	22q12.2	Schwanomma protein (Merlin)
TSC1	9q34.13	Hamartin protein (reacts with Tuberin)
TSC2	16p13.3	Tuberin protein
PTEN	10q23	Stimulates p27; cell cycle arrest (in cytosol)
WT1	11p13	Zinc finger transcription factor
WT2	11p15	
VHL	3p25.3	Inhibits Hypoxia Inducible Factor 2a
MEN1	11q13.1	Menin involved in DNA repair, apoptosis
CPD4/ SMAD4	18q21.2	TGF-β p pathway
DCC	18q21.2	Netrin-1 receptor unable to trigger apoptosis

Common mutations

- Germline
- APC
- FAP
- HFAS
- CHRPE
- BRCA l/2
- HNPCC
- mt DNA dominant in neoplastic clones (promote formation of reactive oxygen species)

Common mutations

- Somatic
- RAS
- p53 with p73 upregulation

  Gastric, esophageal, breast, liver cancers
- p53 with p73 down-regulation

  Hematopoietic
- p53 inactivated by SV40 T antigen and E1B adenovirus product
- Sequestered by HPV E6 protein
- Aflatoxin, benzo(a)pyrene mutate p53.

BRCA genes

- Account for only 1-3% of breast cancers.
- With family history, increase to 20%.
- History of breast and ovarian cancer, 60 to 80%.
- Mutation increases lifetime risk from 12.5% to 50 to 80%.
- Increase lifetime risk of ovarian cancer also.
- Younger ages at presentation.

BRCA genes

- BRCA1 mutation (at 17q21.31)from paternal line associated with earlier presentation of breast cancer than if from maternal line.
- May have basal-like disease (30%).
- BRCA2 (at 13q13.1) is associated with male breast cancer.
- Increased risk of pancreatic cancer in men and women
- Increased risk of prostate cancer

BRCA genes

- Genes show no sequence similarity, but operate in same pathway
- Homologous recombination repair of double stranded DNA breaks.
- Polymorphic mutations.
- Loss of function.
- BRCA1 has high affinity for branched DNA structures.
- It promotes non-homologous end-joining as well as nucleotide excision repair pathways.

BRCA genes

- BRCA1 is phosphorylated in response to DNA damage and may transduce damage signals from checkpoint kinases to effector proteins.
- CHEK 2 activates BRCA1
- Both BRCA1 and BRCA2 interact with RAD51

BRCA genes

- BRCA 1 binds to BRCA2.
- Interacts as well with the estrogen receptor and is involved in X chromosome inactivation.
- BRCA2 binds directly to DNA.
- BRCA1 mutated cells are deficient in the transcription-coupled repair of oxidative damage.
- Inhibition of poly-ADP-ribose polymerase (PARP) disables base excision repair.

BRCA genes

- Estrogen metabolism is abnormal in breast cancer.
- Increased levels of estrogen metabolites such as estrogen-3,4-quinones react with DNA
- Form depurinating adducts that spontaneously dissociate from DNA to form abasic sites
- Thus, generate mutations that must be corrected by the error-prone base excision repair.

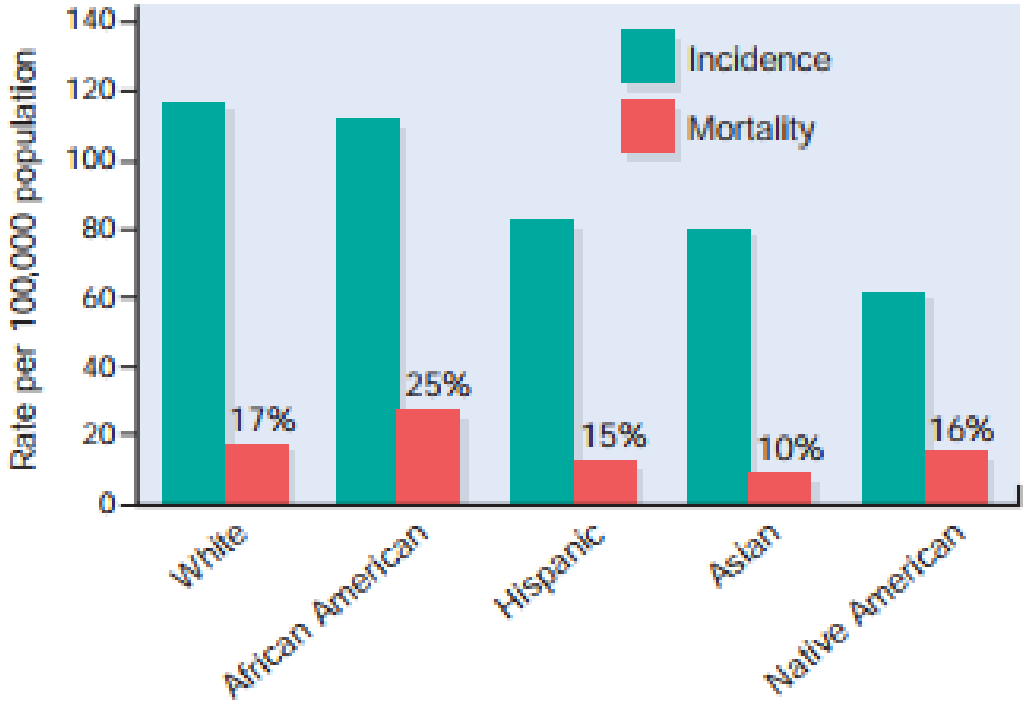
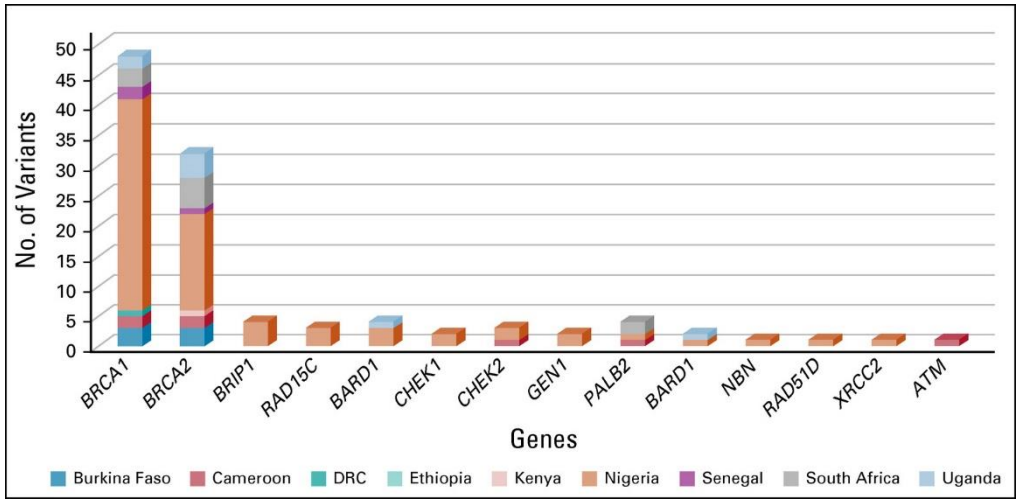


Figure 23-15 Breast cancer incidence and mortality in different ethnic groups (Data from North American Association of Central Cancer Registries). White women have the highest incidence of breast cancer, while African American women have the highest mortality rate. Likely contributors to these differences include socioeconomic factors (better access to care in white women) and biologic factors, particularly the higher incidence of aggressive, high grade, ER-negative tumors in younger African American women.



Genetic Susceptibility to Breast Cancer in Sub-Saharan African Populations
Mahtaab Hayat, Wenlong Carl Chen, Jean-Tristan Brandenburg, Chantal Babb de Villiers, Michèle Ramsay, and Christopher G. Mathew
JCO Global Oncology 2021 :7, 1462-1471

Cell cycle controls genes

- Loss of normal cell cycle control is central to malignant transformation
- Four key regulators of the cell cycle:
- p16/INK4a
- Cyclin D
- CDK4
- RB
- Dysregulated in the vast majority of human cancers.

Table 7-6 Cell Cycle Components and Inhibitors That Are Frequently Mutated in Cancer

Cell Cycle Component	Main Function
Cyclins and Cyclin-Dependent Kinases	
CDK4; D cyclins	Form a complex that phosphorylates RB, allowing the cell to progress through the G_1 restriction point
Cell Cycle Inhibitors	
CIP/KIP family: p21, p27 (CDKN1A-D)	Block the cell cycle by binding to cyclin-CDK complexes p21 is induced by the tumor suppressor p53 p27 responds to growth suppressors such as TGF-β
INK4/ARF family (CDKN2A-C)	p16/INK4a binds to cyclin D-CDK4 and promotes the inhibitory effects of RB p14/ARF increases p53 levels by inhibiting MDM2 activity
Cell Cycle Checkpoint Components	
RB	Tumor suppressive "pocket" protein that binds E2F transcription factors in its hypophosphorylated state, preventing G_1/S transition; also interacts with several transcription factors that regulate differentiation
p53	Tumor suppressor altered in the majority of cancers; causes cell cycle arrest and apoptosis. Acts mainly through p21 to cause cell cycle arrest. Causes apoptosis by inducing the transcription of pro-apoptotic genes such as *BAX*. Levels of p53 are negatively regulated by MDM2 through a feedback loop. p53 is required for the G_1/S checkpoint and is a main component of the G_2/M checkpoint.

CDKN2A gene

- CDKN2A also known as p16/INK4A CDKI.
- Inhibits CYCLIN D/CDK2 phosphorylation of RB, maintaining RB checkpoint.
- Inhibits MDM2
- Preventing destruction of p53 (activation)
- Germline mutation in 5-10% of familial malignant melanoma
- Somatic mutation in 25% of sporadic melanoma

CDKN2A gene

- Point mutations in:
- 75% of pancreas cancers
- up to 70% of glioblastomas
- 50% of esophageal cancers
- 20% of non-small cell lung cancers
- up to 70% of acute lymphoblastic leukemia.
- 20% of non–small-cell lung carcinomas, bladder cancers, and soft tissue sarcomas
- Silenced by hypermethylation in cervical carcinoma

RB gene

- It exists in an active hypo-phosphorylated state in quiescent cells and an inactive hyper-phosphorylated state in cells passing through the G1/S cell cycle transition.
- Bi-allelic loss (germline) is associated with the development of retinoblastoma.
- Loss-of-function mutation associated with osteosarcoma

RB gene

- A shift from the active hypo-phosphorylated state to the inactive hyper-phosphorylated state by gain-of-function mutations that upregulate CDK/cyclin D activity or by loss-of-function mutations that abrogate the activity of CDK inhibitors inhibits RB
- E2F transcription factors are released that drive the expression of genes that are needed for progression to S phase

RB gene

- Simian virus 40 and polyomavirus large T antigens, adenovirus EIA protein, and HPV E7 protein all bind to the hypo-phosphorylated form of RB.
- The binding occurs in the same RB pocket that normally sequesters E2F transcription factors.
- Certain HPV viral types (such as HPV16) that confer a high risk for the development of cervical carcinoma express E7 protein variants with higher affinity for RB than do lower risk viral types.
- As the RB protein is unable to bind the E2F transcription factors, as it is functionally inactivated, the E2F factors are free to cause cell cycle progression.

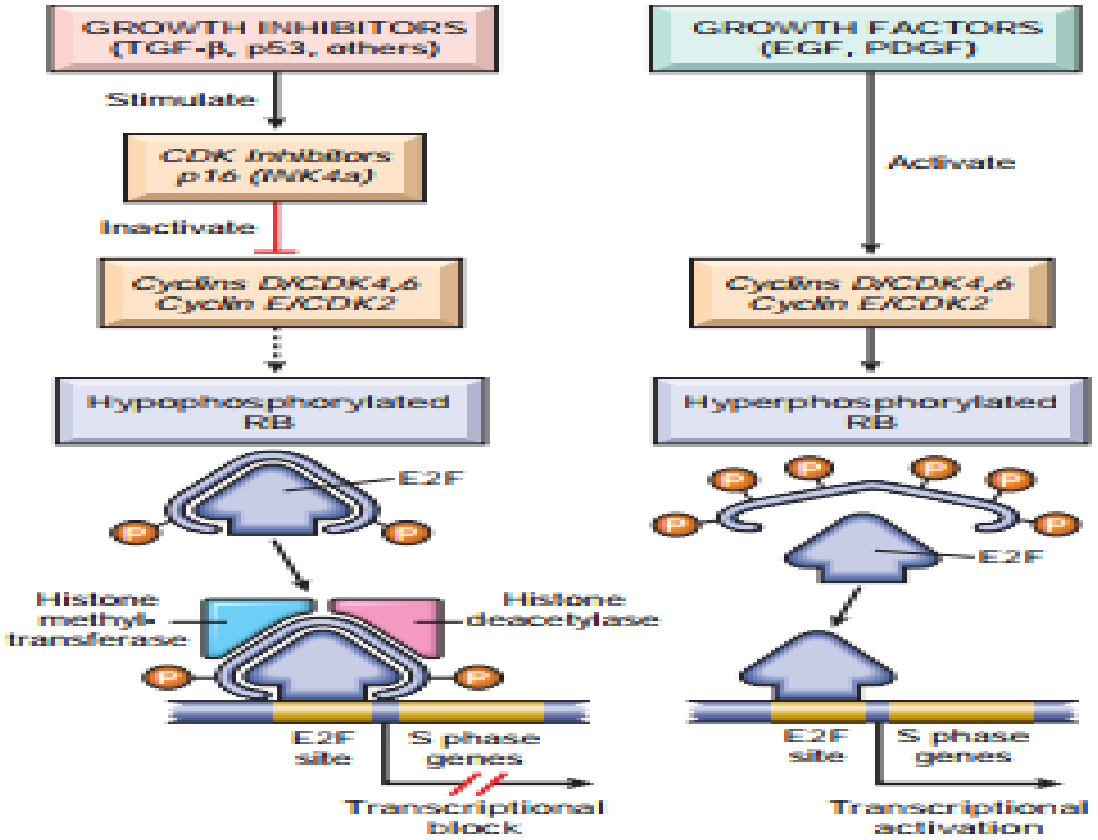


Figure 7-29 The role of RB in regulating the G_1-S checkpoint of the cell cycle. Hypophosphorylated RB in complex with the E2F transcription factors binds to DNA, recruits chromatin-remodeling factors (histone deacetylases and histone methyltransferases), and inhibits transcription of genes whose products are required for the S phase of the cell cycle. When RB is phosphorylated by the cyclin D-CDK4, cyclin D-CDK6, and cyclin E-CDK2 complexes, it releases E2F. The latter then activates transcription of S-phase genes. The phosphorylation of RB is inhibited by cyclin-dependent kinase inhibitors, because they inactivate cyclin-CDK complexes. Virtually all cancer cells show dysregulation of the G_1-S checkpoint as a result of mutation in one of four genes that regulate the phosphorylation of RB; these genes are *RB*, *CDK4*, the genes encoding cyclin D proteins, and *CDKN2A* (p16). TGF-β, transforming growth factor-β.

TP53

- Tumor suppressor gene at 17p13
- Produces nuclear phosphoprotein involved in transcriptional regulation
- N- terminus amino acids bind:
- TATA-binding protein associated factors (TAFs), which attract other proteins needed to initiate gene expression
- MDM2, which inhibits p53, and has the opposite effect as TAF's

TP53

- p53 ensures that cells repair any damaged DNA before cell division by inducing cell cycle arrest to allow time for
- DNA repair
- OR to force the cell to undergo apoptosis via activation of BAX gene

TP53

- There are at least seven negative and three positive feedback loops described
- Six act through the MDM-2 protein to regulate p53 activity
- The p53 circuit communicates with the WNT-β-catenin, IGF-1-AKT, RB-E2F, p38 MAP kinase, cyclin-cdk, p14/19 ARF pathways and the cyclin G-PP2A, and p73 gene products.
- There are three different ubiquitin ligases that can regulate p53 in an autoregulatory manner: MDM-2, Cop-1 and Pirh-2.

TP53

- Wild p53 induces p21 WAF-1, which inhibits cyclin-dependent kinases
- Wild p53 has half-life of only 20 minutes (mutant type has longer half-life)
- Inactivated by SV40 T antigen and E1B adenovirus product
- Sequestered by HPV E6 protein
- Aflatoxin, benzo(a)pyrene mutate p53.
- Over-expression associated with mutated gene product.

TP53

- The function of TP53 gene is usually altered through loss of heterozygosity (LOH), mutations, and rarely by DNA methylation.
- Over 50% of human cancers present inactivated TP53, due loss of function mutations.
- 95% occur within the genomic region encoding the sequence-specific DNA-binding domain of TP53.
- 80% occur in the Zinc finger domains (exons 5-9)
- These mutations disrupt the proper sequence-specific transactivation ability.

TP53

- In non-stressed, healthy cells, p53 is inhibited by MDM2, an enzyme that ubiquitinylates p53, leading to its degradation by the proteasome.
- As a result, p53 is virtually undetectable in normal cells.
- In stressed cells, however, p53 is released from the inhibitory effects of MDM2 via two major mechanisms:
- The key initiators of p53 activation following DNA damage or in cells exposed to hypoxia are two related protein kinases, ataxia-telangiectasia mutated (ATM) and ataxia-telangiectasia and Rad3 related (ATR) genes.

TP53

- Once triggered, both ATM and ATR stimulate the phosphorylation of p53 and MDM2. These posttranslational modifications disrupt the binding and degradation of p53 by MDM2, allowing p53 to accumulate.
- Activation of oncoproteins such as RAS leads to sustained, supraphysiologic signaling through pro-growth pathways such as the MAPK and PI3K/AKT pathways.
- These aberrant signals create cellular stress and lead to increased expression of p14/ARF (encoded by the CDKN2A tumor suppressor gene).

TP53

- p14/ARF binds MDM2 and displaces p53, again allowing p53 levels to rise in the cell.
- Once activated, and p53 levels accumulate in the cell sufficient to activate transcription genes, p53 induces either transient cell cycle arrest, senescence (permanent cell cycle arrest), or programmed cell death (apoptosis).

TP53

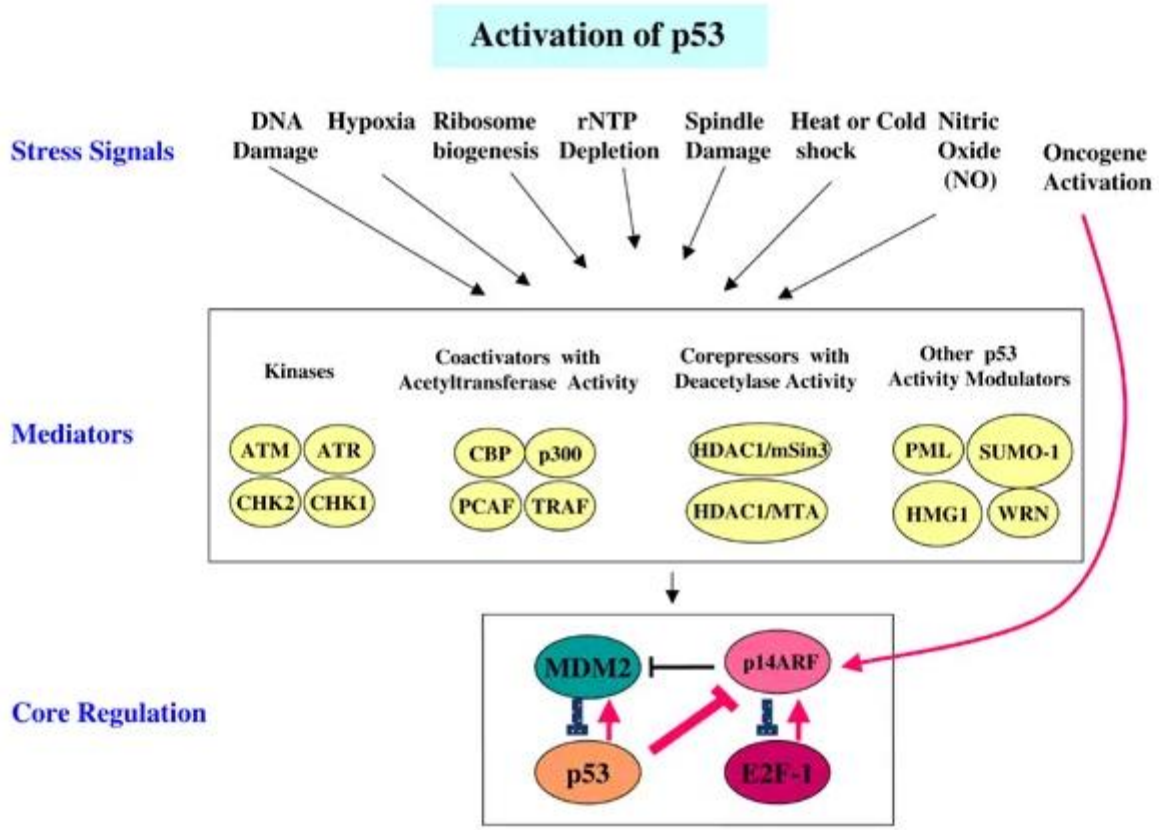
- Rapid onset, p53-mediated cell cycle arrest may be considered a primordial response to DNA damage. It occurs late in the G1 phase and is caused in part by p53-dependent transcription of the CDKN1A gene, which encodes the CDK inhibitor p21.
- p21 inhibits CDK4/cyclin D complexes
- Maintains RB in an active, hypo-phosphorylated state
- Blocks the progression of cells from G1 phase to S phase.

