Molecular and Cell Biology of Tumors

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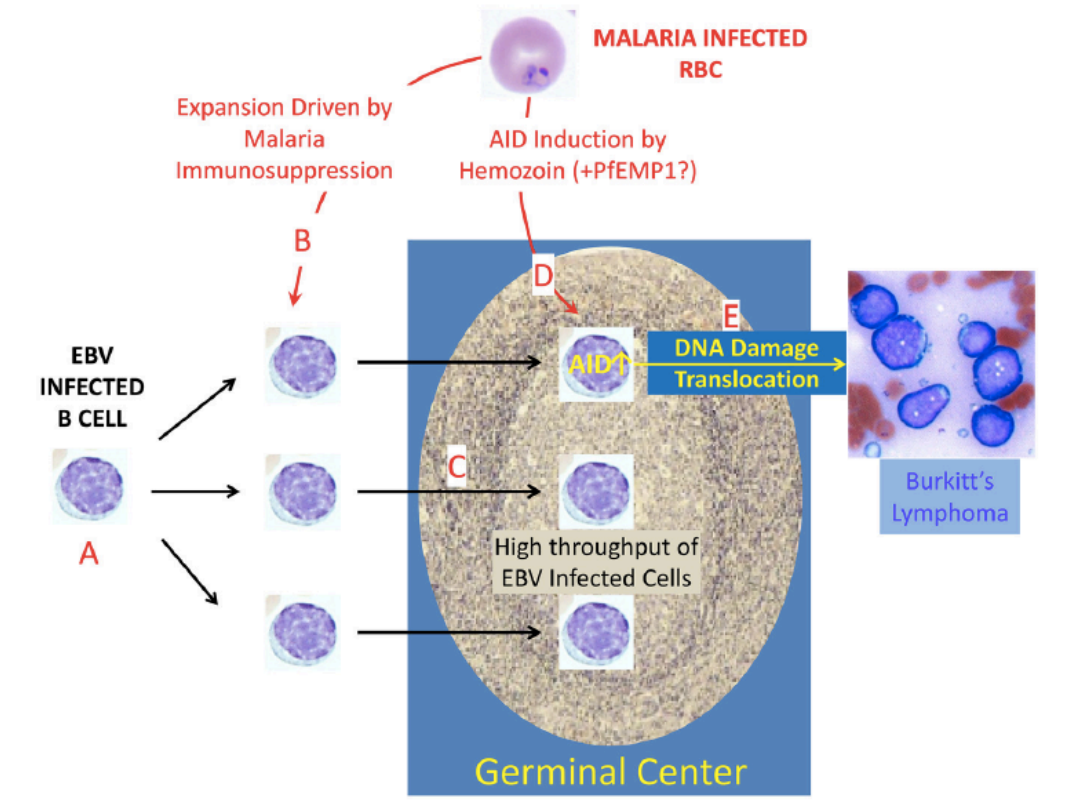
Molecular and Cell Biology of Tumors

4. Cancer predisposition genes I

Endemic Burkitt lymphoma

Why affecting children (unlike sporadic form)?

Chronic malaria ⟶ weaken IS ⟶ sensitivity to EB virus ⟶ eBL with c-Myc translocations



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PEARLS

The Link between *Plasmodium falciparum* Malaria and Endemic Burkitt's Lymphoma—New Insight into a 50-Year-Old Enigma

David Thorley-Lawson[1]*, Kirk W. Deitsch[2], Karen A. Duca[3], Charles Torgbor[1,3]

1 Sackler School of Graduate Biomedical Sciences, Tufts University School of Medicine, Boston, Massachusetts, United States of America, 2 Department of Microbiology and Immunology, Weill Medical College of Cornell University, New York, New York, United States of America, 3 Department of Biochemistry and Biotechnology, Kwame Nkrumah University of Science and Technology (KNUST) and Kumasi Centre for Collaborative Research, Kumasi, Ghana

P. falciparum induces the DNA mutating and double-strand-breaking enzyme **activation-induced cytidine deaminase (AID)**. This is the enzyme that is normally responsible for the somatic hypermutation and classswitch recombination of immunoglobulin genes that occur in B cells. AID occasionally mutate off targets, including oncogenes.

- increase the risk that a B cell will undergo a c-myc translocation and and tolerate the translocation (EBV-infected), synergistically increasing the likelihood that eBL will arise.