TP53

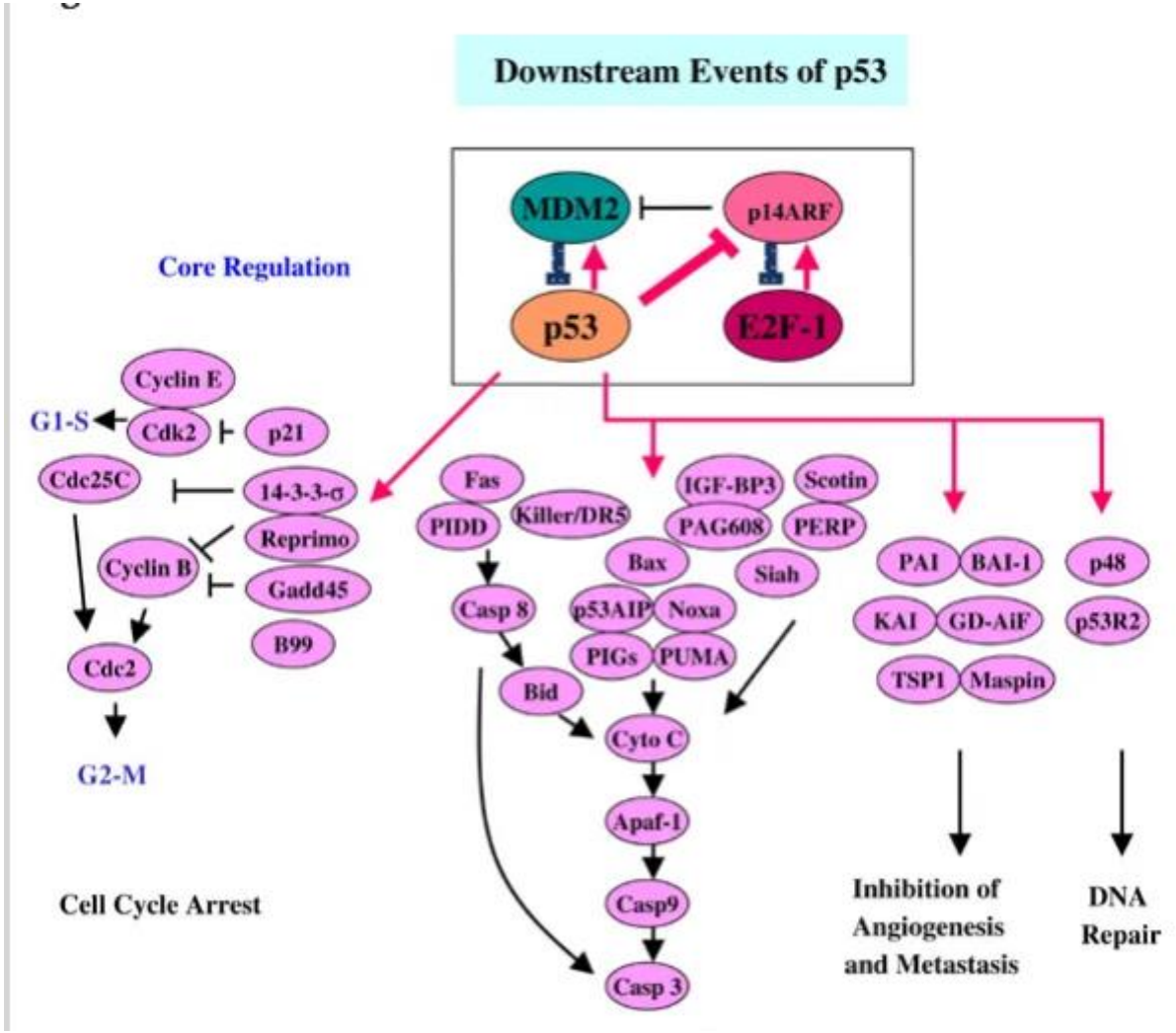
- This pause in cell cycling gives the cells an opportunity to repair DNA damage.
- p53 induces the GADD45 (growth arrest and DNA damage) gene , that enhances DNA repair.
- If DNA damage is repaired successfully, the signals responsible for p53 stabilization cease and p53 levels fall, releasing the cell cycle block. The cells may then revert to a normal state.

TP53

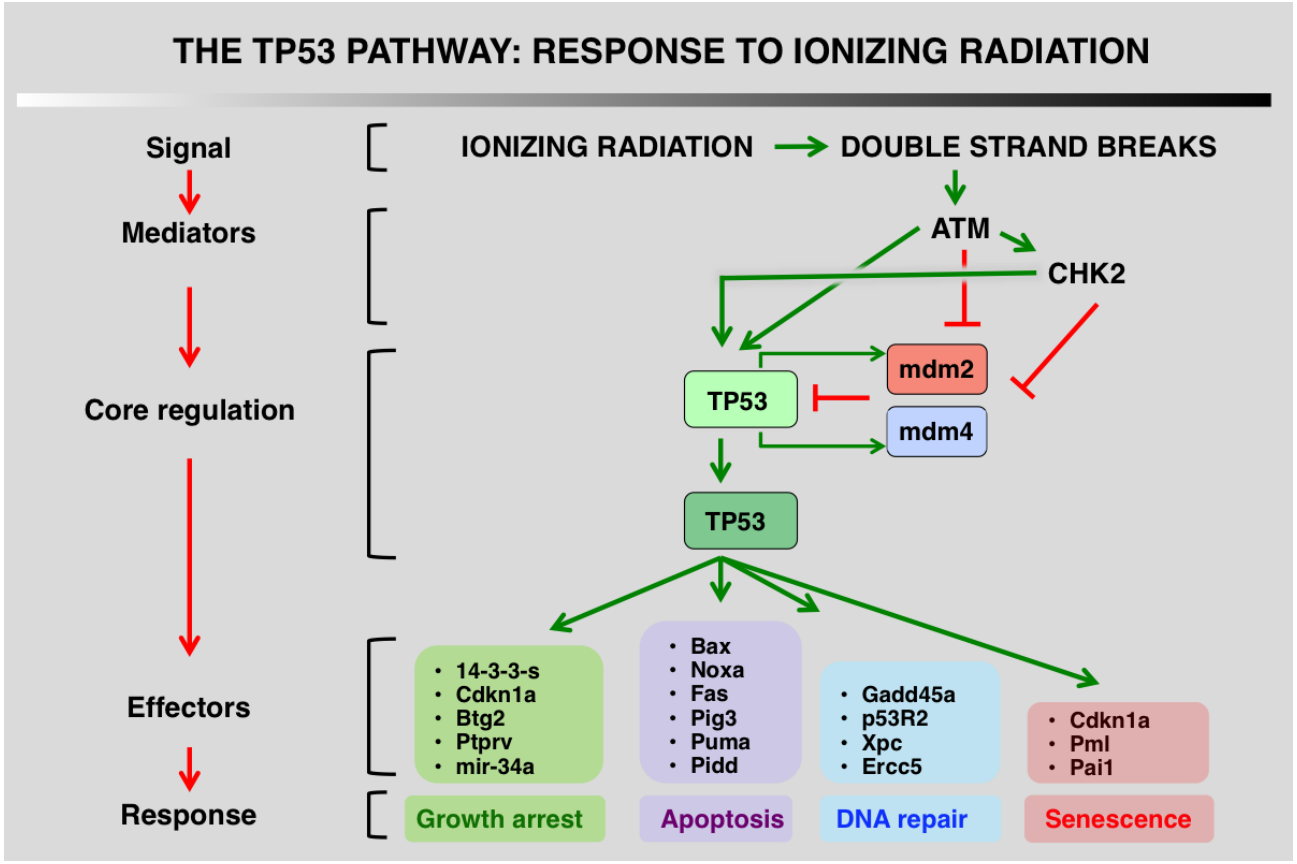
- It may be the level and duration of pp53 action that determines whether the cell enters senescence of apoptosis.
- One plausible idea is that senescence is the product of epigenetic changes that result in the formation of heterochromatin at key loci, including genes that are required for progression of cells from the G1 phase into S phase.
- It appears that the affinity of p53 for its binding sites in the promoters and enhancers of DNA repair genes is higher than its affinity for binding sites in pro-apoptotic genes.



Harris, S., Levine, A. The p53 pathway: positive and negative feedback loops. Oncogene 24 (2005); 2899–2908. Figure 1. https://doi.org/10.1038/sj.onc.1208615 Accessed 03/17/2020



Harris, S., Levine, A. The p53 pathway: positive and negative feedback loops. Oncogene 24 (2005); 2899–2908. Figure 2. https://doi.org/10.1038/sj.onc.1208615 Accessed 03/17/2020



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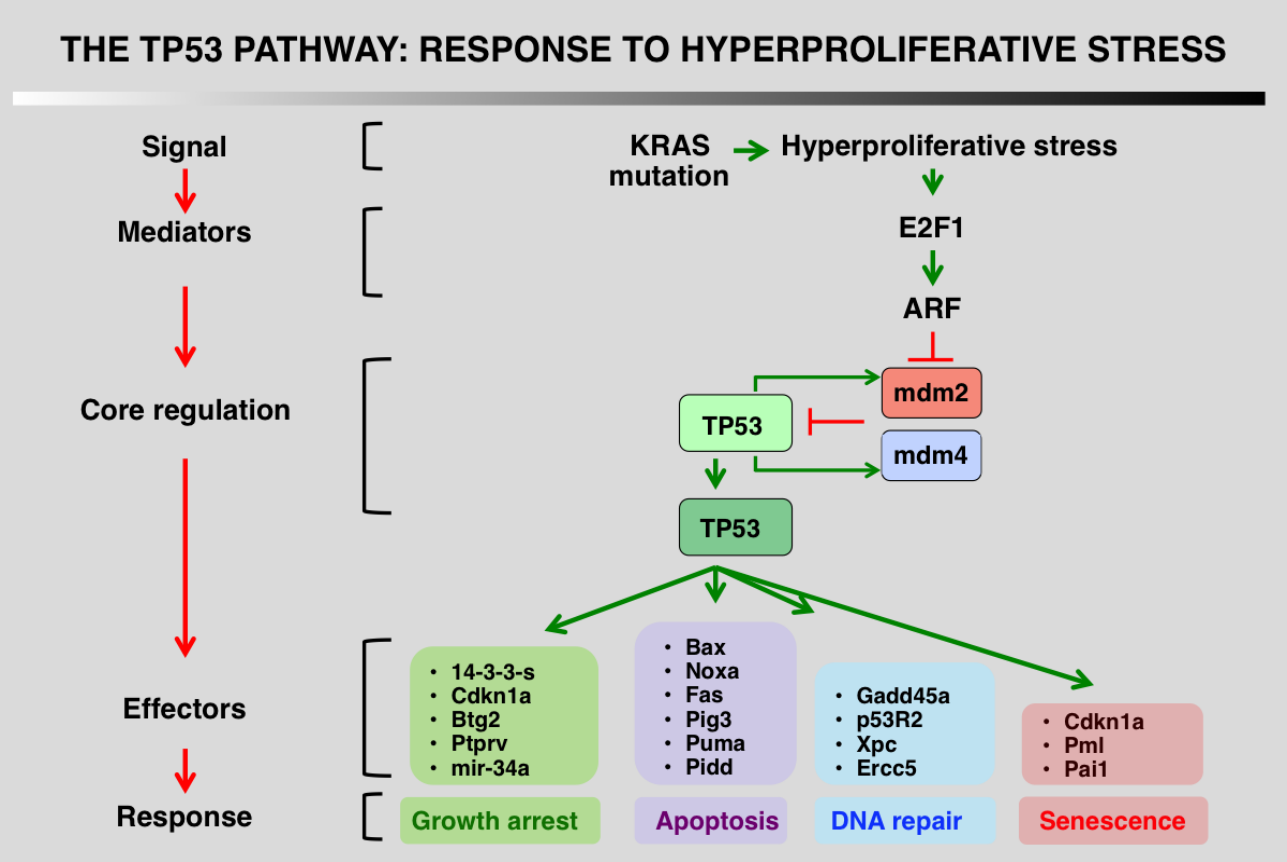

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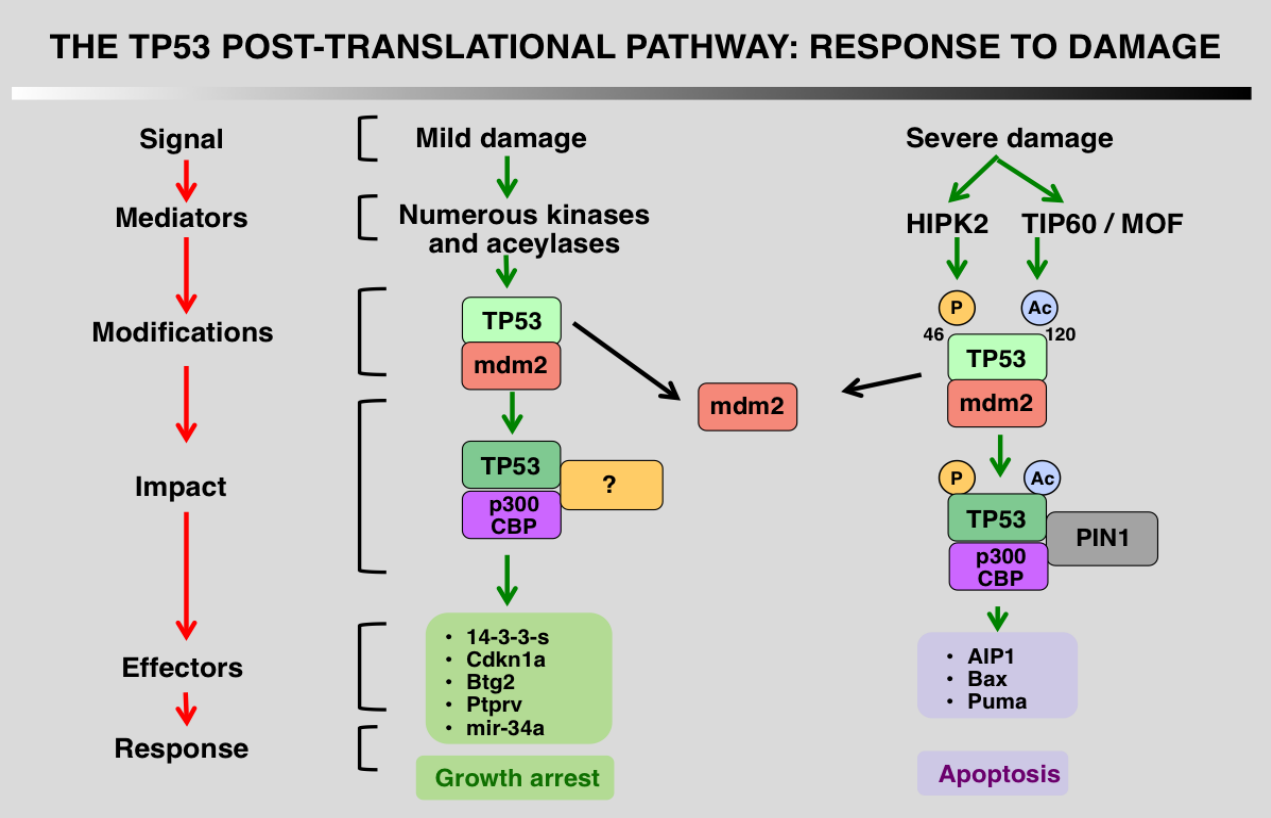

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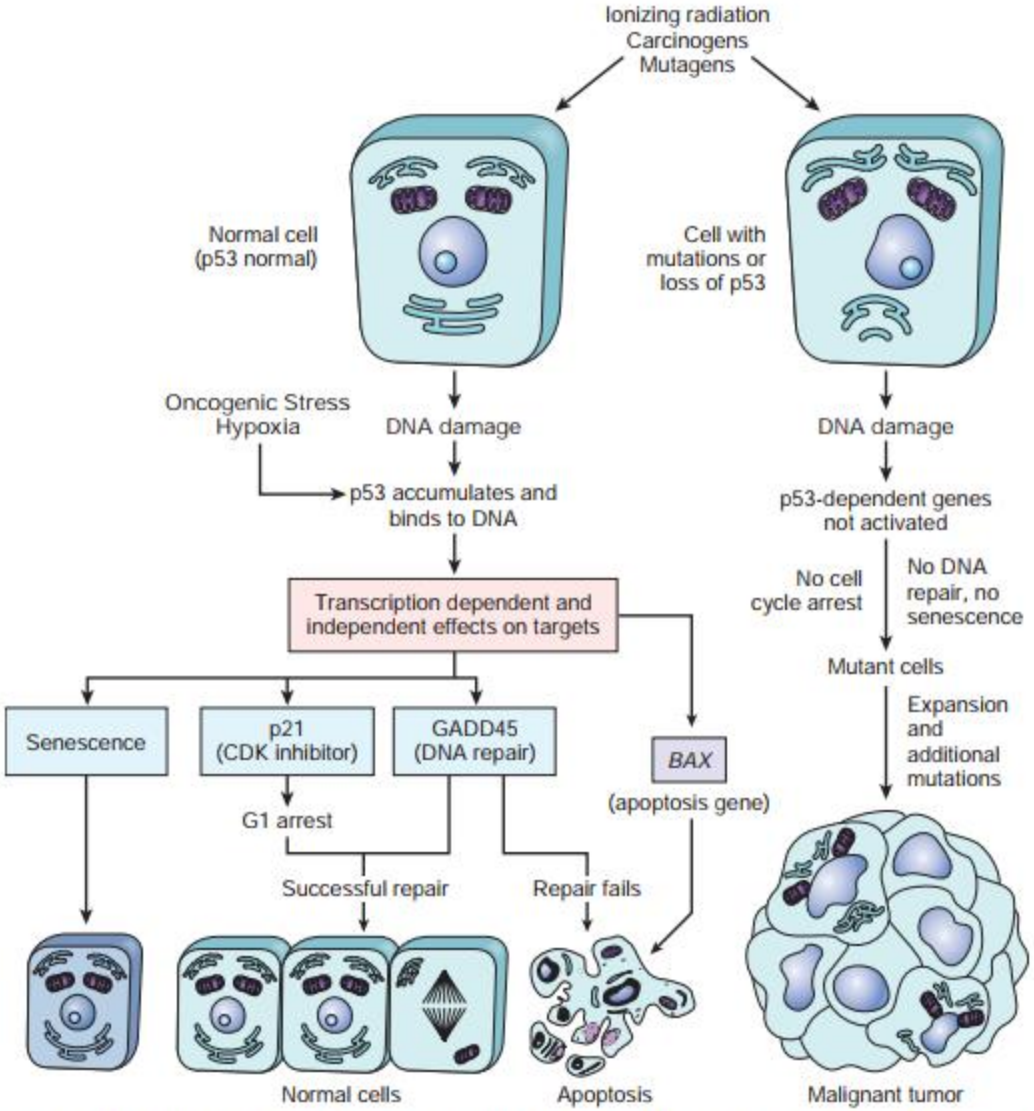
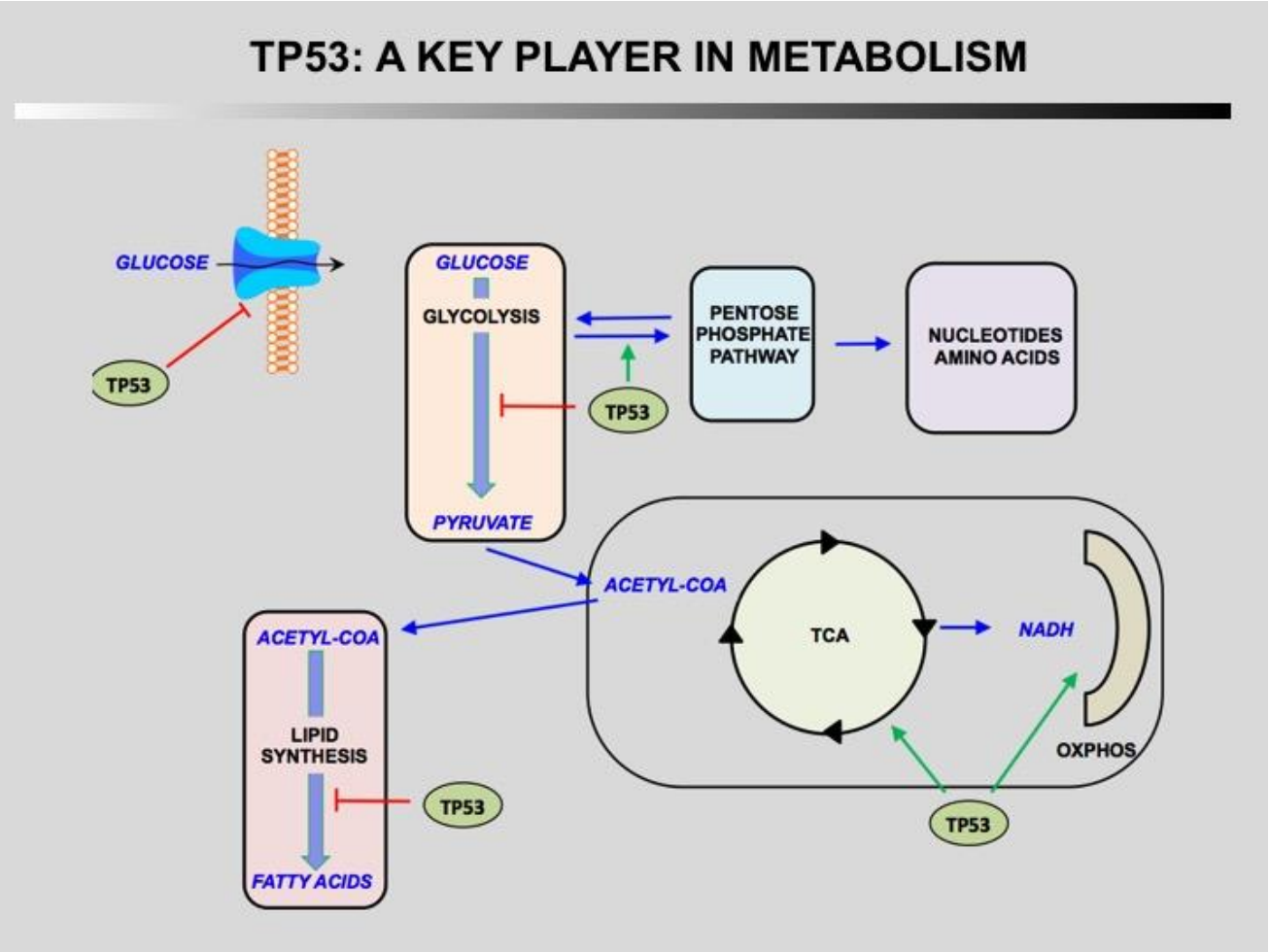


Figure 7-30 The role of p53 in maintaining the integrity of the genome. Activation of normal p53 by DNA-damaging agents or by hypoxia leads to cell cycle arrest in G_1 and induction of DNA repair by transcriptional upregulation of the cyclin-dependent kinase inhibitor *CDKN1A* (encoding the cyclin-dependent kinase inhibitor p21) and the *GADD45* genes. Successful repair of DNA allows cells to proceed with the cell cycle; if DNA repair fails, p53 triggers either apoptosis or senescence. In cells with loss or mutations of the p53 gene, DNA damage does not induce cell cycle arrest or DNA repair, and genetically damaged cells proliferate, giving rise eventually to malignant neoplasms.