Thorley-Lawson D et PLoS Pathog. 2016 Jan 21;12(1):e1005331.

Endemic Burkitt lymphoma

Why affecting children (unlike sporadic form)?

Chronic malaria → weaken IS → sensitivity to EB virus → eBL

Plasmodium falciparum − DNA rearrangements and immunosuppression

EBV − resistance to apoptosis (in case of identified DNA lesions as translocations) and expansion of target cell population (higher likelihood of translocation event)

Int. J. Cancer: **124,** 1745–1755 (2009)
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MINI REVIEW

Epstein-Barr virus and the pathogenesis of Burkitt's lymphoma: More questions than answers

Georg W. Bornkamm*

Helmholtz Zentrum München, German Research Center for Environmental Health, Institute of Clinical Molecular Biology and Tumor Genetics, München, Germany

Exposure to Holoendemic Malaria Results in Suppression of Epstein-Barr Virus–Specific T Cell Immunosurveillance in Kenyan Children

Ann M. Moormann,[1] Kiprotich Chelimo,[4] Peter O. Sumba,[4] Daniel J. Tisch,[2] Rosemary Rochford,[3] and James W. Kazura[1]

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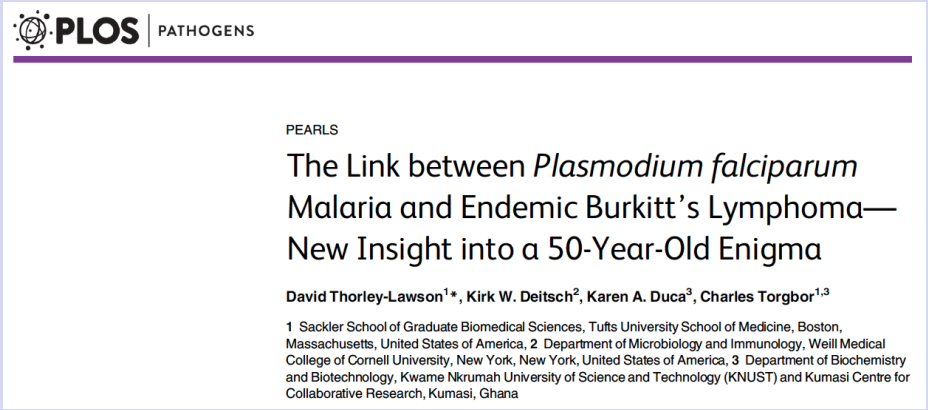
Endemic Burkitt lymphoma

Why affecting children (unlike sporadic form)?