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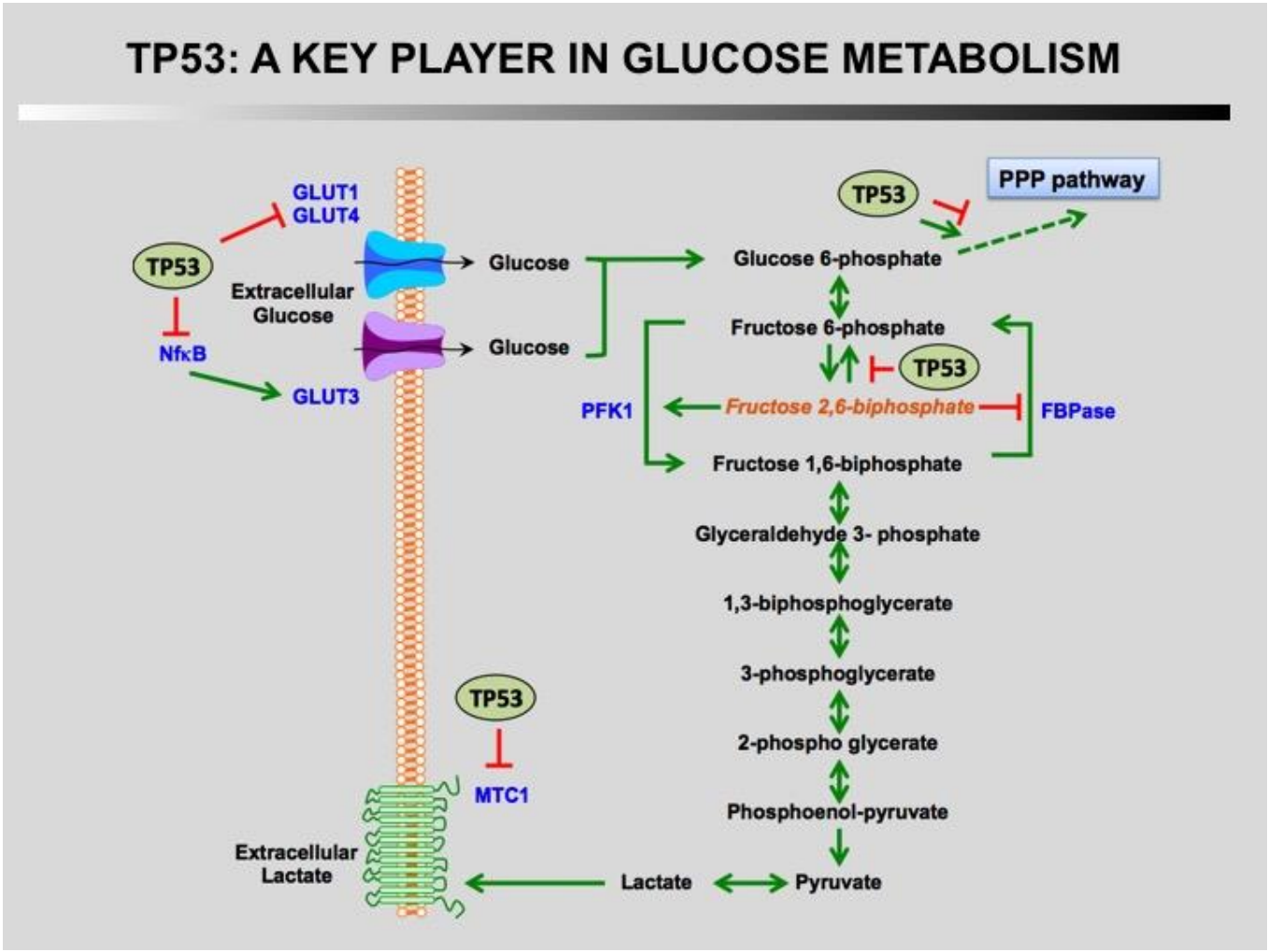

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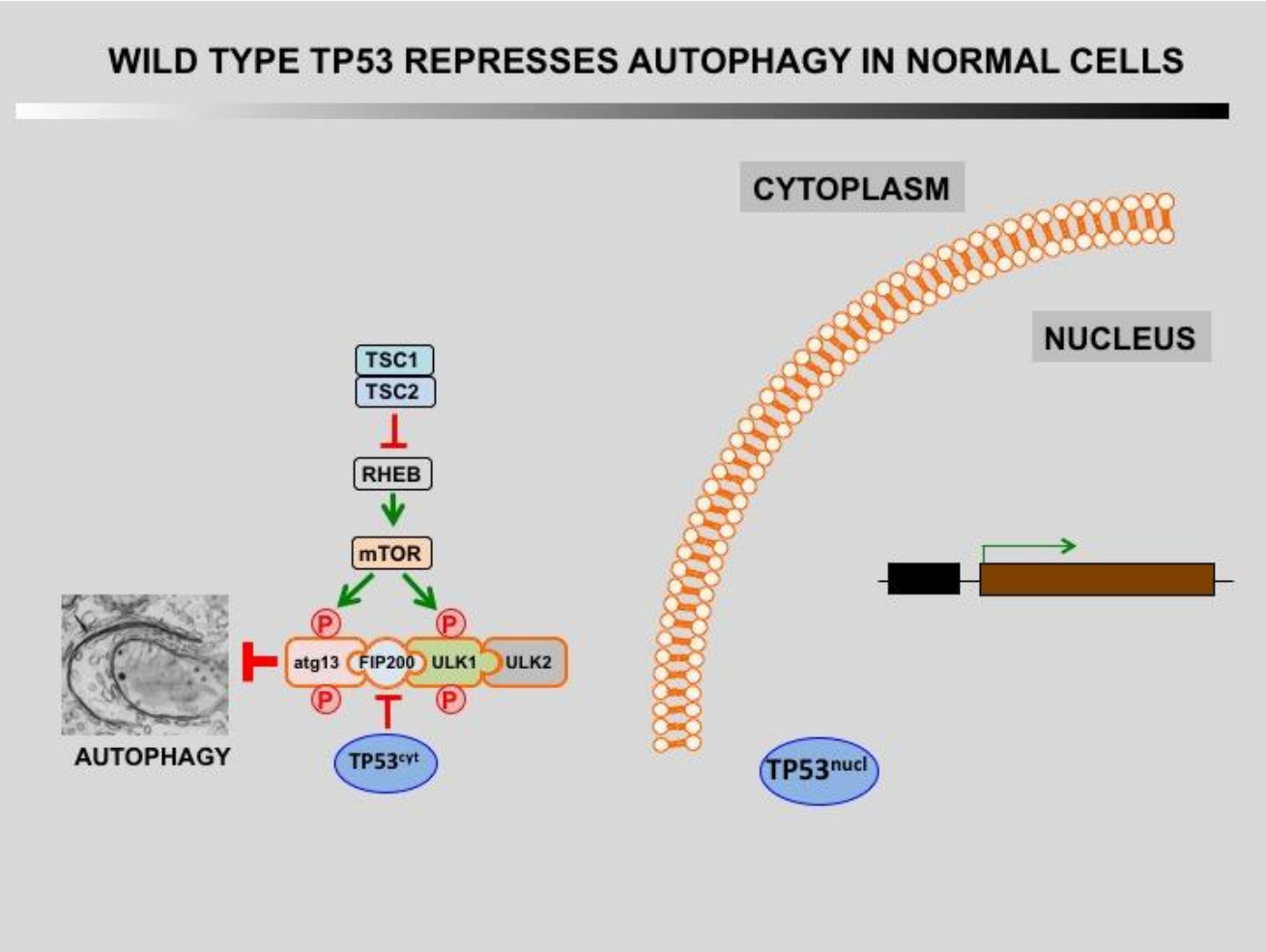

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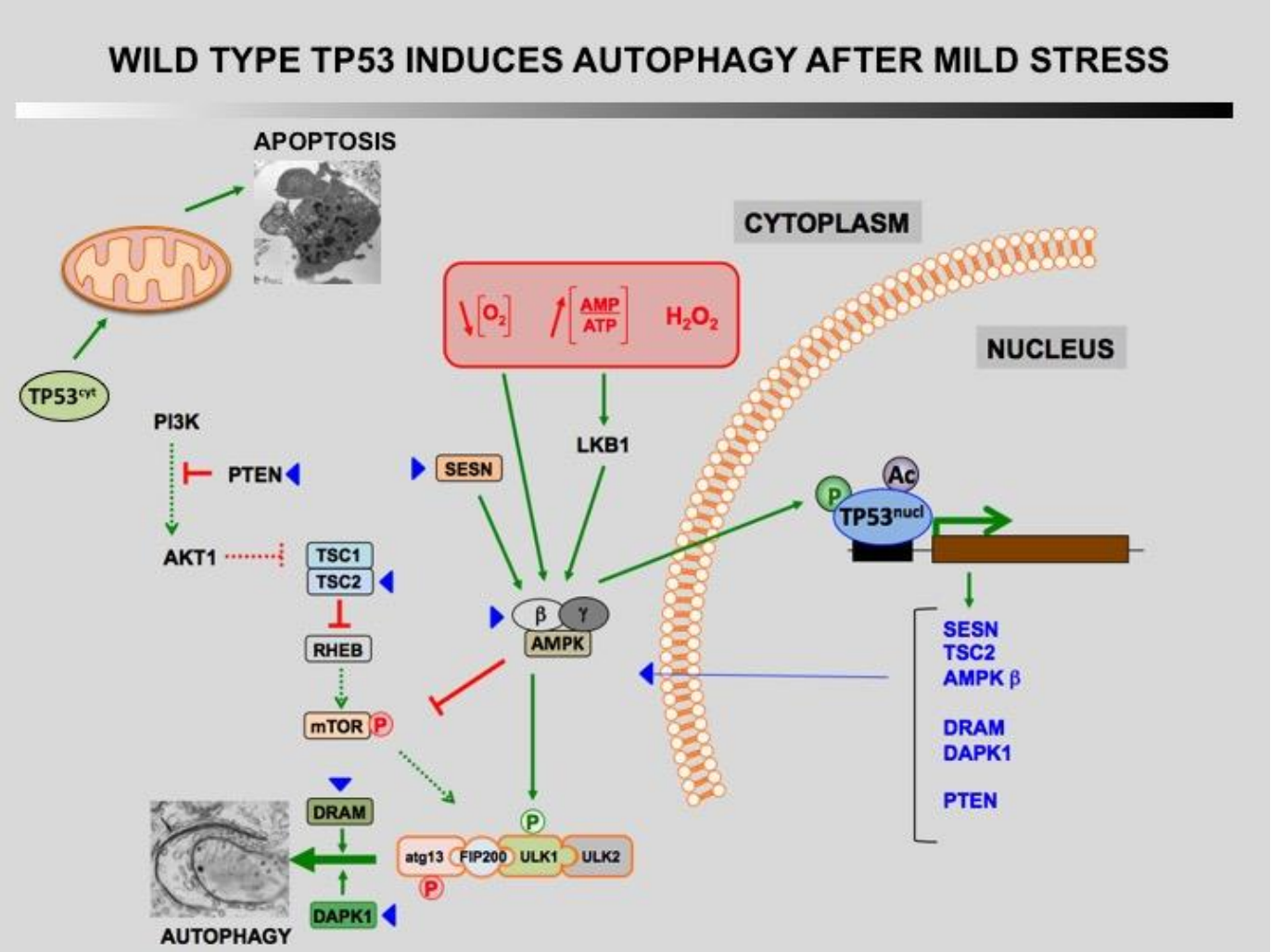

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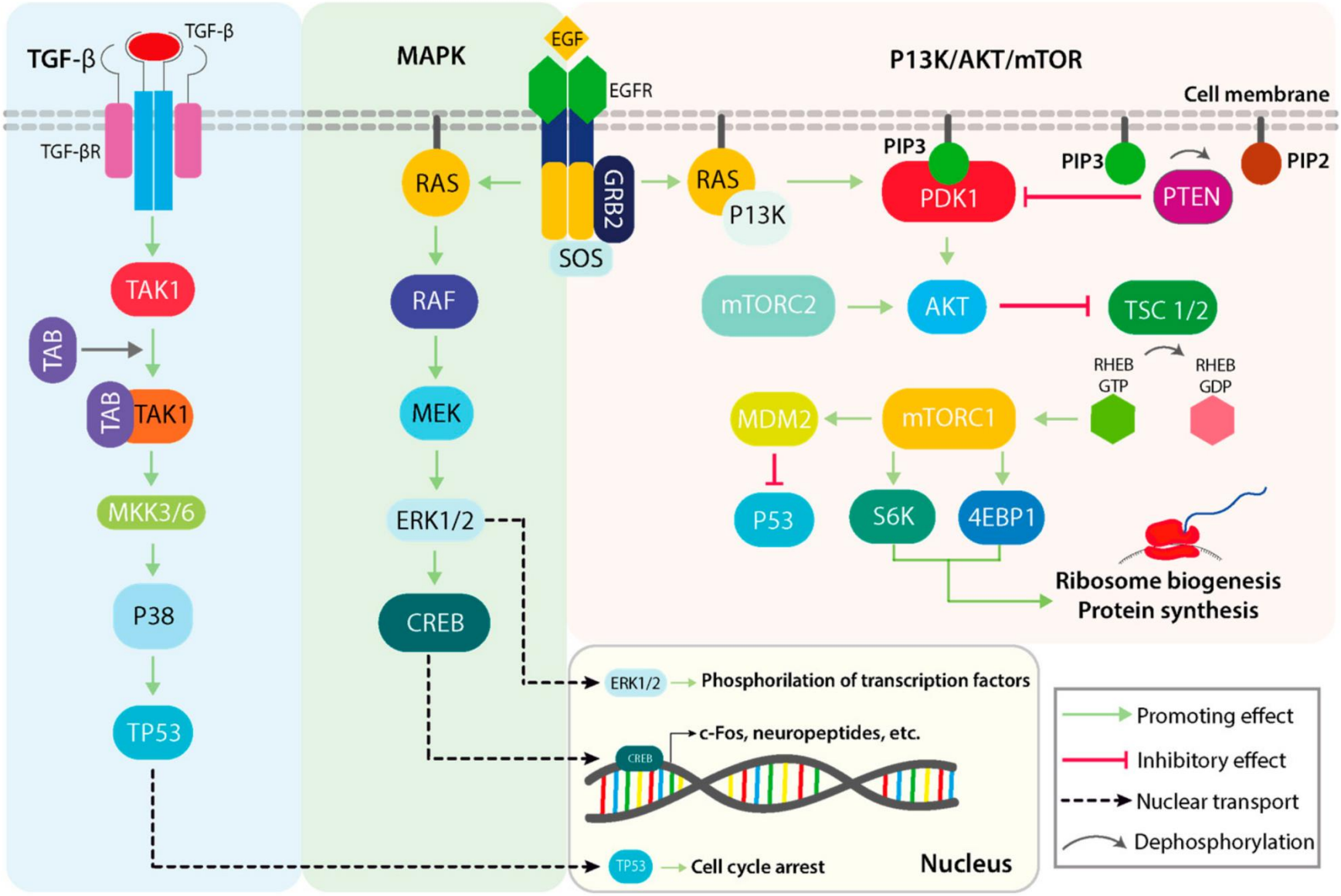
Table 7-7 Selected Tumor Suppressor Genes and Associated Familial Syndromes and Cancers, Sorted by Cancer Hallmarks*

Gene	Protein	Function	Familial Syndromes	Sporadic Cancers
Inhibitors of Mitogenic Signaling Pathways				
APC	Adenomatous polyposis coli protein	Inhibitor of WNT signaling	Familial colonic polyps and carcinomas	Carcinomas of stomach, colon, pancreas; melanoma
NF1	Neurofibromin-1	Inhibitor of RAS/MAPK signaling	Neurofibromatosis type 1 (neurofibromas and malignant peripheral nerve sheath tumors)	Neuroblastoma, juvenile myeloid leukemia
NF2	Merlin	Cytoskeletal stability, Hippo pathway signaling	Neurofibromatosis type 2 (acoustic schwannoma and meningioma)	Schwannoma, meningioma
PTCH	Patched	Inhibitor of Hedgehog signaling	Gorlin syndrome (basal cell carcinoma, medulloblastoma, several benign tumors)	Basal cell carcinoma, medulloblastoma
PTEN	Phosphatase and tensin homologue	Inhibitor of PI3K/AKT signaling	Cowden syndrome (variety of benign skin, GI, and CNS growths; breast, endometrial, and thyroid carcinoma)	Diverse cancers, particularly carcinomas and lymphoid tumors
SMAD2, SMAD4	SMAD2, SMAD4	Component of the TGFβ signaling pathway, repression of MYC and CDK4 expression, inducers of CDK inhibitor expression	Juvenile polyposis	Frequently mutated (along with other components of the TGFβ signaling pathway) in colonic and pancreatic carcinoma
Inhibitors of Cell Cycle Progression				
RB	Retinoblastoma (RB) protein	Inhibitor of G1/S transition during cell cycle progression	Familial retinoblastoma syndrome (retinoblastoma, osteosarcoma, other sarcomas)	Retinoblastoma; osteosarcoma; carcinomas of breast, colon, lung
CDKN2A	p16INK4a and p14ARF	p16: Negative regulator of cyclin-dependent kinases; p14, indirect activator of p53	Familial melanoma	Pancreatic, breast, and esophageal carcinoma, melanoma, certain leukemias
Inhibitors of "Pro-growth" Programs of Metabolism and Angiogenesis				
VHL	Von Hippel Lindau (VHL) protein	Inhibitor of hypoxia-induced transcription factors (e.g., HIF1α)	Von Hippel Lindau syndrome (cerebellar hemangioblastoma, retinal angioma, renal cell carcinoma)	Renal cell carcinoma
STK11	Liver kinase B1 (LKB1) or STK11	Activator of AMPK family of kinases; suppresses cell growth when cell nutrient and energy levels are low	Peutz-Jeghers syndrome (GI polyps, GI cancers, pancreatic carcinoma and other carcinomas)	Diverse carcinomas (5%–28% of cases, depending on type)
SDHB, SDHD	Succinate dehydrogenase complex subunits B and D	TCA cycle, oxidative phosphorylation	Familial paragangliomas, familial pheochromocytoma	Paragangliomas
Inhibitors of Invasion and Metastasis				
CDH1	E-cadherin	Cell adhesion, inhibition of cell motility	Familial gastric cancer	Gastric carcinoma, lobular breast carcinoma
Enablers of Genomic Stability				
TP53	p53 protein	Cell cycle arrest and apoptosis in response to DNA damage	Li-Fraumeni syndrome (diverse cancers)	Most human cancers
DNA Repair Factors				
BRCA1, BRCA2	Breast cancer-1 and breast cancer-2 (BRCA1 and BRCA2)	Repair of double-stranded breaks in DNA	Familial breast and ovarian carcinomas; carcinomas of male breast; chronic lymphocytic leukemia (BRCA2)	Rare
MSH2, MLH1, MSH6	MSH1, MLH1, MSH6	DNA mismatch repair	Hereditary nonpolyposis colon carcinoma	Colonic and endometrial carcinoma
Unknown Mechanisms				
WT1	Wilms tumor-1 (WT1)	Transcription factor	Familial Wilms tumor	Wilms tumor, certain leukemias
MEN1	Menin	Transcription factor	Multiple endocrine neoplasia-1 (MEN1; pituitary, parathyroid, and pancreatic endocrine tumors)	Pituitary, parathyroid, and pancreatic endocrine tumors

*Some tumor suppressors impact multiple cancer phenotypes (e.g., p53 affects cell cycle progression, genomic stability, susceptibility to cell death, and cellular metabolism); only a subset of major effects are given for each tumor suppressor gene listed. TCA, tricarboxylic acid.



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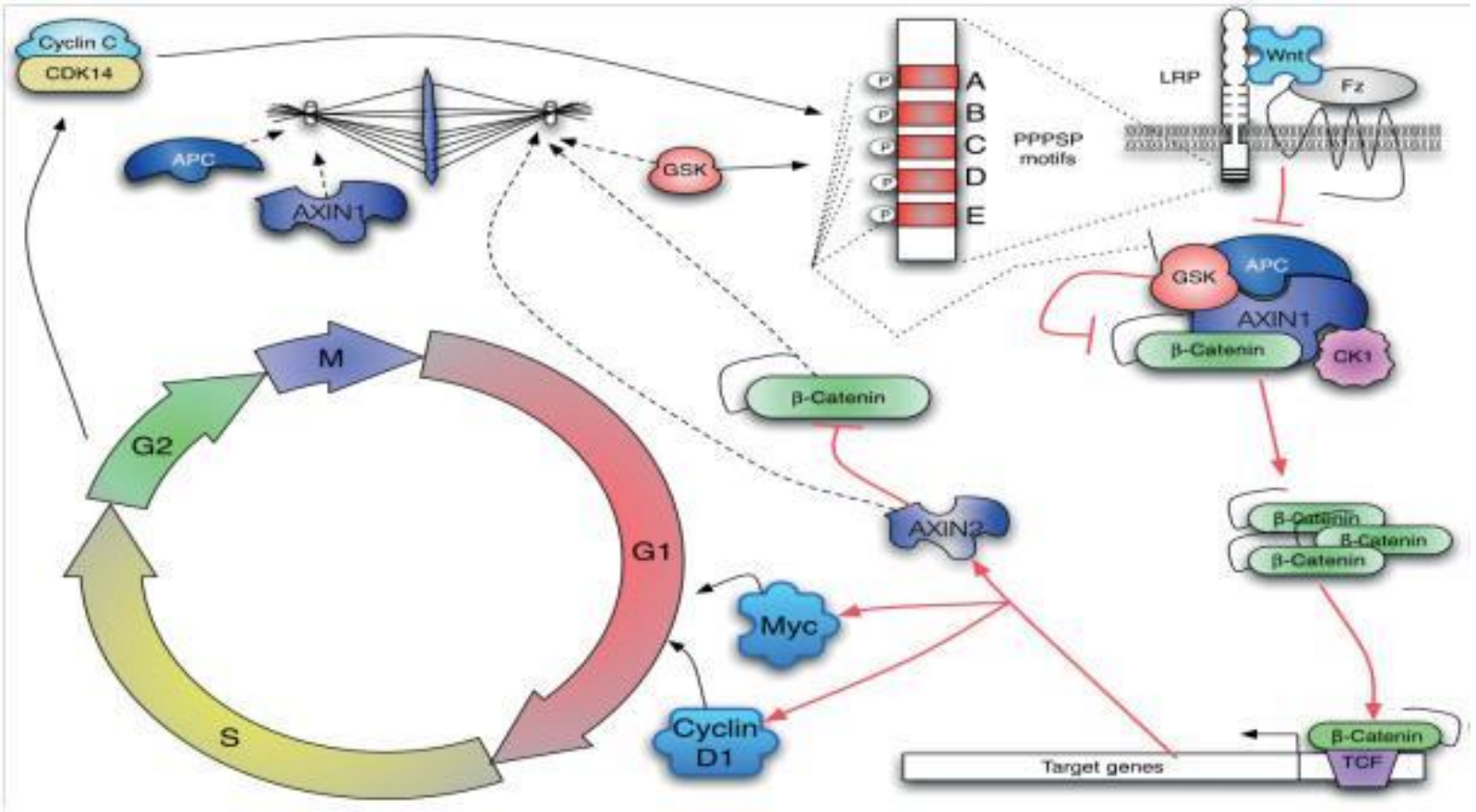

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WNT/β-catenin

- Plays a critical role in embryonic development and maintenance of homeostasis in mature tissues, in particular intestinal epithelial regeneration.
- Secreted extracellular glycoproteins.
- Targets are frizzled (FRZ) and low density lipoprotein receptors (LDLR5 or 6).

WNT/β-catenin

- Binding activates:
- (a) WNT/β-catenin pathway
- (b) Planar cell polarity pathway
- (c) WNT/Ca^{2+} pathway
- (d) WNT/PKA pathway.
- c-MET and β-catenin upregulation associated with epithelial-mesenchymal transformation.

WNT/β-catenin

- The β-catenin destruction complex is composed of a tumor suppressor protein encoded by the APC gene, AIN, CKI, and GSK3.
- In the absence of receptor binding, the destruction complex binds to newly synthesized β-catenin protein
- Rapidly degraded by the ubiquitin–proteasome pathway through phosphorylation of the serine/threonine rich region of β-catenin.
- Stem cell proliferation is promoted.

WNT/β-catenin

- Receptor binding by WNT ligands inactivates the β-catenin destruction complex, resulting in accumulation of β-catenin.
- β-catenin is translocated into the nucleus to form a complex with TCF/LEF, a transcription factor, leading to transcriptional activation of c-MYC and CYCLIN D.
- Stem cell proliferation is blocked.
- PITX2 is a bicoid related transcription factor promoting methylation, induced by β-catenin, and required for cell-type-specific development.

WNT/β-catenin

- Gain of function mutations present in:
- 50% hepatoblastomas
- 20% hepatocellular carcinomas

E-cadherin

- β-catenin binds to the cytoplasmic tail of E-cadherin, a cell surface protein that maintains intercellular adhesiveness. Loss of cell-cell contact, such as in a wound or injury to the epithelium, disrupts the interaction between E-cadherin and β-catenin, and also promotes increased translocation of β-catenin to the nucleus.
- Loss of E-cadherin allows easy disaggregation of cells, promoting invasion and metastasis.
- Usually reduced as a result of activating mutations of β-catenin.

Mismatch repair genes

- Lynch Syndrome
- Hereditary nonpolyposis colorectal cancer
- 1 in 279 persons
- High risk for ovarian and endometrial cancer
- Autosomal dominant.
- Germline defects in hMSH2 (at 2p21), hMLH1 (at 3p21), hPMS1 (at 2p32.2)1, and hPMS2 (at 7p22.1).
- Mutation in type II receptor for TGF-β contributes to loss of growth inhibition.
- EPCAM gene adjacent to MSH2 may inactivate MSH2.

TGF-β

- TGF-β signaling is initiated by binding of TGF-β ligands to type II TGF-β receptors.
- Upon ligand binding, TGFBR2 recruits and phosphorylates TGFBR1, which then phosphorylates two downstream transcription factors, SMAD2 and SMAD3.
- These form a hetero-oligomeric complex with SMAD4 and translate to the nucleus, inhibiting transcription factors c-JUN, c-MYC, as well as promoting CYCLIN associated proteins CYCLIN D1,CDK4, p21, p27, p15, and RB.

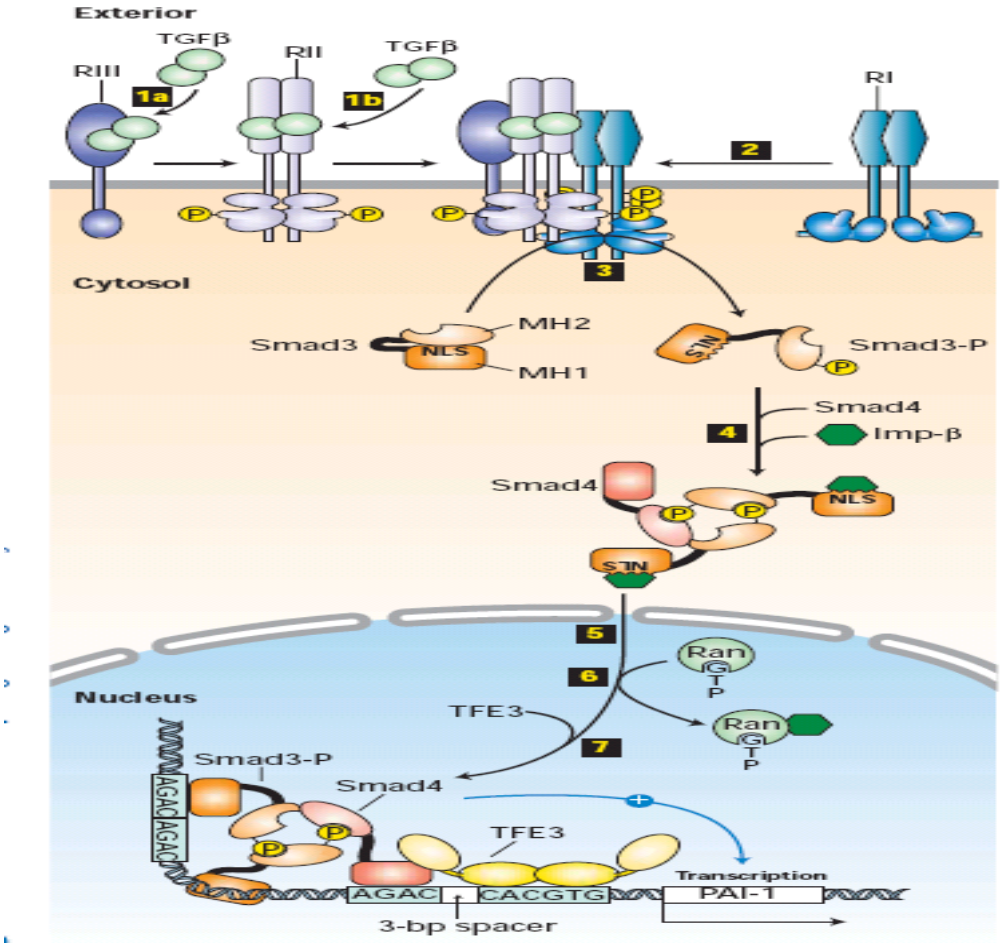
TGF-β

- TGF-β is not simply a tumor suppressor
- Also directly stimulates the production of several mitogenic growth factors, such as TGF-α, fibroblast growth factor, and EGF.
- In addition, SMAD-independent pathways, including RAS/RAF/MAPK, JNK, and PI3K/AKT, can be activated, all of which can drive the carcinogenic process.
- TGF-β has also been shown to promote angiogenesis as well as regulate cell adhesion, motility, and the extracellular matrix.

TGF-β

- Loss of function mutations in TGF-β II receptor lead to over expression of STAT3, over expression of WNT-α and increased HGF, leading to immunosuppression locally.
- Leads to metastasis
- The precise way by which TGF-β can go from a tumor suppressor to a tumor promoter remains to be characterized but no doubt depends on the particular cellular context and milieu.
- Colon, stomach, and endometrial cancers (TGF-β)
- Pancreatic cancers (SMAD)

TGF-β pathway



NOTCH

- The NOTCH signaling pathway plays a critical role in the proliferation of intestinal epithelium.
- Five membrane-bound NOTCH ligands have been identified: Jagged1, Jagged2, Delta-like (DLL) 1, DLL 3, and DLL 4.
- Under physiologic conditions, binding of NOTCH ligands to their cognate transmembrane receptors (NOTCH 1–4) initiates proteolytic cleavage of the receptors by α-secretase and γ-secretase (ADAM/TACE) to release the intracellular domain of the NOTCH receptor

NOTCH

- The cleaved NOTCH receptors (NICD) translocate into the nucleus and form complexes with RBP-jκ (CSL or CBF-1) and induce transcriptional activation of Notch-target genes such as hairy/enhancer of split (Hes1), a basic helix-loop-helix transcription factor, as well as MYC, p21, and TGF-β.
- HH, NOTCH 1 mutations in pancreatic cancer
- NOTCH, WNT mutations in colon cancer.
- NOTCH 1 affects cytosolic estrogen receptor, ERB2
- NOTCH 4 triggers PKC
- NOTCH 3, PETN (inhibits PI3K/AKT pathway).
- DLL-4 is required to initiate angiogenesis.

Hedgehog

- The HH pathway is critical for normal development and patterning of various organs, including the gut epithelium.
- There are three HH homologues in humans: Indian (IHH), Sonic (SHH), and Desert (DHH).
- The receptor for HH ligands is the Patched protein (PTCH), which suppresses the activity of Smoothened (SMOH), a G protein–coupled receptor-like receptor.

Hedgehog

- Binding of HH ligands to PTCH1 activates SMOH mediated activation of GLI transcription factors
- Interaction then regulates the expression of several HH target genes
- CYCLIN D, CYCLIN E, MYC, VEGF, IGF1, HIP.

Hedgehog

- Hedgehog mutations that inactivate PTCH, activate SMOH.
- Germline mutation in basal cell nevus syndrome (Gorlin)
- 20-50% of sporadic basal cell carcinomas (UV related)
- 10-25% of medulloblastoma
- Hedgehog mutations that upregulate ligand expression are found in cancers of the colon and pancreas.
- Regulatory control of the GLI transcription factor (and stem cell renewal) also involves interaction with the polycomb gene BM-1.

Epithelial growth factor receptor

- The human epithelial growth factor receptor family includes:
- HER1 (EGFR, ERBB-1)
- HER2 (ERBB-2)
- HER3 (ERBB-3)
- HER4 (ERBB-4).

Epithelial growth factor receptor

- The natural ligands for EGFR include:
- EGF
- TGF-α
- Amphiregulin and heregulin
- Heparin-binding EGF
- β-cellulin.

Epithelial growth factor receptor

- On ligand binding, the EGFR can either undergo receptor dimerization by binding to a second EGFR molecule
- OR preferentially forms a heterodimer with others members of the HER family, with the greatest affinity to ERBB-2.
- Downstream of EGFR:
- PTSG2 gene (COX-2) at 1q31.1, induced by cytokine; produces arachidonic acid
- Blocked by calcitrol (active vitamin D)
- Inhibitor binds to peroxisome-retinoid X receptor cis-recognition sequence in nucleolus; block transcription

RAS

- RAS is a member of the monomeric small family of guanine nucleotide-binding proteins.
- The RAS superfamily of proteins plays a critical role in transmitting key extracellular signals into intracellular signal transduction cascades.
- Epithelial growth factors
- RAS proteins possess GDP, GTP-binding and intrinsic GTPase activities, allowing them to switch between active (GTP-bound) and inactive (GDP-bound) conformations.
- RAS, MAPK, PI3K/AKT are the three most important downstream signaling arms

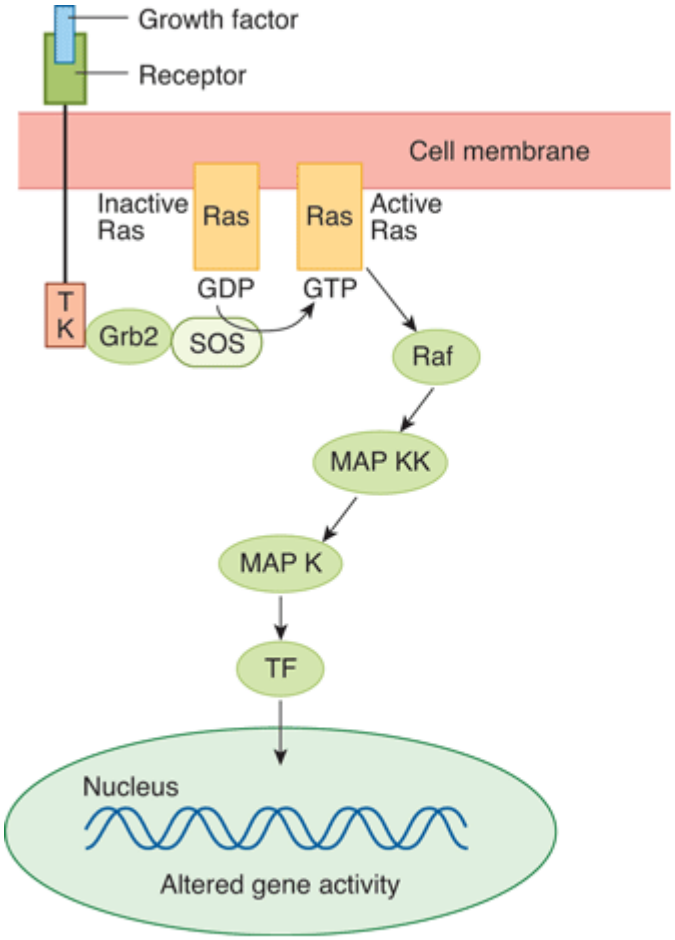
RAS

- The RAS family of proteins contains a CAAX motif in the C-terminus, which serves as a substrate for post-translational lipid modification of its cysteine residue.
- After lipid modification through palmitoylation, RAS proteins are transferred and attached to the plasma membrane through their covalently bound farnesyl and palmitoyl moieties.
- Three RAS genes have been identified (H, N, K) at 5p21.
- Neurofibromin, the protein product of the NF1 gene, contains a GTPase-activating domain that acts as a brake on RAS signaling.

RAS

- RAS proteins activate the RAF/MEK/ERK signaling cascade
- Mediates cell growth and cell cycle entry via phosphorylation of key transcription factors:
- c-FOS and MYC
- Phosphorylation of the RSK (ribosomal protein S6 kinase) and MNK (MAPK-interacting serine/threonine kinase)
- Activation of the PI3K/AKT pathway.
- RAF inhibition overcome by stromal cell production of hepatocyte growth factor (HGF) and MET activation.
- Activates MAPK/AKT by alternate path.

RAS/RAF/MAPK pathway



There is cross-talk between this pathway and the cAMP pathway, as well as cross-talk with the IP_3–DAG pathway.

Xanthine oxidoreductase activates MAPK in apoptosis.

Fig. 2-30 Accessed 08/01/2010

RAS

- K-RAS activates PI_3K
- Activates AKT by PIP_3 to PIP_2,
- Activates PTEN.
- Phospholipid and second messenger activation.
- Activates PLC by PIP_2 to IP_3, leading to Ca^{2+} release
- DAG activates PKC.
- K-RAS
- Activates RAF
- Activates MEK
- Activates ERK
- Activates ELK1

RAS

- GTP cascade.
- K-RAS
- Activates TIAM1
- Activates RAC (utilizing GTP)
- Activates PAK
- GDP regeneration completes cycle
- K-RAS
- Activates RAS (GEF)
- Activates RAS (utilizing GTP)
- Activates TBK1
- GDP regeneration completes cycle

RAS

- Point mutations are found in :
- 90% of pancreatic adenocarcinomas
- 90% of cholangiocarcinomas
- 50% of colon, endometrial, and thyroid cancers
- 30% of myeloid leukemias
- 30% of lung adenocarcinomas

RAS

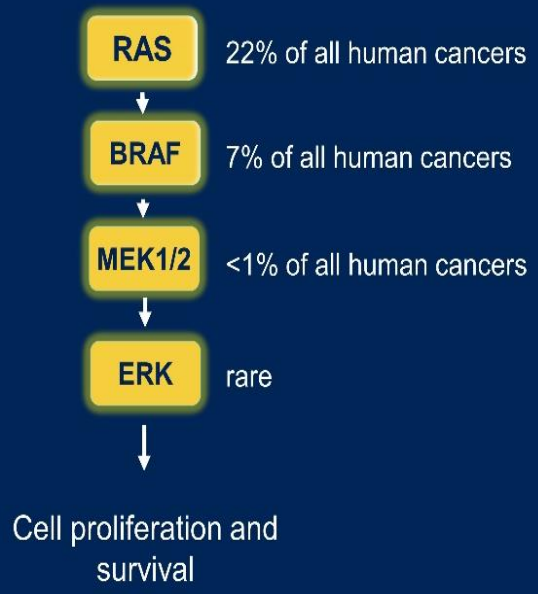
- Patients with K-RAS mutation have a poorer prognosis and are resistant to EGFR-tyrosine kinase inhibitors
- Do not overlap with EFGR, ALK, or ROS1 mutations
- Resistance to RAS G12Ci may be polyclonal and not signal rewiring

Cancers with High Frequency of MAPK Pathway Mutations

RAS
- Hairy cell leukemia
- Melanoma
- Papillary thyroid

BRAF
- Hairy cell leukemia
- Melanoma
- Langerhans Cell Histiocytosis
- Papillary thyroid

MEK1/2
- Colorectal
- Glioma
- Langerhans Cell Histiocytosis
- Ovarian



BRAF

- BRAF (at 7q34) is a serine/threonine protein kinase that sits at the top of a cascade of other serine/threonine kinases of the MAPK family.
- Point mutations found in:
- 100% of hairy cell leukemias
- 80% of benign nevi
- >60% of melanomas
- The V600E mutation is associated with resistance to platinum chemotherapy

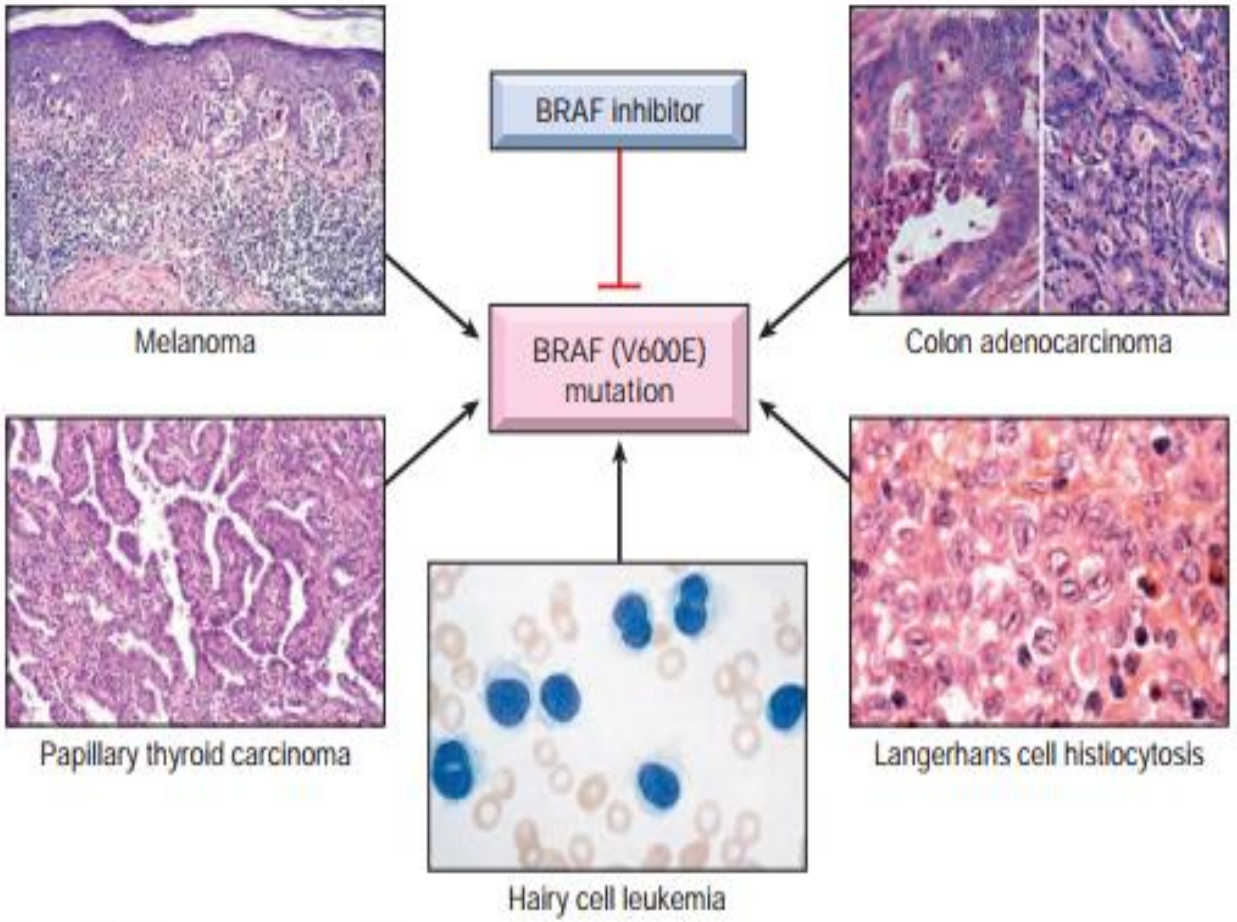


Figure 7-52 Diverse tumor types with a common molecular pathogenesis.

P13/AKT

- The PI3K signaling cascade plays an integral role in regulating several key cellular processes:
- Protein synthesis
- Glucose metabolism
- Cell survival and growth
- Proliferation
- Cell repair
- Cell migration
- Angiogenesis.

P13/AKT

- Signaling is modulated
- Growth factors
- EGF, insulin-like growth factor 1, fibroblast growth factor
- Steroid hormones
- Vitamins
- Integrins
- Intracellular Ca^2
- RAS-dependent MAPK pathway.

P13/AKT

- PIP_3 is activated
- Bind and activate proteins with PH domains (e.g.,PDK1), localizing them to the cell membrane.
- AKT is a known effector of PI3K
- A serine/threonine kinase family
- The mammalian target of rapamycin (mTOR) is a central regulator of G_1 cell cycle protein synthesis that precedes commitment to normal cell cycle proliferation.
- Inhibition deregulates P13/AKT and is anti-proliferative.

P13K/AKT

- Activation of AKT (by PDK1)
- Leads to enhanced cell growth and proliferation through down-regulation of p21 and p27
- Through increased translation and stabilization of CYCLIN D1
- Through activation of the mTOR pathway.
- The process of cell survival is mediated through:
- Inhibition of the pro-apoptotic BAD
- Inhibition of the forkhead transcription factors (FHKR) that activate apoptosis-associated genes
- Activation of NF-κB transcriptional activity.

PI3K/AKT

- AKT1 activity has been shown to enhance the secretion of matrix metalloproteinases and induce epithelial to mesenchymal transition.
- Increased expression of AKT2 appears to play an important role in upregulating the expression of β1 integrins and the process of cellular adhesion and motility.
- AKT also activates caspase 9 and BAD, affecting cell survival.
- Polycomb group proteins inactivate AKT.

SRC kinase

- Family of non-receptor protein-tyrosine kinases
- c-SRC, c-YES, FYN, LYN, LCK, HCK, BLK, FGR, YRK
- Play a central role in cell division, survival, motility, adhesion, invasion, and angiogenesis.
- Activated in response to external cellular signals that promote proliferation, survival, motility, and invasion
- Through activation of cytokine receptors, receptor protein-tyrosine kinases, G protein–coupled receptors, and integrins.
- SRC activation common in hormone responsive breast cancer; likely explains bone metastases.