Chronic malaria ⟶ weaken IS ⟶ sensitivity to EB virus ⟶ eBL

(Fig 1). The increased combinatorial risk of these two events explains the increased prevalence of eBL in *P. falciparum*-endemic areas, but many questions remain. These include why eBL is specifically linked only with *P. falciparum* and not the other species that cause malaria in humans (discussed in detail below), the origins of sporadic BL (which is not linked with *P. falciparum* and is frequently EBV-negative), why eBL is predominantly a cancer of children, and why the tumor is located in very specific anatomical regions that tend to change with age (the jaw in children and the abdomen in adults). Nevertheless, these experiments represent an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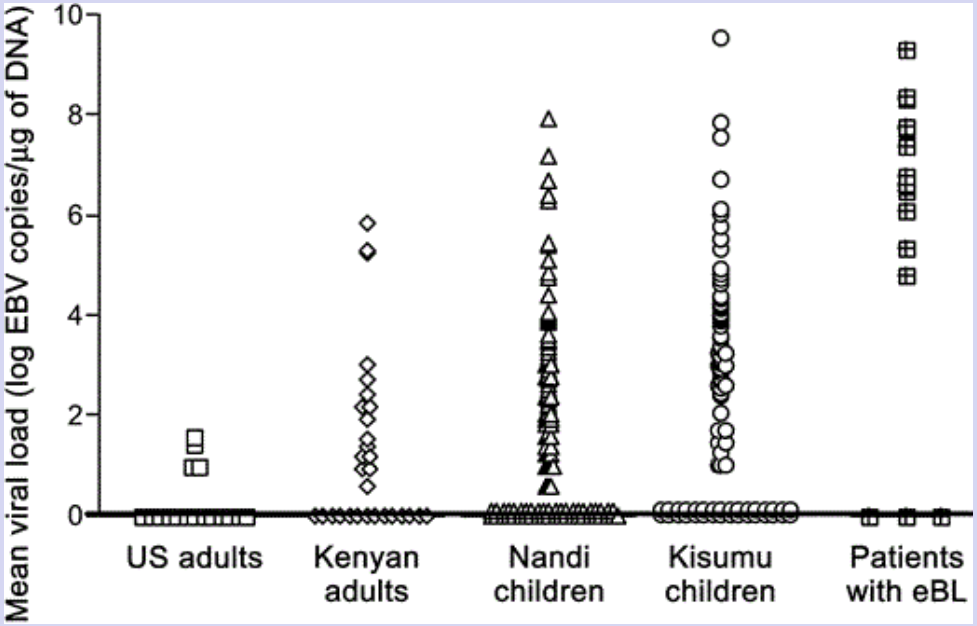
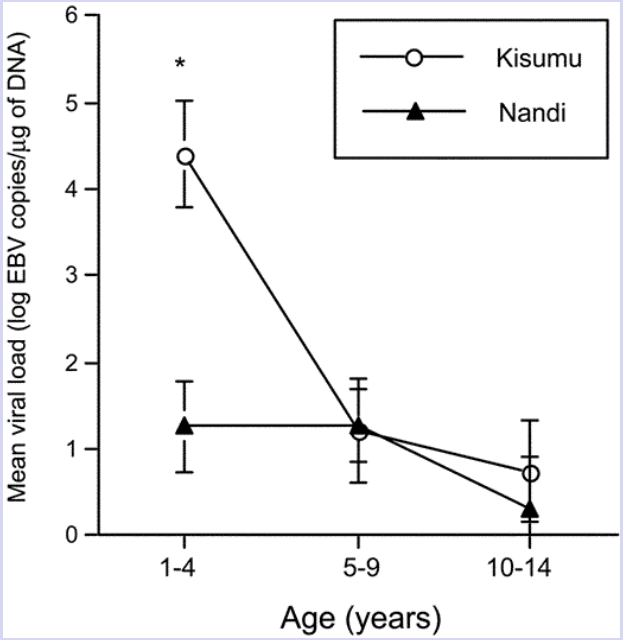
Endemic Burkitt lymphoma

Why affecting children (unlike sporadic form)?

Perennial, intense reinfections − holoendemic regions − Kisumu − high eBL





In developing countries, EBV typically acquired in early childhood

Reactivated latent EBV − by weak immune system as a result of recurrent malaria = outgrowth of EBV+ B-lymphocytes

Moormann AM et al, J Infect Dis. 2005 Apr 15;191(8):1233-8.

Hereditary papillary renal cancer (c-*met*)

some **RASopathies**
(*ras*, *raf*, *MAPK*,..)

Multiple endocrine neoplasia type 2 (*RET*)