NF-κB pathway

- NF-κB connects with c-FLIP to FAS (death receptor pathway)
- BCL-2
- NOXA decreases as does MCL-1/BIM/BAK
- Stimulates SMAC
- Inhibits SMAC/cytochrome c release in mitochondrion directly
- As does A1 when activated by NF-κB
-

NF-κB pathway

- XIAP inhibits caspase 9
- IGF-1R stimulates AKT
- IL6 activates MAPK and heat shock protein response
- Disrupts cell cycle progression and protein folding response

mTOR pathways

- PI3K leads to AKT/mTOR and Cyclin D1
- PTEN inhibits AKT
- AKT inhibits p27 (p27 inhibits Cyclin D1), GSK3, BAD
- IGF-IR, VEGFR-1,2,3, EGFR, HER2 activate PI3K/AKT/mTOR
- Lead to growth, angiogenesis, cell division (EGF, TGF-α, neuregulin ligand to EGFR)
- MET activates HER3
- Activates PI3K /AKT/mTOR .
- Resistant to radiation.

mTOR pathways

- AKT phosphorylates and inhibits TSC2
- TSC2 usually forms a heterodimer with TSC1 that inhibits a RAS homologue (Rheb) necessary for mTOR activation
- LKB1 in the cell energy sensing pathway stimulates an AMP activated kinase (AMPK) that activates TSC1-TSC2 heterodimer and inhibits mTOR activation.

mTOR pathways

- Cellular nutrient and energy levels and redox levels influence the PI3K/AKT pathway
- Calorie restriction as well as metformin use upregulate AMPK and also function as radiosensitizers.

mTOR pathways

- Downstream mTOR targets include:
- c-MYC, Cyclin D1, ELF2 protein kinase.
- HIF-1, VEGF, and eukaryocytic initiation factor 4A, B, and G
- mTORC2 involved in cytoskeletal organization and actin remodeling
- Also activates AKT
- mTORC1 involved in cell growth and proliferation;

MYC

- The MYC proto-oncogene belongs to the immediate early response genes, which are rapidly and transiently induced by RAS/MAPK signaling following growth factor stimulation of quiescent cells.
- Virtually all pathways that regulate growth impinge on MYC through one or more of these mechanisms.
- Several SNPs/SNVs that are strongly linked to an elevated risk of cancers, such as prostate and ovarian carcinoma, fall within a large region devoid of recognizable genes that lies next to MYC at 8q24.21.

Table 1

Selected Myc target genes with relevance to metabolic activity of transformed cells[27, 100]

Target Gene	Regulation	Pathway	Functional Relevance
Cyclin A2, Cyclin D2, Cyclin E1	Up	Growth Factor Response	Response to mitogenic stimuli
Enolase, LDH-A	Up	Glycolysis	Metabolic Transformation
Serine hydroxymethyl transferase	Up	C1 Metabolism	Anaplerosis
EIF4E, Ribosomal Proteins L3, L6, S15A	Up	Translation Initiation	Global increase in translation
Ornithine Decarboxylase, prothymosin-α, HMG1/Y	Up	Transformation	Anchorage-independent growth
Iron-regulatory protein-2 H-ferritin, transferrin receptor	Up	Iron metabolism	Required for Myc-induced proliferation
Nucleolin, NM23, Nucleophosmin	Up	Cellular proliferation	Required for RNA and DNA synthesis
$p21^{CIP1}$	Down	DNA damage response	Differentiation
$p15^{INK4B}$	Down	TGFβ pathway	Resistance to growth arrest
N-cadherin, Integrins	Down	Cell adhesion	Metastatic Potential

Miller, DM, Thomas, SD, Islam, A, Muench, D, Sedoris, K, “c-Myc and Cancer Metabolism,” Clin Cancer Res. 2012 Oct 15; 18(20): 5546–5553. doi: 10.1158/1078-0432.CCR-12-0977

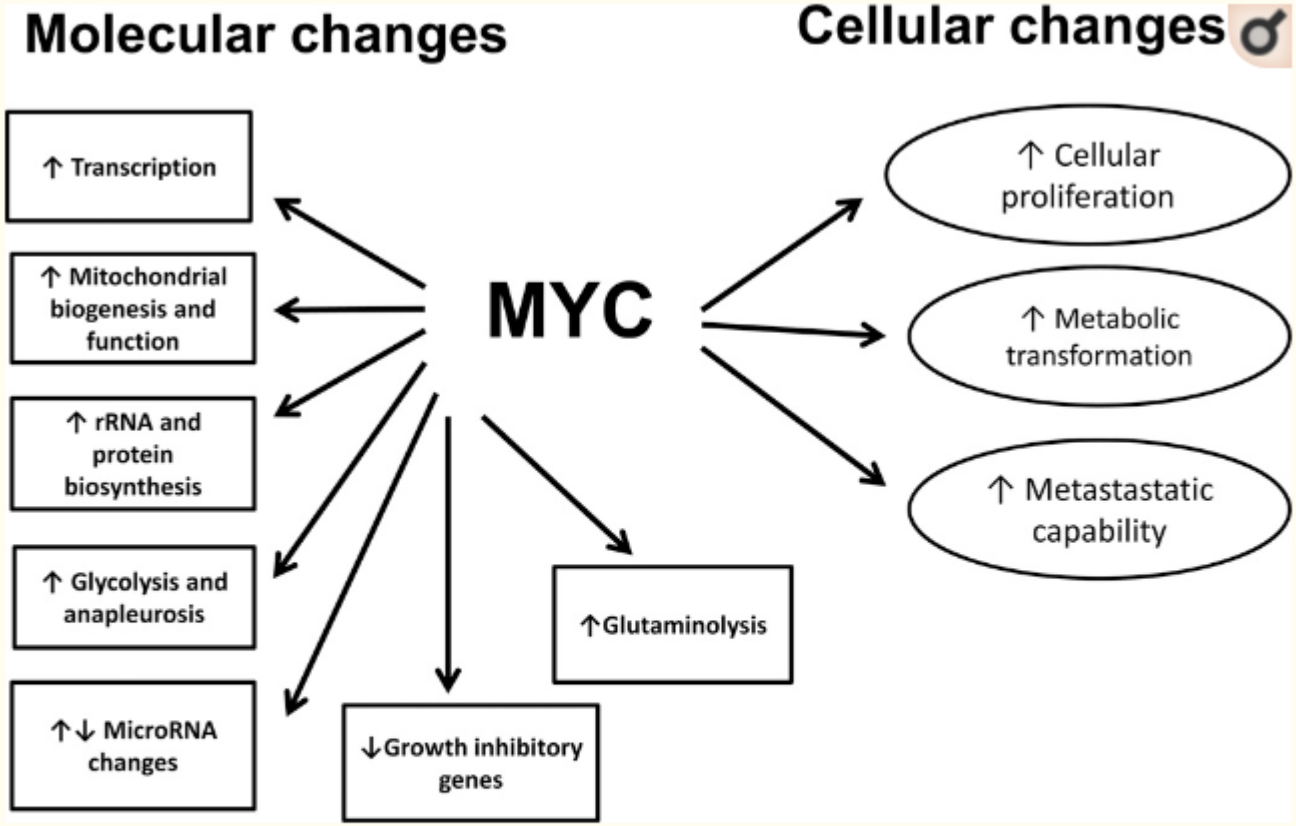


Figure 2

Pleiotropic Effects of c-Myc Expression. The c-myc gene has a variety of molecular and cellular effects (which are closely related). These effects result from myc-mediated changes in large gene families which drive cellular functions. Microarray studies have shown that these changes occur in concert and have major effects on cellular function.

Miller, DM, Thomas, SD, Islam, A, Muench, D, Sedoris, K, "c-Myc and Cancer Metabolism," Clin Cancer Res. 2012 Oct 15; 18(20): 5546–5553. doi: 10.1158/1078-0432.CCR-12-0977

MYC

- MYC target genes directly involved in cell cycle progression include cyclin D:
- MYC also upregulates the expression of rRNA genes and rRNA processing, enhancing the assembly of ribosomes needed for protein synthesis.
- MYC upregulates a program of gene expression that leads to metabolic reprogramming and the Warburg Effect

MYC

- Multiple glycolytic enzymes and factors involved in glutamine metabolism which contribute to the generation of metabolic intermediates that are needed for synthesis of macromolecules are activated
- Rapidly growing tumors frequently have MYC mutations.
- Burkitt's lymphoma (translocation)
- MYC also upregulates expression of telomerase
- MYC is required to reprogram somatic cells into pluripotent stem cells

MYC

- MYC is amplified in many breast, colon, and lung carcinomas.
- The functionally identical N-MYC and L-MYC genes are also amplified in 25% of neuroblastomas and small cell cancers of the lung, respectively.
- Constitutive RAS/MAPK signaling (many cancers), Notch signaling (several hematologic cancers), Wnt signaling (colon carcinoma), and Hedgehog signaling (medulloblastoma) all transform cells in part through upregulation of MYC.

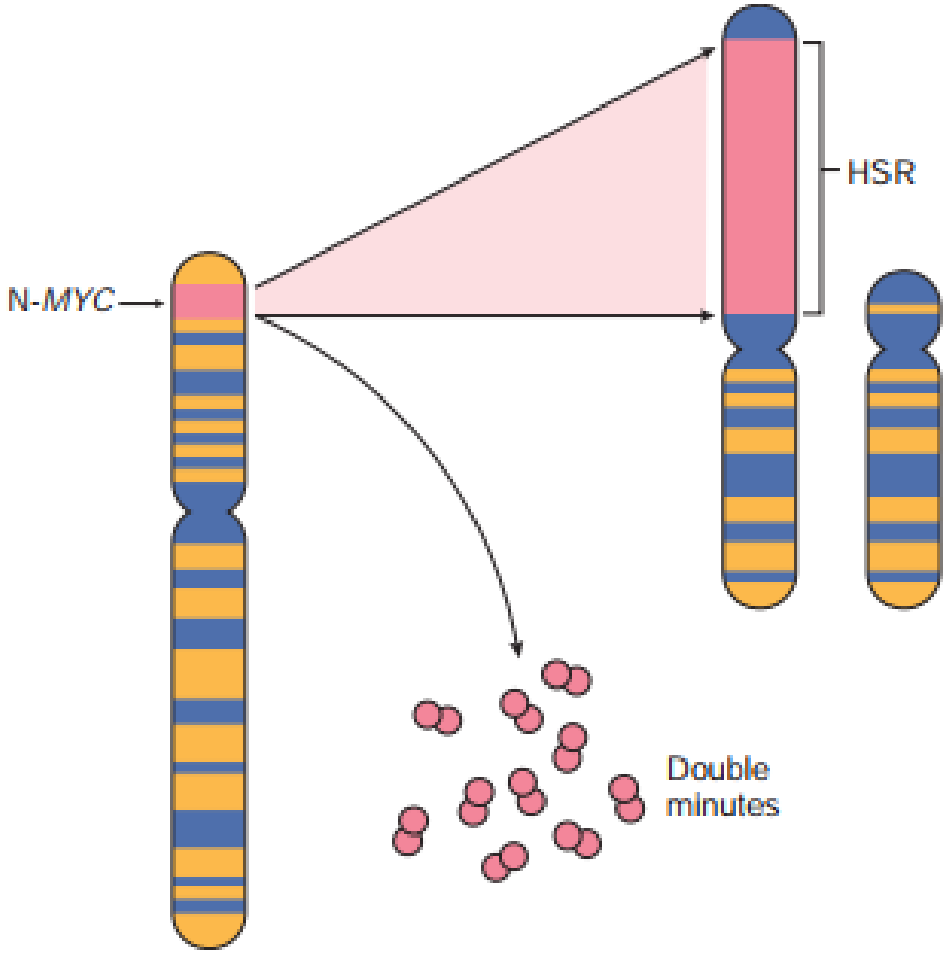


Figure 7-27 Amplification of the *NMYC* gene in human neuroblastomas. The *NMYC* gene, normally present on chromosome 2p, becomes amplified and is seen either as extra chromosomal double minutes or as a chromosomally integrated, homogeneous staining region (HSR). The integration involves other autosomes, such as 4, 9, or 13. (Modified from Brodeur GM: Molecular correlates of cytogenetic abnormalities in human cancer cells: implications for oncogene activation. In Brown EB (ed): Progress in Hematology, Vol 14. Orlando, FL, Grune & Stratton, 1986, p 229-256.)

Other gene expression

- Mild down-regulation of tumor suppressor gene may also lead to neoplasia (PETN).
- PETN acts as brake on P13K/AKT and RAS pathways.
- VHL gene over-expression leads to increased transcription of hypoxia inducible factor (HIF) 1α which leads to increase in transcription of VEGF, PDGF.
- HIF1 activates PKM2
- In tumor cells, upregulates HIF1.
- May explain why tumor cells preferentially undergo aerobic glycolysis (Warburg effect)

Other gene expression

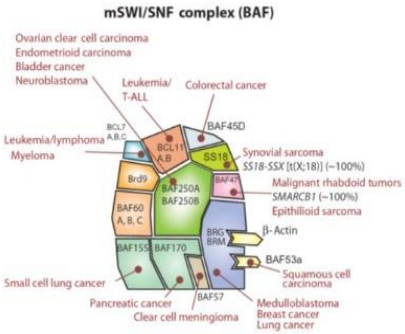
- VEGF induces FLK1 (and neuronal attraction)
- VEGF and FGF create an angiogenic gradient that stimulates the proliferation of endothelial cells and guides the growth of new vessels toward the tumor.
- VEGF also increases the expression of ligands that activate the Notch signaling pathway
- Which regulates the branching and density of the new vessels.
- VEGF1 motility
- VEGF2 proliferation
- VEGF3 lymphangiogenesis.

Other gene expression

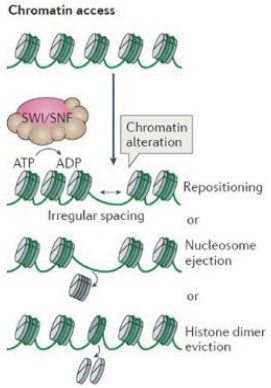
- 85% GIST have kit mutation; 10% GDFR.
- As in NF1, BRAF transcription leads to over expression of IGF1R.
- Mantle cell lymphoma (t11,14) CYCLIN D1 adjacent to heavy chain gene
- Activates CYCLIN D1.
- FLT3 R internal tandem duplications found in 35% acute myelogenous leukemia.
- Polycomb group proteins repress transcription of c-MYC and BMI-1.

SWI/SNF (BAF) Complex

- Multi-protein chromatin remodeling complex
- Mediates nucleosomal repositioning and ejection
- Employs ATP hydrolysis to disrupt histone-DNA contacts
- ATPase subunits include SMARCA2 (BRM) and SMARCA4 (BRG1)
- SWI/SNF complex is mutated in upwards of 20% of cancers

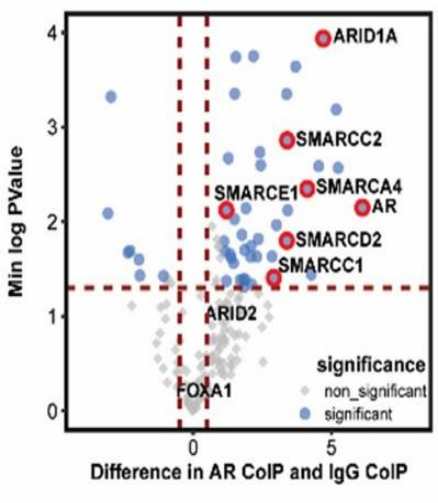


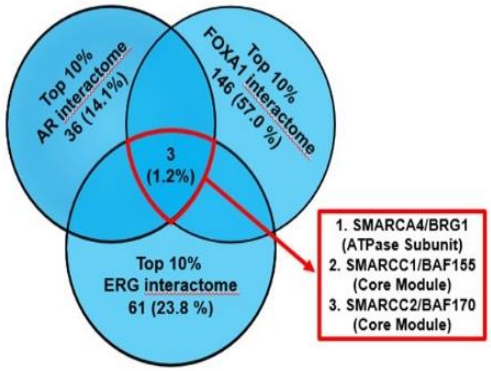
Kadoch and Crabtree, *Science Advances* 2015



Calpier et al, Nature Reviews Molecular Cell Biology, 2017

Meta-analysis of AR, FOXA1 and ERG interactomes revealed SWI/SNF as a common chromatin co-factor





- AR-interacting proteins identified by RIME (S Stelloo, et al. *Oncogene* 2017)
- FOXA1-interacting proteins identified by IP-Mass Spec (in-house)
- ERG-interacting proteins identified by SILAC (Gabriel J. Sandoval, et al. *Mol Cell* 2018)

Selected important proto-oncogenes

Functional category	Oncogene	Mechanism	Types of cancer
Transcription (nuclear regulatory proteins)	**c-MYC** **n-MYC** **l-MYC**	**translocation** **amplification** **amplification**	**Burkitt's lymphoma** **Neuroblastoma** **Small cell lung cancer**
Growth factors			
	PDGFB	**over expression**	**Astrocytoma, Osteosarcoma**
	TGFA	**over expression**	**Astrocytoma, Liver**
	HGF	**over expression**	**Thyroid**
Fibroblast	**FGF3**	**amplification**	**Bladder, Breast, Melanoma**
	HST1	**over expression**	**Stomach**

Selected important proto-oncogenes

Functional category	Oncogene	Mechanism	Types of cancer
Growth factor receptor	ERB-B (HER2/neu)	over expression	Non-small cell lung cancer, head and neck cancer, and gliobastoma (ERB1); Breast and Ovary, other adenocarcinomas (ERB2)
	FLT3	amplification point mutation	Breast, Ovary
	RET	point mutation	CML MEN 2, medullary carcinoma of thyroid (familial)
	PDGFRB	over expression translocation	Glioma
		point mutation	CML
	c-KIT	point mutation	GIST, GIST, Seminoma, Leukemia

Selected important proto-oncogenes

Functional category	Oncogene	Mechanism	Types of cancer
Signal transduction	SRC		Sarcoma
GTP binding	k-RAS	point mutation	Colon, Non-small cell lung cancer, Pancreas
	h-RAS	point mutation	Bladder, Kidney
	n-RAS	point mutation	Leukemia
Non-receptor tyrosine kinase	ABL	translocation	CML, ALL
	JAK2	point mutation	Polycythemia vera, Myelofibrosis
RAS/RAF/MAPK	BRAF	point mutation	Melanoma
WNT	β-catenin	point mutation	Hepatoblastoma, hepatocellular cancer

Selected important proto-oncogenes

Functional category	Oncogene	Mechanism	Types of cancer
Cell cycle control	**BCL-1,** **CYCLIN D1** **CYCLIN E**	**translocation** **amplification** **over expression**	**Mantle cell lymphoma** **Breast, Esophagous** **Breast**
Cyclin-dependent kinase	**CDK4**	**translocation** **over expression** **amplification**	**Breast** **Glioblastoma, Melanoma**
	CDKN2a	**point mutation**	**Melanoma, Pancreas, Glioblastoma, ALL, Esophagous**
Apoptosis block (cytochrome c affected)	**BCL-2**	**over expression**	**B-cell Lymphoma**

Epigenetic changes

- A cancer cell's lineage is generated by epigenetic modifications that produce a pattern of gene expression that characterizes that particular cell type.
- Lineage restricted cancer genes only act within epigenetic contexts in which key oncogenic targets are controlled by these genes.

Epigenetic changes

- Epigenetic changes include:
- Histone modifications catalyzed by enzymes associated with chromatin regulatory complexes
- DNA methylation
- DNA methyltransferases
- CDKN2A silenced by local hyper-methylation of DNA
- Looping of enhancer elements onto gene promoters
- T-cell leukemia

Table 7-9 Examples of Epigenomic Regulatory Genes that are Mutated in Cancer

Gene(s)	Function	Tumor (Approximate Frequency of Mutation)
DNMT3A	DNA methylation	Acute myeloid leukemia (20%)
MLL1	Histone methylation	Acute leukemia in infants (90%)
MLL2	Histone methylation	Follicular lymphoma (90%)
CREBBP/EP300	Histone acetylation	Diffuse large B cell lymphoma (40%)
ARID1A	Nucleosome positioning/chromatin remodeling	Ovarian clear cell carcinoma (60%), endometrial carcinoma (30%–40%)
SNF5	Nucleosome positioning/chromatin remodeling	Malignant rhabdoid tumor (100%)
PBRM1	Nucleosome positioning/chromatin remodeling	Renal carcinoma (30%)

microRNA

- MicroRNAs (miRNAs) are small regulatory RNAs produced by DICER proteins that regulate gene expression in development and adaptive responses to the environment.
- DICER (14q32.2) is a gene that encodes an endonuclease that is required for the processing and production of functional mIRs.
- The degree of base pairing between a miRNA and its target messenger RNA seems to determine whether the regulation occurs through cleavage or translation inhibition

microRNA

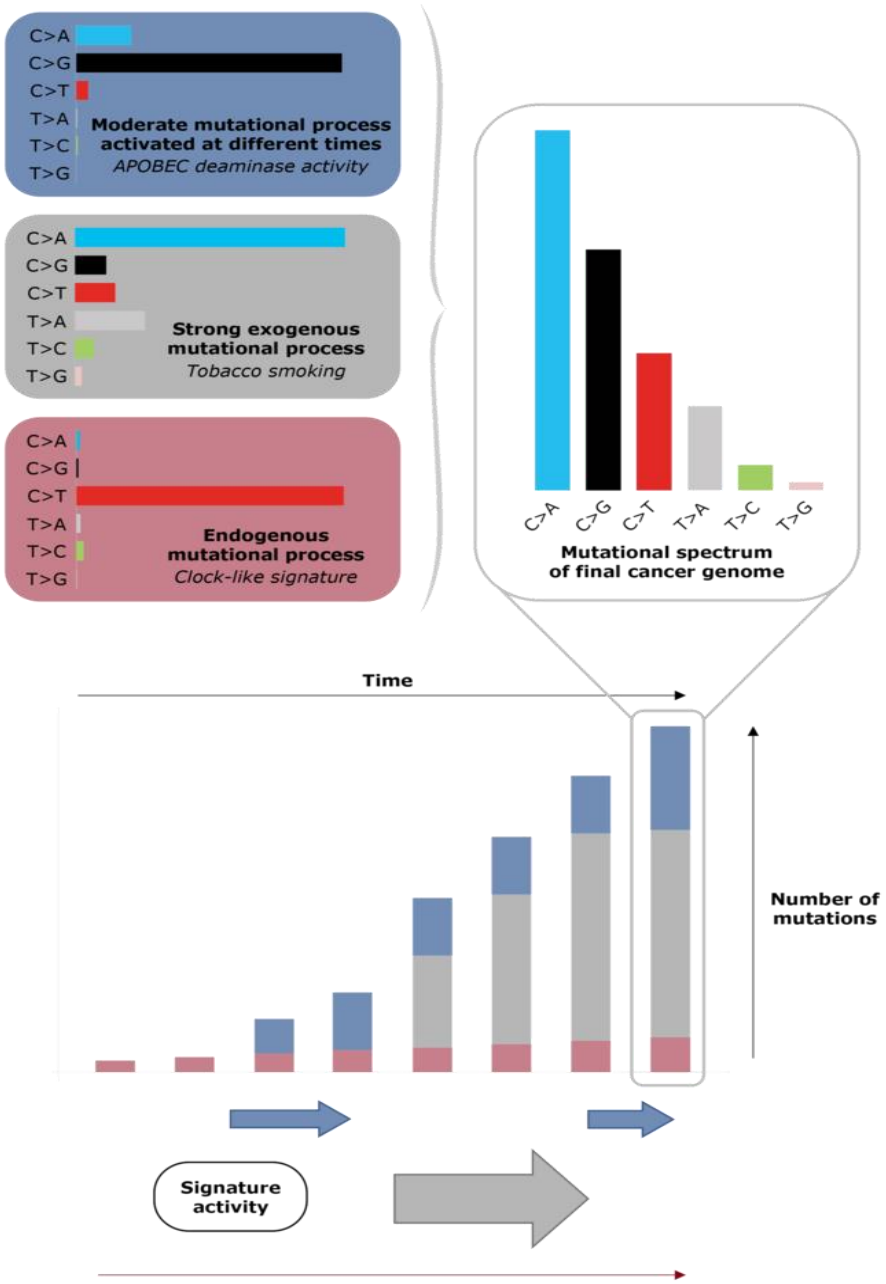
- miRs mediate sequence-specific inhibition of messenger RNA (mRNA) translation through the action of the RNA-induced silencing complex (RISC).
- miR-200 promotes epithelial-mesenchymal transitions
- miR-155 is overexpressed in many human B cell lymphomas and indirectly upregulates a large number of genes that promote proliferation, including MYC.