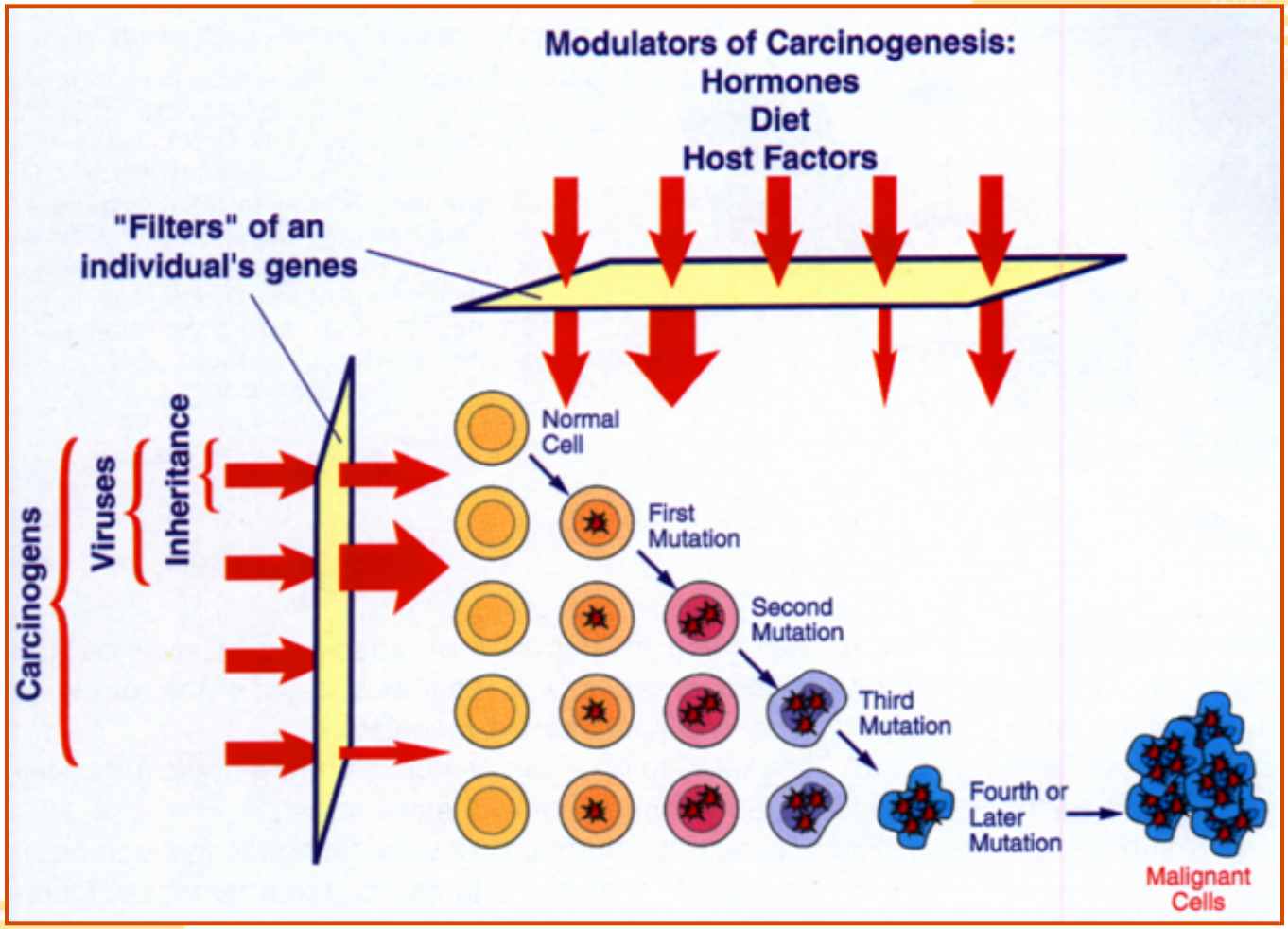
Familial
medullary thyroid carcinoma (*RET*)

Familial gastrointestinal stromal tumors (c-*kit*)

Complex process of cancerogenesis

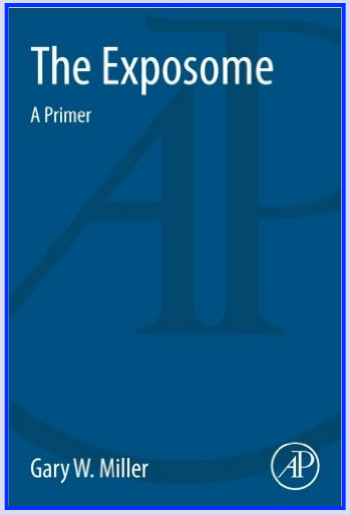
- Tumors develop via accumulation of **genetic alterations**. Some may be inherited, other (somatic) may be result from exposure of carcinogens or oncogenic viruses.
- Frequent contact with carcinogens does not automatically and immediately cause cancer development...
- **Modulators** of cancerogenesis involve hormons, diet, and other endogenous factors
- Carcinogen exposure and effect of modulators are „filtered“ by individual variants of specific genes that either eliminate, weaken or enforce their impact.
- Understanding of interactions of genes and environment needed for elucidation of cancerogenesis.