microRNA

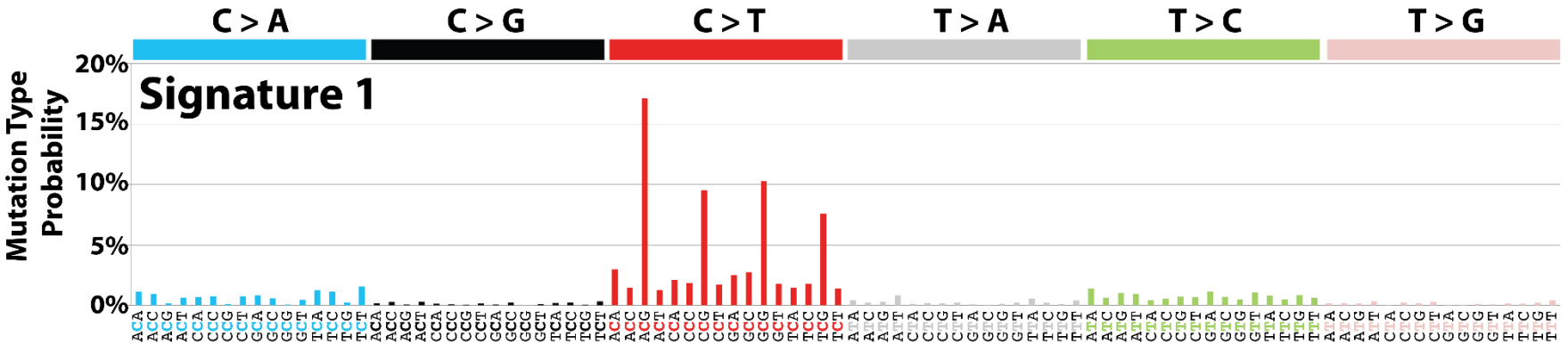
- Deletions of miR-15 and miR-16, are among the most frequent genetic lesions in chronic lymphocytic leukemia
- Their loss leads to upregulation of the anti-apoptotic protein BCL-2.
- Other DICER abnormalities are noted in the pleuropulmonary blastoma family predisposition syndrome

Other non-coding RNAs

- Noncoding RNAs fall into several classes:
- piwi-interacting RNAs (piRNAs), the most common type of small noncoding RNA, which (like miRs) are believed to have a role in post-transcriptional gene silencing
- snoRNAs, which are important in maturation of rRNA and the assembly of ribosomes
- Long intervening noncoding RNAs (lincRNAs), some of which regulate the activity of chromatin “writers,” the factors that modify histones and thereby control gene expression

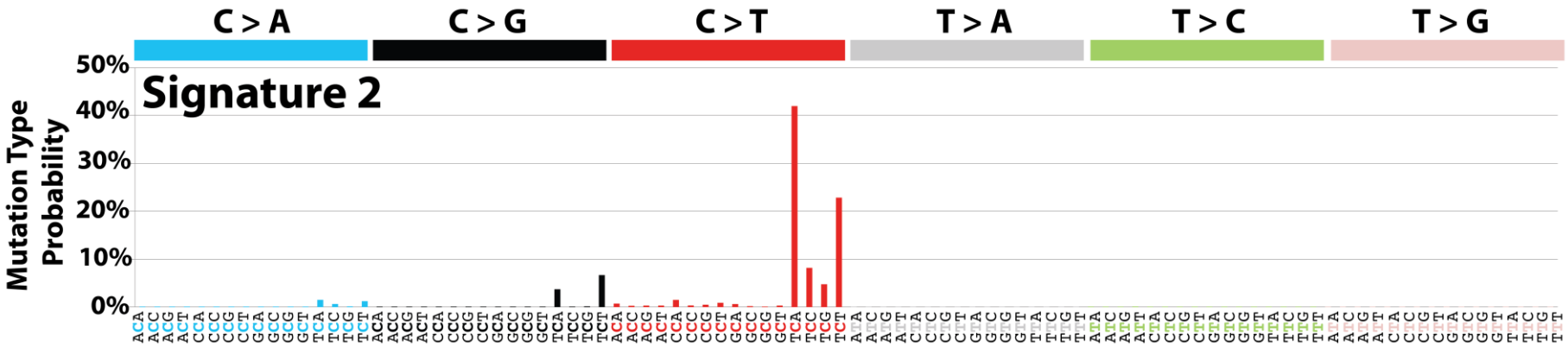


https://cancer.sanger.ac.uk/cosmic/signatures_v2.tt



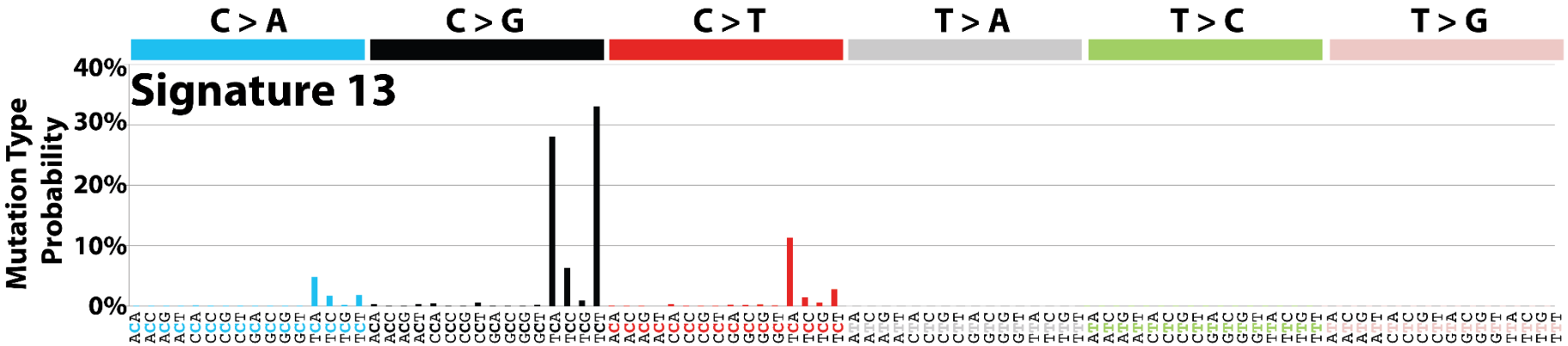
Cancer types:
Signature 1 has been found in all cancer types and in most cancer samples.
Proposed etiology:
Signature 1 is the result of an endogenous mutational process initiated by spontaneous deamination of 5-methylcytosine.
Additional mutational features:
Signature 1 is associated with small numbers of small insertions and deletions in most tissue types.
Comments:
The number of Signature 1 mutations correlates with age of cancer diagnosis.

https://cancer.sanger.ac.uk/cosmic/signatures_v2.tt



Cancer types:
Signature 2 is most commonly found in cervical and bladder cancers.
Proposed etiology:
Signature 2 has been attributed to activity of the AID/APOBEC family of cytidine deaminases.
Additional mutational features:
Transcriptional strand bias of mutations has been observed in exons, but is not present or is weaker in introns.
Comments:
Activation of AID/APOBEC cytidine deaminases is due to viral infection, retrotransposon jumping or to tissue inflammation.

https://cancer.sanger.ac.uk/cosmic/signatures_v2.tt



Cancer types:

Signature 13 is most commonly found in cervical and bladder cancers.

Proposed etiology:

Signature 13 has been attributed to activity of the AID/APOBEC family of cytidine deaminases.

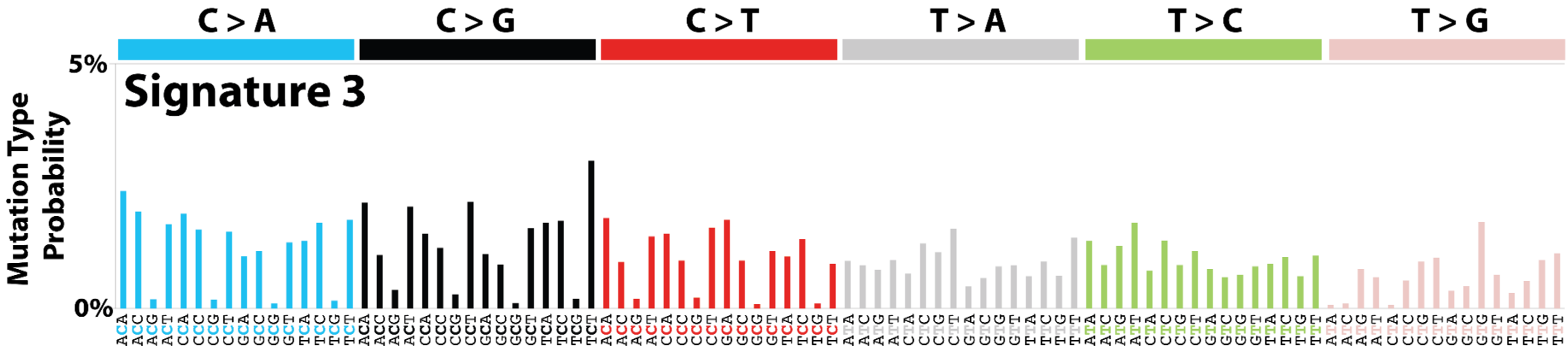
Additional mutational features:

Transcriptional strand bias of mutations has been observed in exons, but is not present or is weaker in introns.

Comments:

Activation of AID/APOBEC cytidine deaminases is due to viral infection, retrotransposon jumping or to tissue inflammation.

https://cancer.sanger.ac.uk/cosmic/signatures_v2.tt



Cancer types:

Signature 3 has been found in breast, ovarian, and pancreatic cancers.

Proposed etiology:

Signature 3 is associated with failure of DNA double-strand break-repair by homologous recombination.

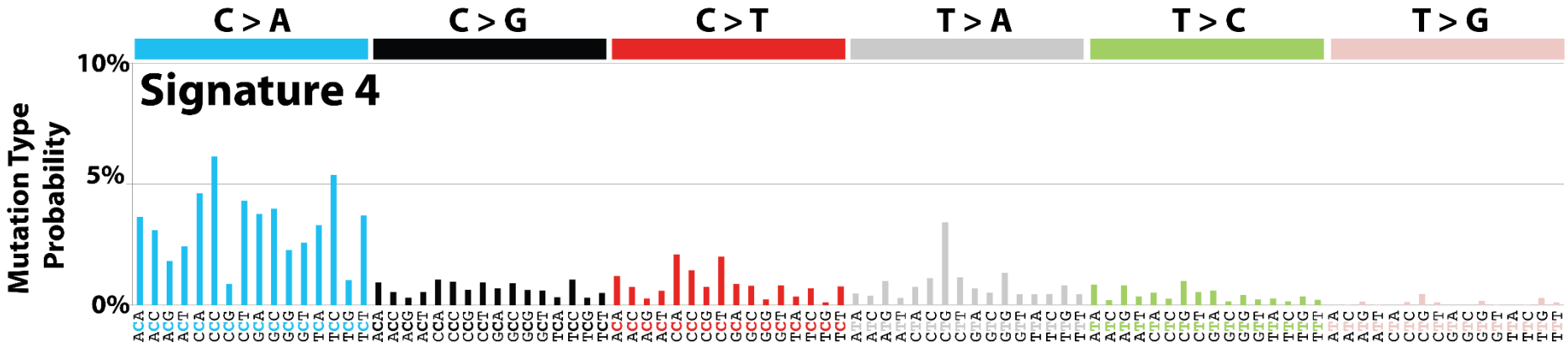
Additional mutational features:

Signature 3 associates strongly with elevated numbers of large (longer than 3bp) insertions and deletions with overlapping microhomology at breakpoint junctions.

Comments:

Signature 3 is strongly associated with germline and somatic BRCA1 and BRCA2 mutations in breast, pancreatic, and ovarian cancers. In pancreatic cancer, responders to platinum therapy usually exhibit Signature 3 mutations.

https://cancer.sanger.ac.uk/cosmic/signatures_v2.tt



Cancer types:

Signature 4 has been found in head and neck cancer, liver cancer, lung adenocarcinoma, lung squamous carcinoma, small cell lung carcinoma, and esophageal cancer.

Proposed etiology and comment:

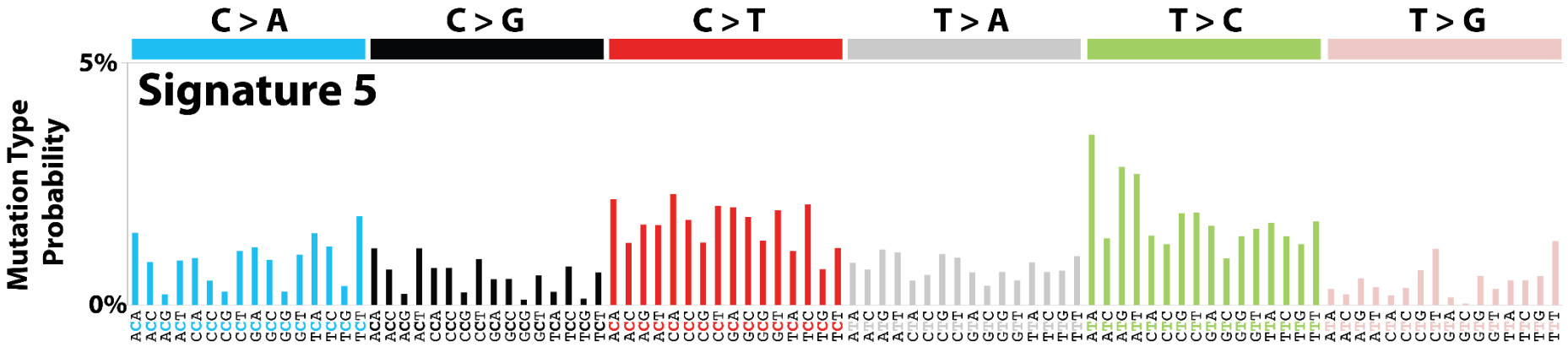
Signature 4 is associated with smoking and its profile is similar to the mutational pattern observed in experimental systems exposed to tobacco carcinogens (e.g., benzo[a]pyrene).

Not seen in tobacco chewers.

Additional mutational features:

Signature 4 exhibits transcriptional strand bias for C>A mutations, compatible with the notion that damage to guanine is repaired by transcription-coupled nucleotide excision repair. Signature 4 is also associated with CC>AA dinucleotide substitutions.

https://cancer.sanger.ac.uk/cosmic/signatures_v2.tt



Cancer types:

Signature 5 has been found in all cancers.

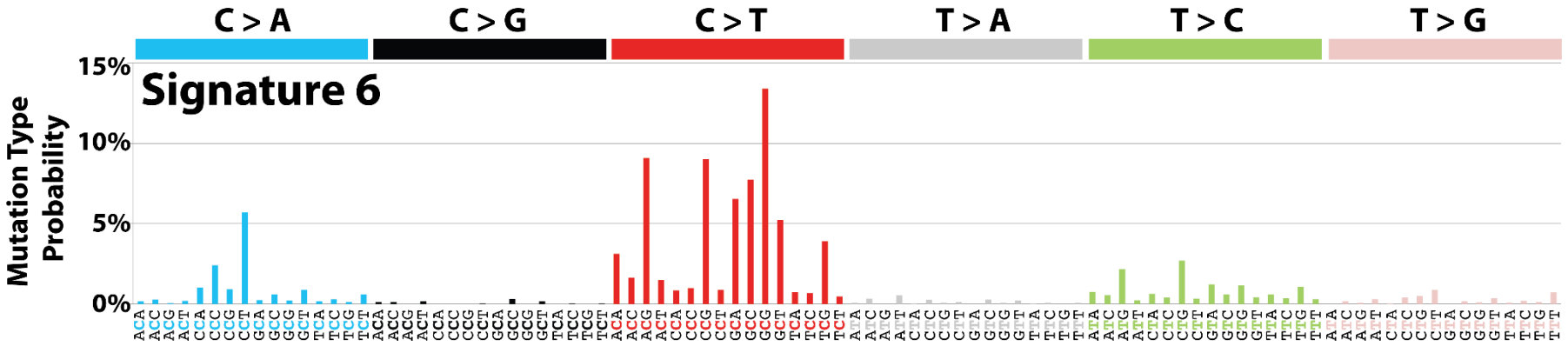
Proposed etiology:

Unknown

Additional mutational features:

Signature 5 exhibits transcriptional strand bias for T>C substitutions at ApTpN context.

https://cancer.sanger.ac.uk/cosmic/signatures_v2.tt



Cancer types:

Signature 6 is most common in colorectal and uterine cancers.

Proposed etiology:

Signature 6 is associated with defective DNA mismatch repair and is found in microsatellite unstable tumors.

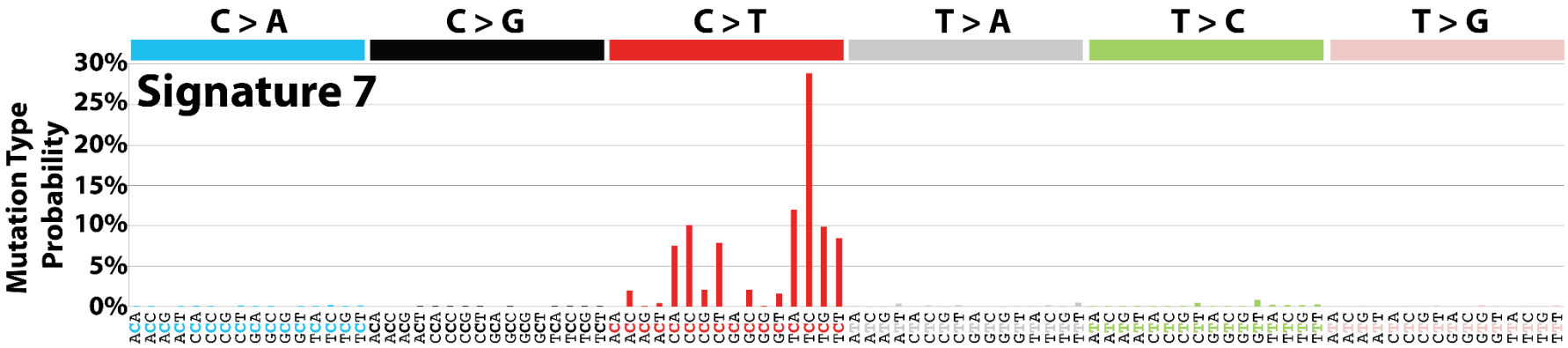
Additional mutational features:

Signature 6 is associated with high numbers of small (shorter than 3bp) insertions and deletions at mono/polynucleotide repeats.

Comments:

Signature 6 is one of four mutational signatures associated with defective DNA mismatch repair and is often found in the same samples as Signatures 15, 20, and 26

https://cancer.sanger.ac.uk/cosmic/signatures_v2.tt



Cancer types:

Signature 7 has been found predominantly in skin cancers and in cancers of the lip categorized as head and neck or oral squamous cancers.

Proposed etiology:

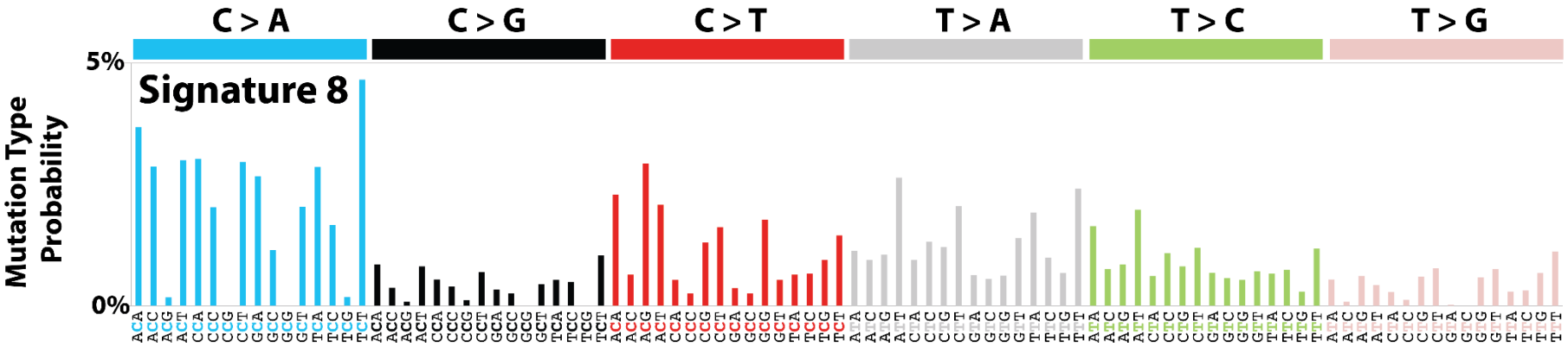
Signature 7 is likely due to ultraviolet light exposure.

Additional mutational features:

Signature 7 is associated with large numbers of CC>TT dinucleotide mutations at dipyrimidines.

Additionally, Signature 7 exhibits a strong transcriptional strand-bias indicating that mutations occur at pyrimidines (viz., by formation of pyrimidine-pyrimidine photodimers) and these mutations are being repaired by transcription-coupled nucleotide excision repair.

https://cancer.sanger.ac.uk/cosmic/signatures_v2.tt



Cancer types:

Signature 8 has been found in breast cancer and medulloblastoma.

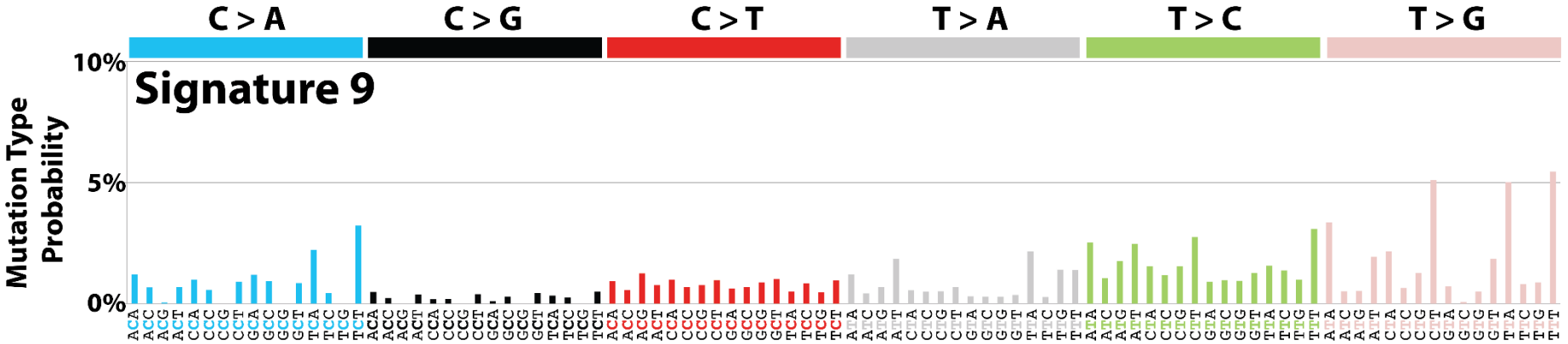
Proposed etiology:

Unknown.

Additional mutational features:

Signature 8 exhibits weak strand bias for C>A substitutions and is associated with double nucleotide substitutions, notably CC>AA

https://cancer.sanger.ac.uk/cosmic/signatures_v2.tt



Cancer types:

Signature 9 has been found in chronic lymphocytic leukaemias and malignant B-cell lymphomas.

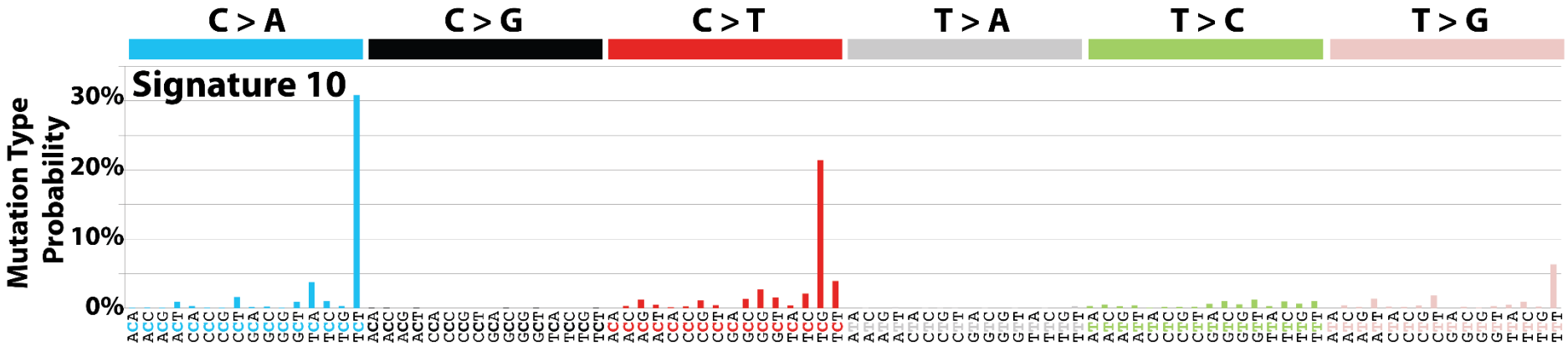
Proposed etiology:

Signature 9 is characterized by a pattern of mutations that has been attributed to polymerase η, which is implicated with the activity of AID during somatic hypermutation.

Comments:

Chronic lymphocytic leukaemias that possess immunoglobulin gene hypermutation (IGHV-mutated) have elevated numbers of mutations attributed to Signature 9 compared to those that do not have immunoglobulin gene hypermutation.

https://cancer.sanger.ac.uk/cosmic/signatures_v2.tt



Cancer types:

Signature 10 has been found most notably in colorectal and uterine cancer, usually generating huge numbers of mutations in small subsets of samples.

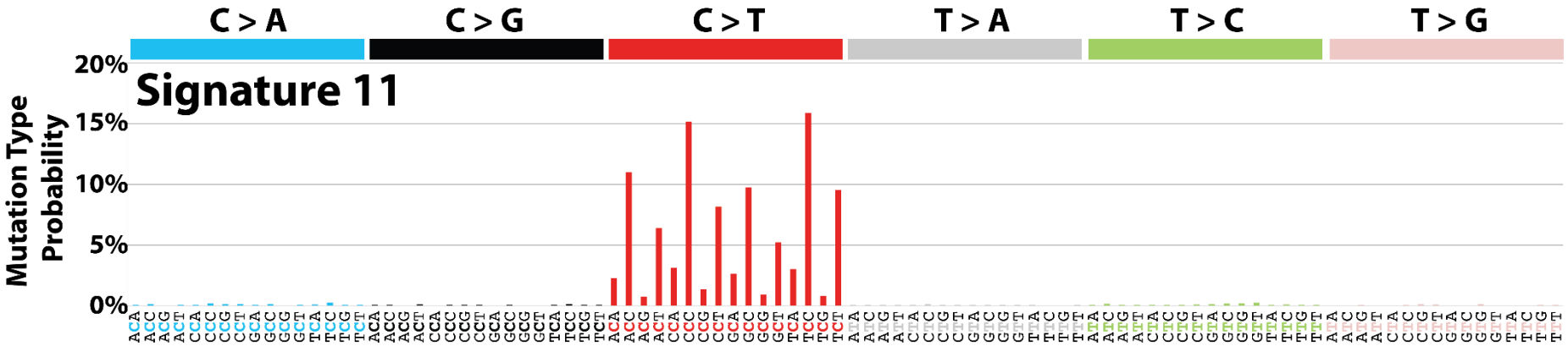
Proposed etiology:

It has been proposed that the mutational process underlying this signature is altered activity of the error-prone polymerase POLE. The presence of large numbers of Signature 10 mutations is associated with recurrent POLE somatic mutations, viz., Pro286Arg and Val411Leu.

Additional mutational features:

Signature 10 exhibits strand bias for C>A mutations at TpCpT context and T>G mutations at TpTpT context.

https://cancer.sanger.ac.uk/cosmic/signatures_v2.tt



Cancer types:

Signature 11 has been found in melanoma and glioblastoma.

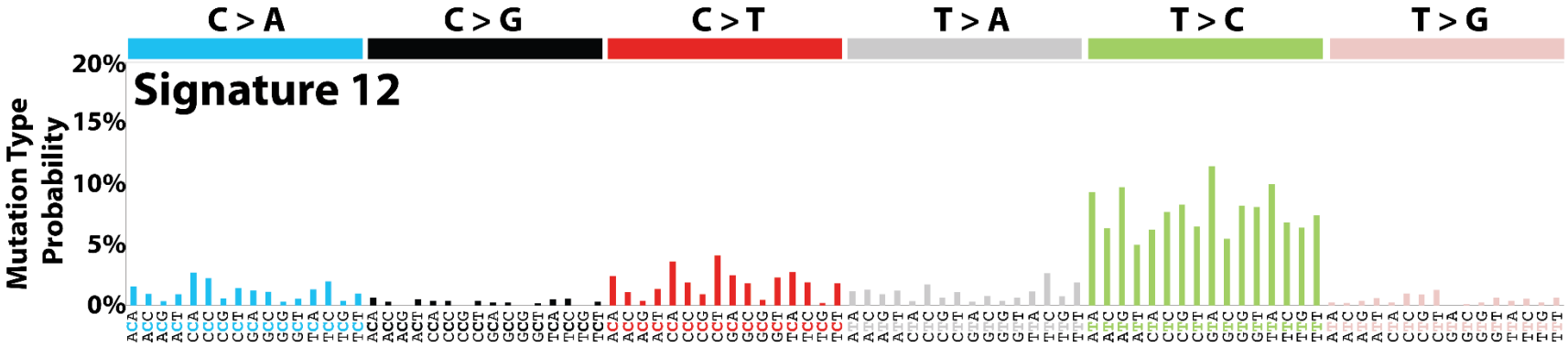
Proposed etiology:

Signature 11 exhibits a mutational pattern resembling that of alkylating agents, particularly temozolomide

.**Additional mutational features:**

Signature 11 exhibits a strong transcriptional strand-bias for C>T substitutions indicating that mutations occur on guanine and that these mutations are effectively repaired by transcription-coupled nucleotide excision repair.

https://cancer.sanger.ac.uk/cosmic/signatures_v2.tt



Cancer types:

Signature 12 has been found in liver cancer.

Proposed etiology:

Unknown.

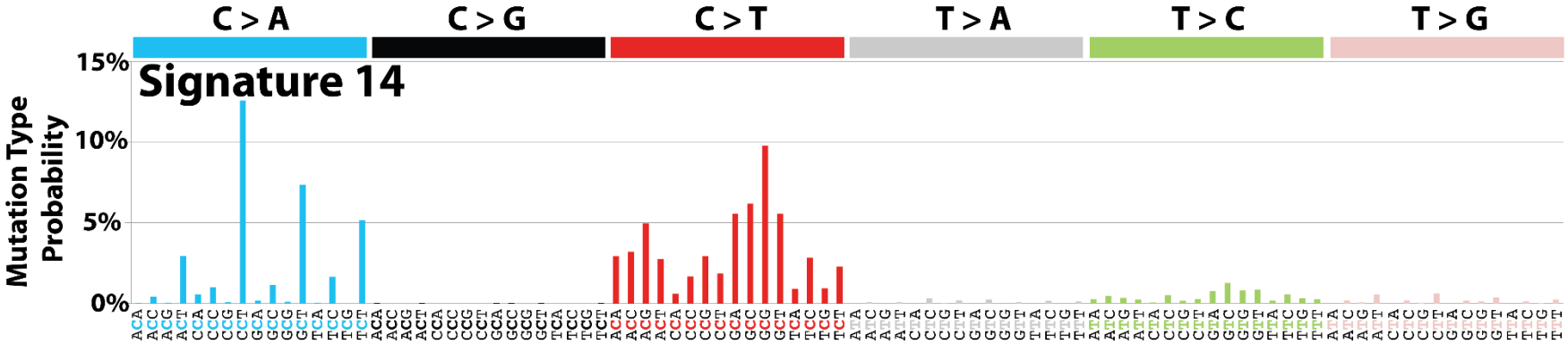
Additional mutational features:

Signature 12 exhibits a strong transcriptional strand-bias for T>C substitutions.

Comments:

Signature 12 usually contributes a small percentage (<20%) of the mutations observed in a liver cancer sample.

https://cancer.sanger.ac.uk/cosmic/signatures_v2.tt



Cancer types:

Signature 14 has been found in uterine cancer.

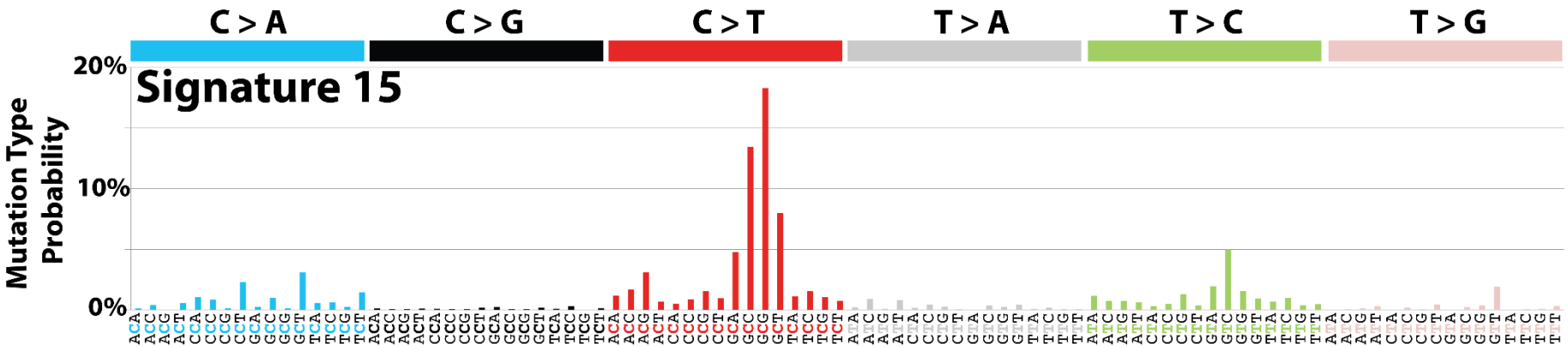
Proposed etiology:

Unknown.

Comments:

Signature 14 generates very high numbers of somatic mutations (>200 mutations per MB) in all samples in which it has been observed

https://cancer.sanger.ac.uk/cosmic/signatures_v2.tt



Cancer types:

Signature 15 has been found in several stomach cancers and a single small cell lung carcinoma.

Proposed etiology:

Signature 15 is associated with defective DNA mismatch repair.

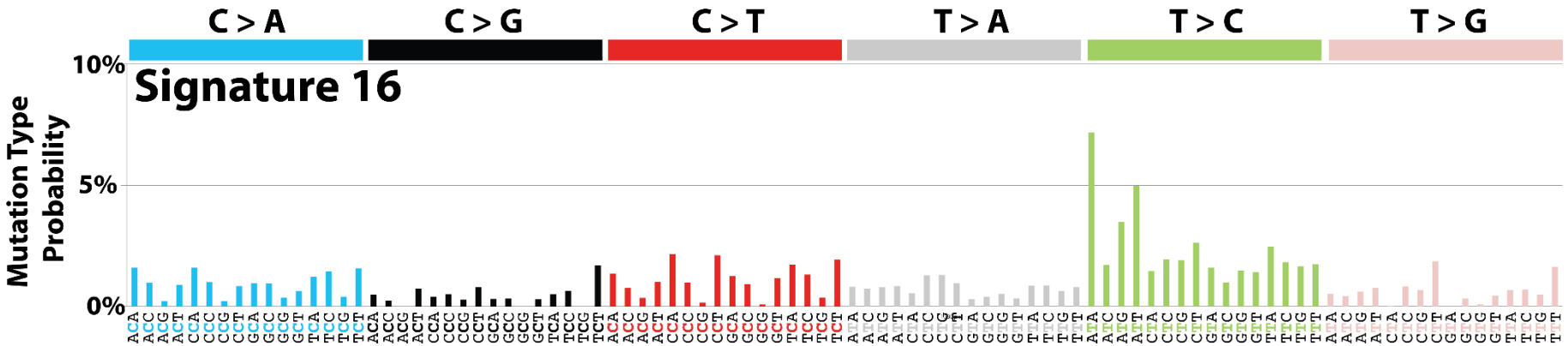
Additional mutational features:

Signature 15 is associated with high numbers of small (shorter than 3bp) insertions and deletions at mono/polynucleotide repeats.

Comments:

Signature 15 is one of four mutational signatures associated with defective DNA mismatch repair and is often found in the same samples as Signatures 6, 20, and 26.

https://cancer.sanger.ac.uk/cosmic/signatures_v2.tt



Cancer types:

Signature 16 has been found in liver cancer.

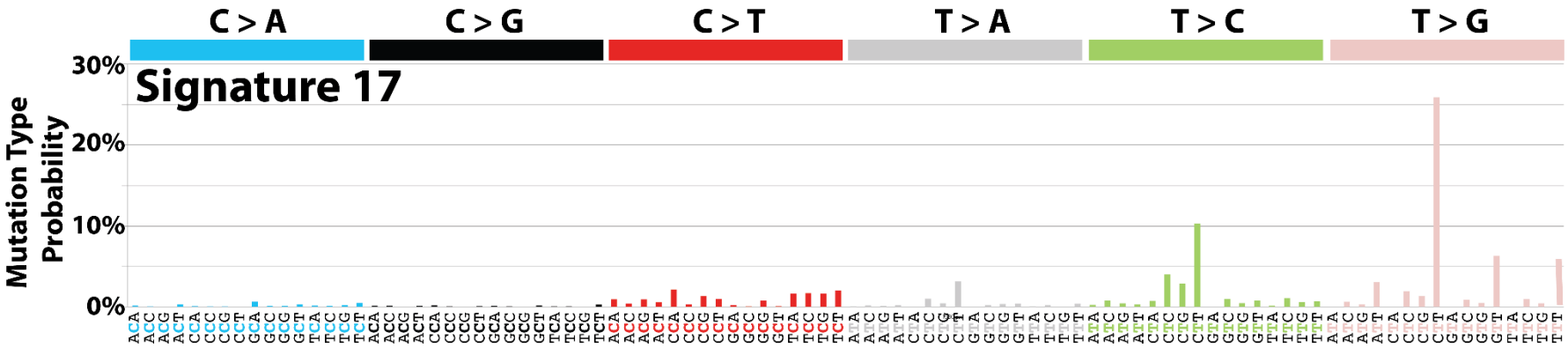
Proposed etiology:

Unknown.

Additional mutational features:

Signature 16 exhibits an extremely strong transcriptional strand bias for T>C mutations at ApTpN context, with T>C mutations occurring almost exclusively on the transcribed strand.

https://cancer.sanger.ac.uk/cosmic/signatures_v2.tt



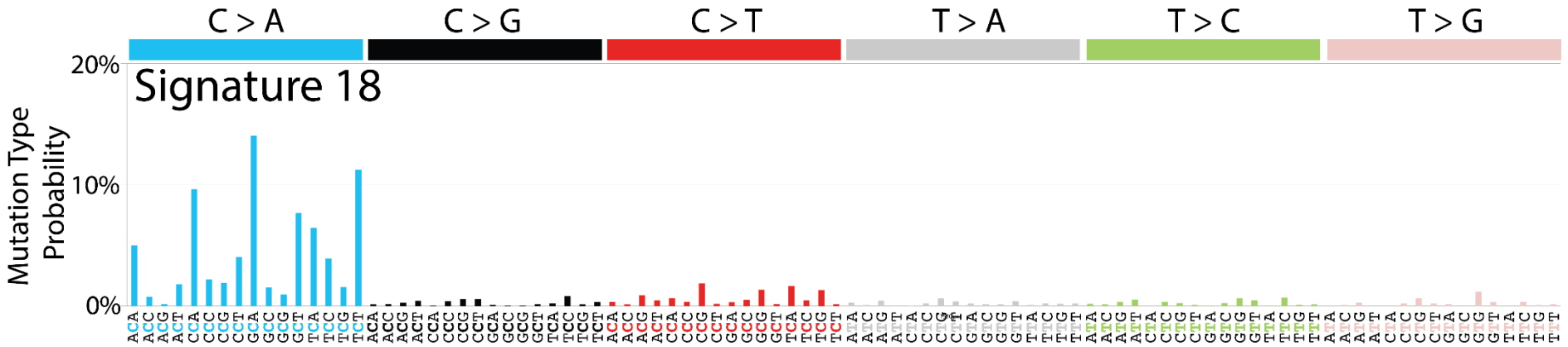
Cancer types:

Signature 17 has been found in esophagus cancer, breast cancer, liver cancer, lung adenocarcinoma, B-cell lymphoma, stomach cancer and melanoma.

Proposed etiology:

Unknown.

https://cancer.sanger.ac.uk/cosmic/signatures_v2.tt



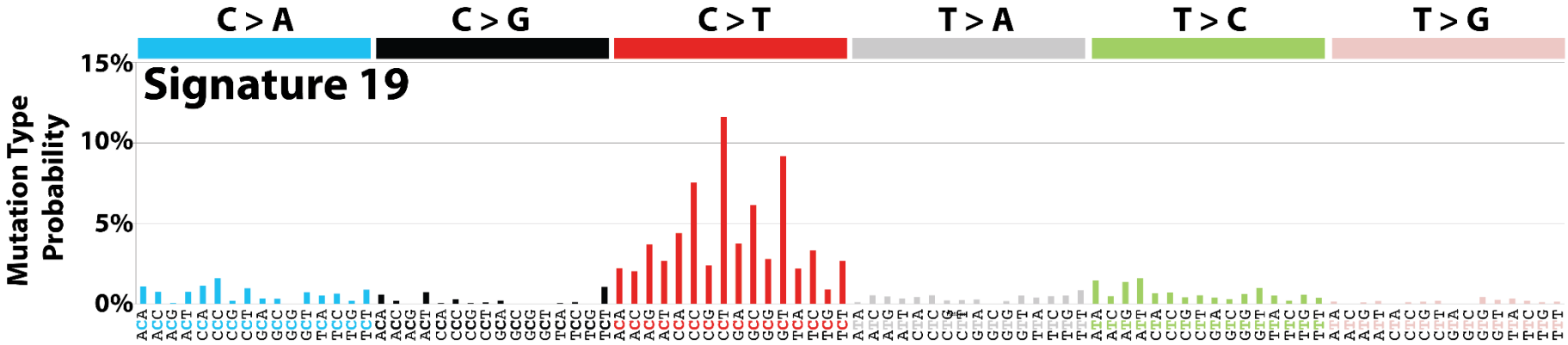
Cancer types:

Signature 18 has been found commonly in neuroblastoma.

Proposed etiology:

Unknown

https://cancer.sanger.ac.uk/cosmic/signatures_v2.tt



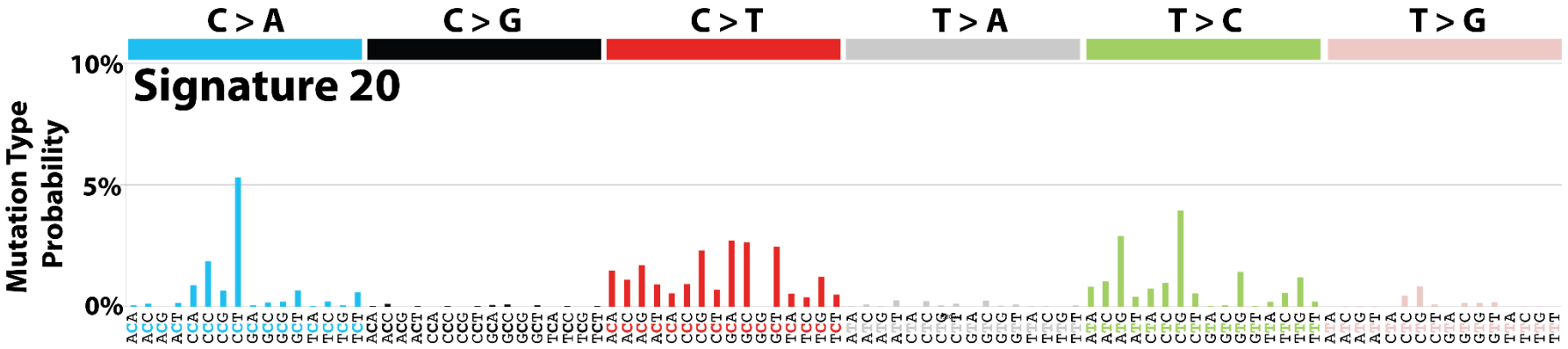
Cancer types:

Signature 19 has been found only in pilocystic astrocytoma.

Proposed etiology:

Unknown

https://cancer.sanger.ac.uk/cosmic/signatures_v2.tt



Cancer types:

Signature 20 has been found in stomach and breast cancers.

Proposed etiology:

Signature 20 is believed to be associated with defective DNA mismatch repair.

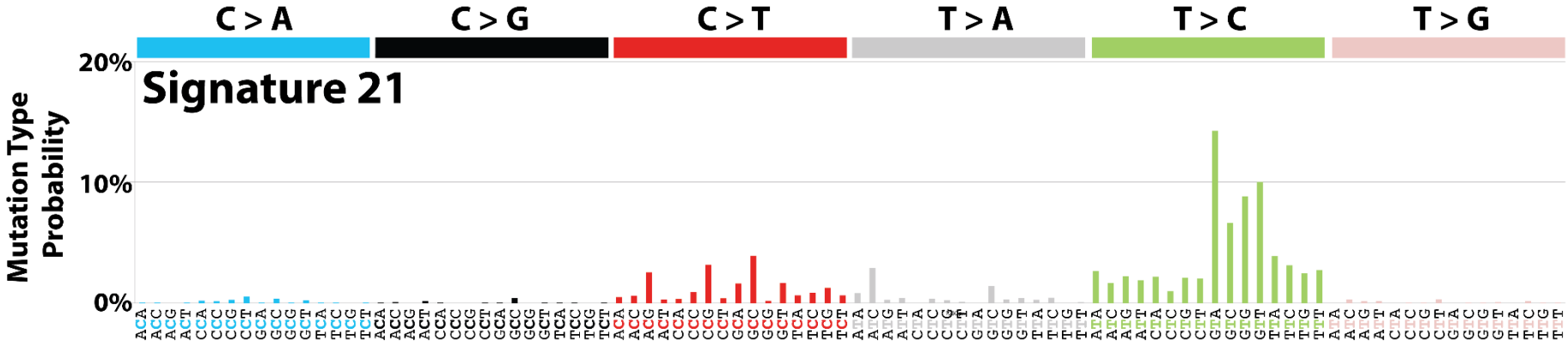
Additional mutational features:

Signature 20 is associated with high numbers of small (shorter than 3bp) insertions and deletions at mono/polynucleotide repeats.

Comments:

Signature 20 is one of four mutational signatures associated with defective DNA mismatch repair and is often found in the same samples as Signatures 6, 15, and 26.

https://cancer.sanger.ac.uk/cosmic/signatures_v2.tt



Cancer types:

Signature 21 has been found only in stomach cancer.

Proposed etiology:

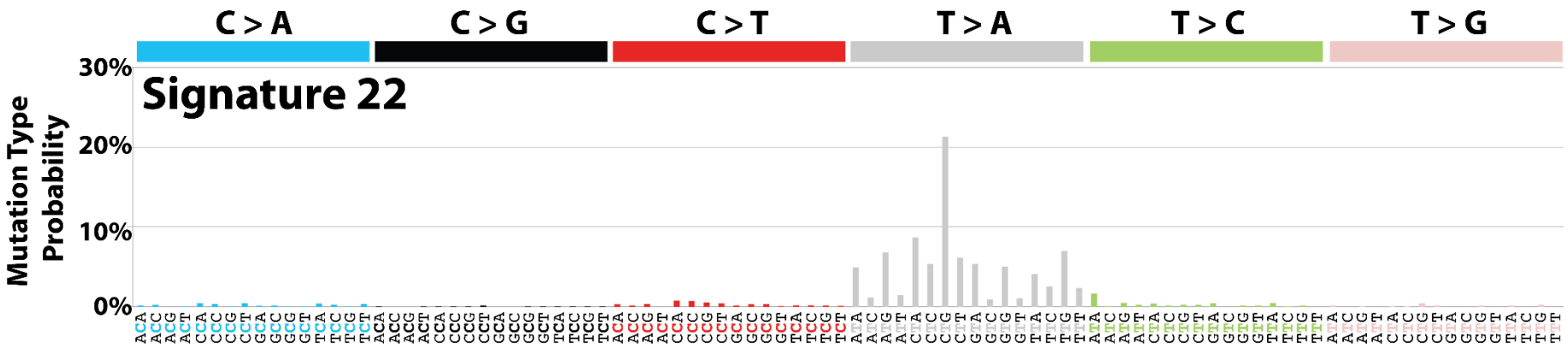
Unknown.

Comments:

The mutational pattern of Signature 21 is somewhat similar to the one of Signature 26. Additionally, Signature 21 is found only in samples that also have Signatures 15 and 20.

Signature 21 is probably also related to microsatellite unstable tumors

https://cancer.sanger.ac.uk/cosmic/signatures_v2.tt



Cancer types:

Signature 22 has been found in urothelial (renal pelvis) carcinoma and liver cancers.

Proposed etiology:

Signature 22 has been found in cancer samples with known exposures to aristolochic acid.

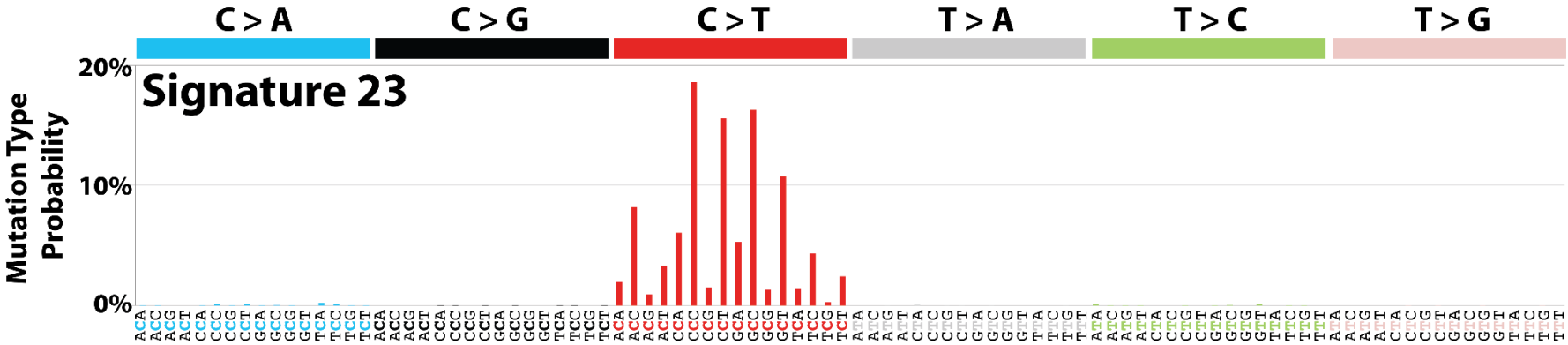
Additional mutational features:

Signature 22 exhibits a very strong transcriptional strand bias for T>A mutations indicating adenine damage that is being repaired by transcription-coupled nucleotide excision repair.

Comments:

Signature 22 has a very high mutational burden in urothelial carcinoma; however, its mutational burden is much lower in liver cancers.

https://cancer.sanger.ac.uk/cosmic/signatures_v2.tt



Cancer types:

Signature 23 has been found only in a liver cancer.

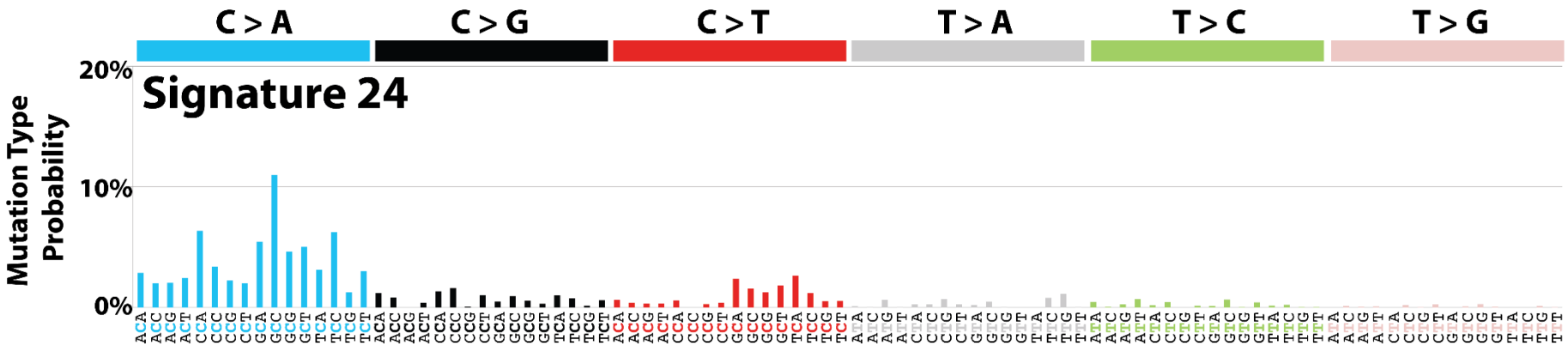
Proposed etiology:

Unknown

Additional mutational features:

Signature 23 exhibits very strong transcriptional strand bias for C>T mutations.

https://cancer.sanger.ac.uk/cosmic/signatures_v2.tt



Cancer types:

Signature 24 has been found only in a subset of liver cancers.

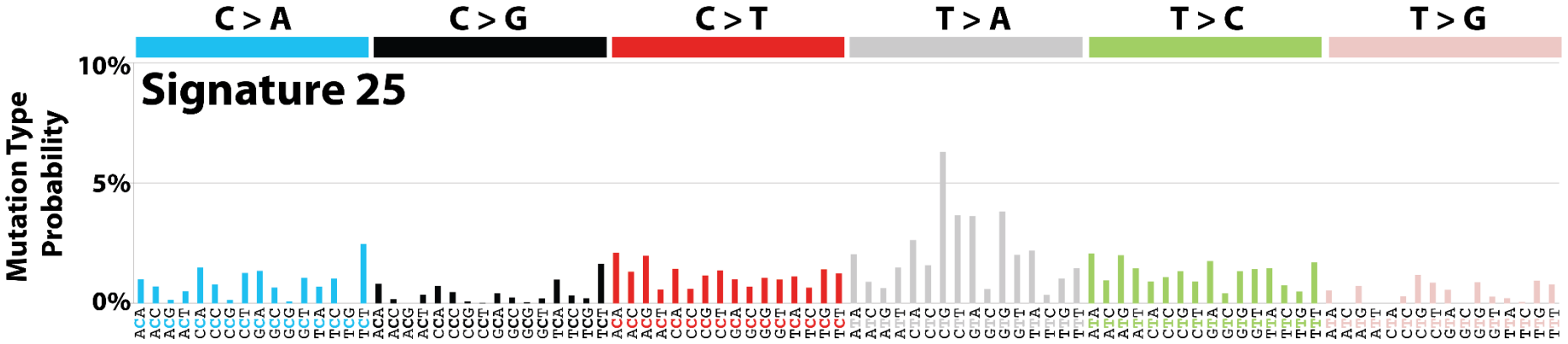
Proposed etiology:

Known exposure to aflatoxin.

Additional mutational features:

Signature 24 exhibits a very strong transcriptional strand bias for C>A mutations indicating guanine damage that is being repaired by transcription-coupled nucleotide excision repair.

https://cancer.sanger.ac.uk/cosmic/signatures_v2.tt



Cancer types:

Signature 25 has been found in cell lines of Hodgkin lymphomas.

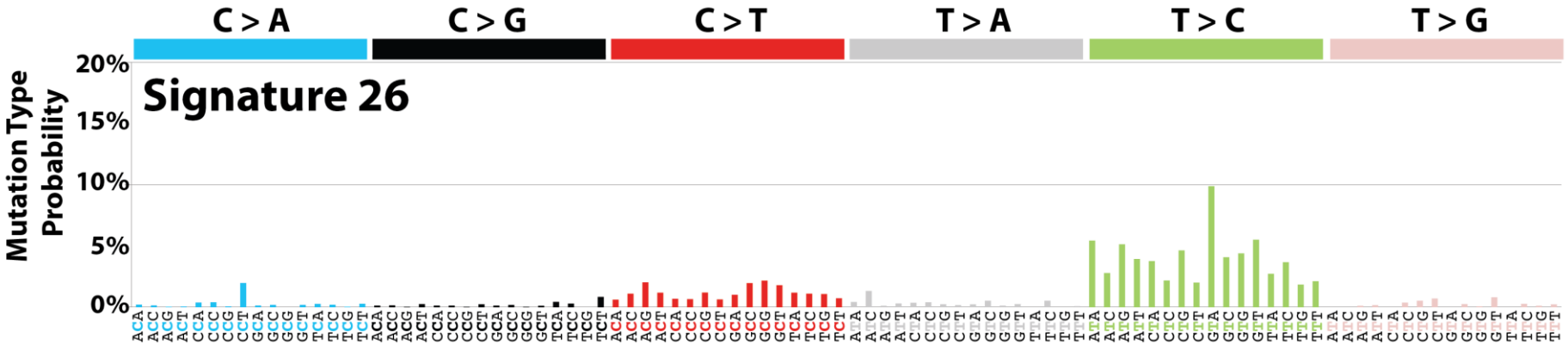
Proposed etiology:

Unknown

Additional mutational features:

Signature 25 exhibits transcriptional strand bias for T>A mutations.

https://cancer.sanger.ac.uk/cosmic/signatures_v2.tt



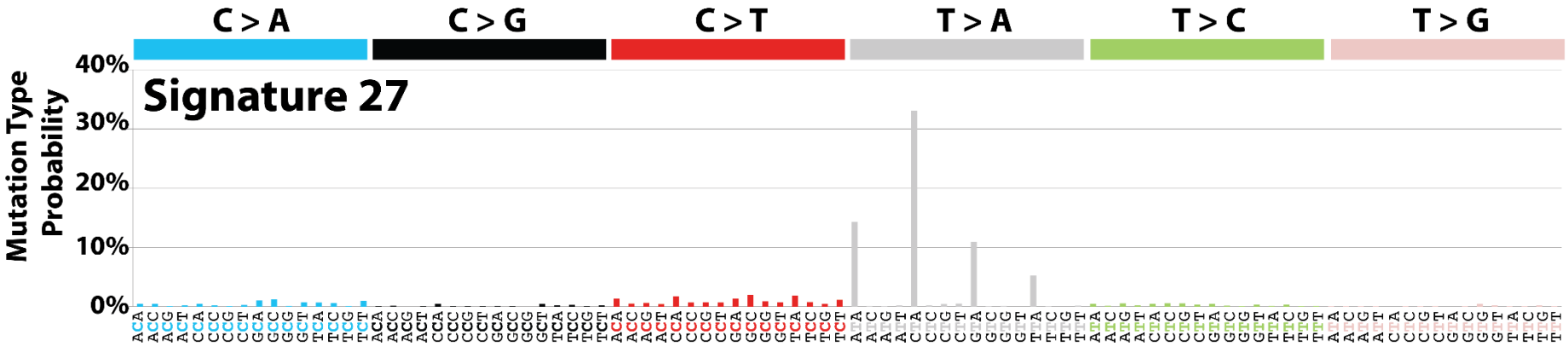
Cancer types:
Signature 26 has been found in breast cancer, cervical cancer, stomach cancer and uterine carcinoma.

Proposed etiology:
Signature 26 is believed to be associated with defective DNA mismatch repair.

Additional mutational features:
Signature 26 is associated with high numbers of small (shorter than 3bp) insertions and deletions at mono/polynucleotide repeats.

Comments:
Signature 26 is one of four mutational signatures associated with defective DNA mismatch repair and is often found in the same samples as Signatures 6, 15 and 20.

https://cancer.sanger.ac.uk/cosmic/signatures_v2.tt



Cancer types:

Signature 27 has been observed in a subset of kidney clear cell carcinomas.

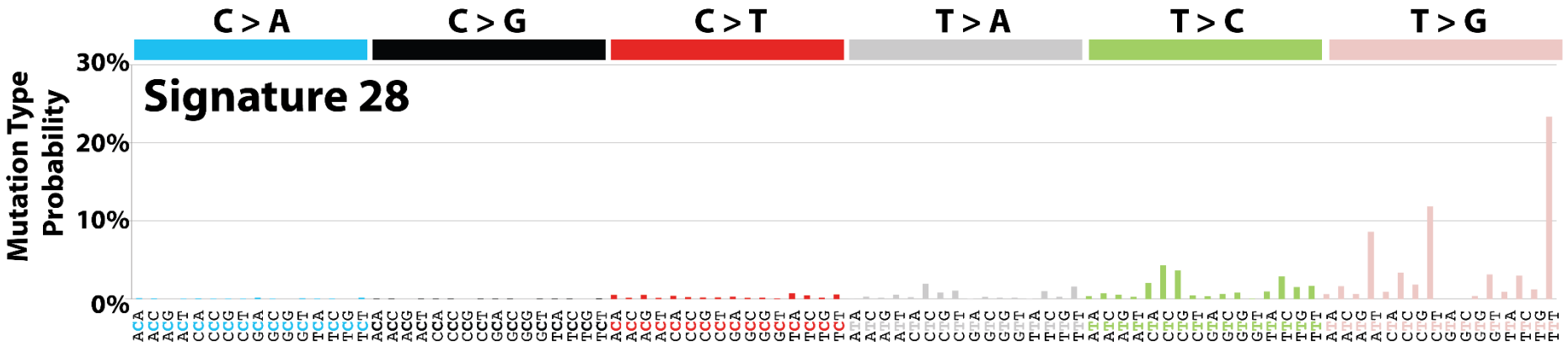
Proposed etiology:

Unknown.

Additional mutational features:

Signature 27 exhibits very strong transcriptional strand bias for T>A mutations. Signature 27 is associated with high numbers of small (shorter than 3bp) insertions and deletions at mono/polynucleotide repeats.

https://cancer.sanger.ac.uk/cosmic/signatures_v2.tt



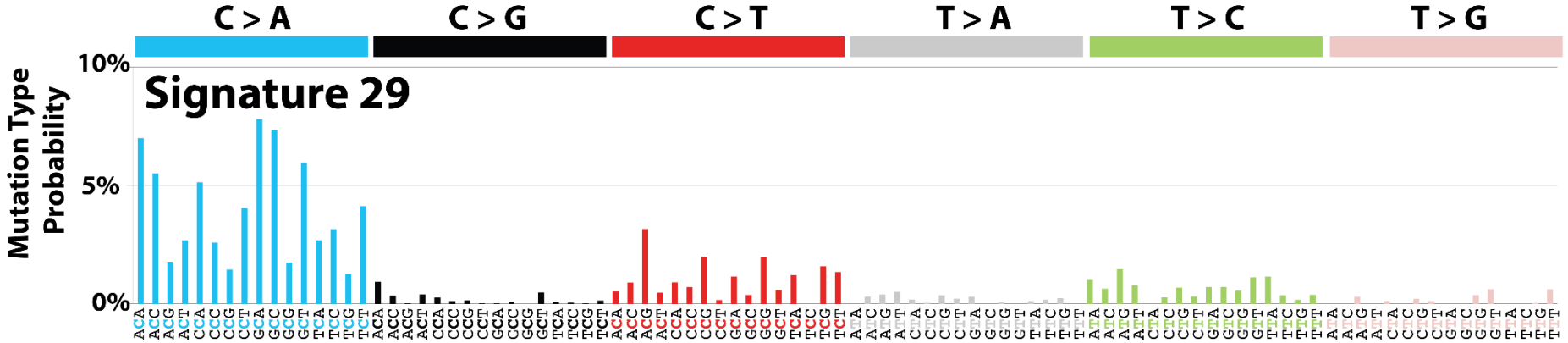
Cancer types:

Signature 28 has been observed in a subset of stomach cancers.

Proposed etiology:

Unknown.

https://cancer.sanger.ac.uk/cosmic/signatures_v2.tt



Cancer types:

Signature 29 has been observed only in gingivo-buccal oral squamous cell carcinoma.

Proposed etiology and Comment:

Tobacco chewing habit.

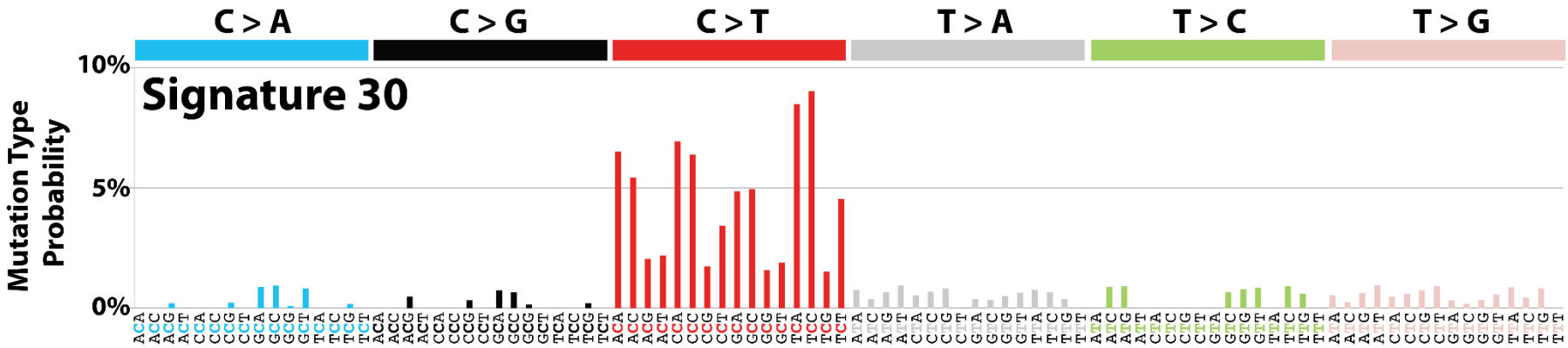
The pattern differs from that of smokers (Signature 4)

Additional mutational features:

Signature 29 exhibits transcriptional strand bias for C>A mutations indicating guanine damage that is most likely repaired by transcription-coupled nucleotide excision repair.

Signature 29 is also associated with CC>AA dinucleotide substitutions.

https://cancer.sanger.ac.uk/cosmic/signatures_v2.tt



Cancer types:
Signature 30 has been observed in a small set of breast cancers.
Proposed etiology and Comment:
Unknown.

https://cancer.sanger.ac.uk/cosmic/signatures_v2.tt