Complex process of cancerogenesis

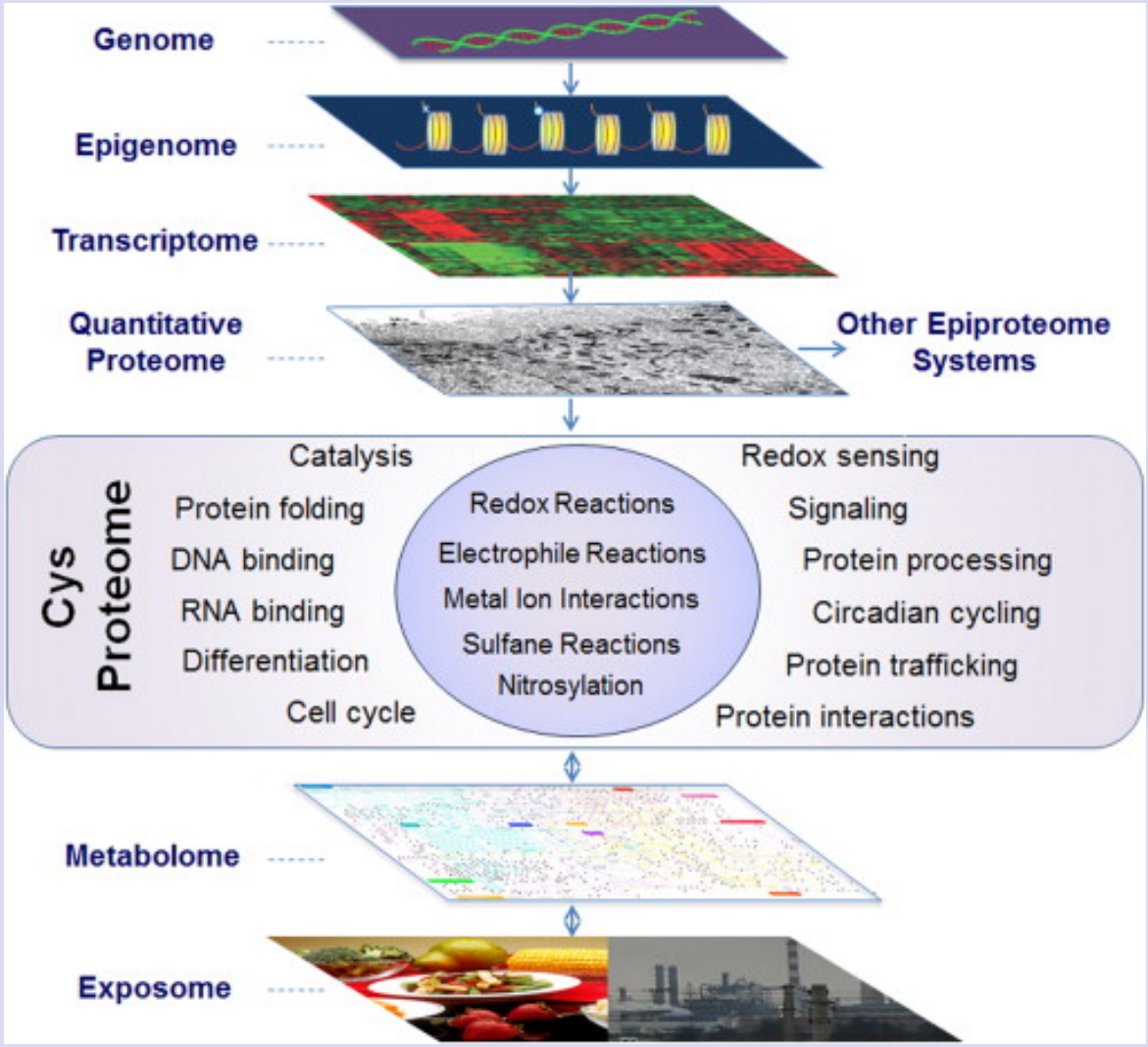


Exposome

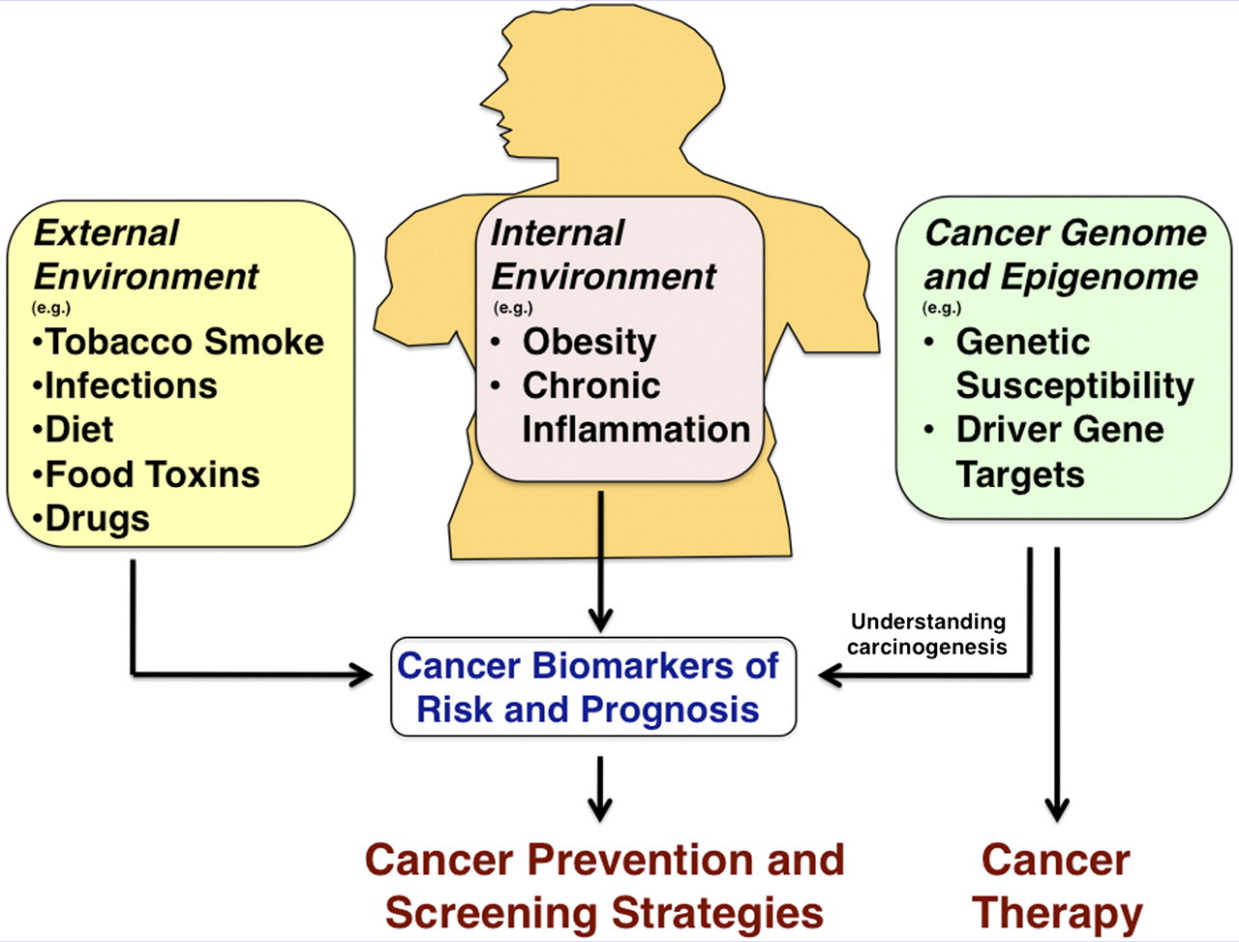
- „The cumulative measure of environmental influences and associated biological responses throughout the lifespan including exposures from the environment, diet, behavior, and endogenous processes.“



Exposome


Genome
Epigenome
Transcriptome
Quantitative Proteome
Other Epiproteome Systems
Cys Proteome
Catalysis
Protein folding
DNA binding
RNA binding
Differentiation
Cell cycle
Redox Reactions
Electrophile Reactions
Metal Ion Interactions
Sulfane Reactions
Nitrosylation
Redox sensing
Signaling
Protein processing
Circadian cycling
Protein trafficking
Protein interactions
Metabolome
Exposome

Exposome


External Environment
(e.g.)
•Tobacco Smoke
•Infections
•Diet
•Food Toxins
•Drugs
Internal Environment
(e.g.)
• Obesity
• Chronic Inflammation
Cancer Genome and Epigenome
(e.g.)
• Genetic Susceptibility
• Driver Gene Targets
Understanding carcinogenesis
Cancer Biomarkers of Risk and Prognosis
Cancer Prevention and Screening Strategies
Cancer Therapy

Cancer predisposition genes

1. Differences in metabolism of carcinogens („filters“)

(e.g. cytochrom P-450, CYP2D6, CYP1A1, N-acetyltransferase - NAT, glutathion-S-transferase M1 – GSTM1,…)

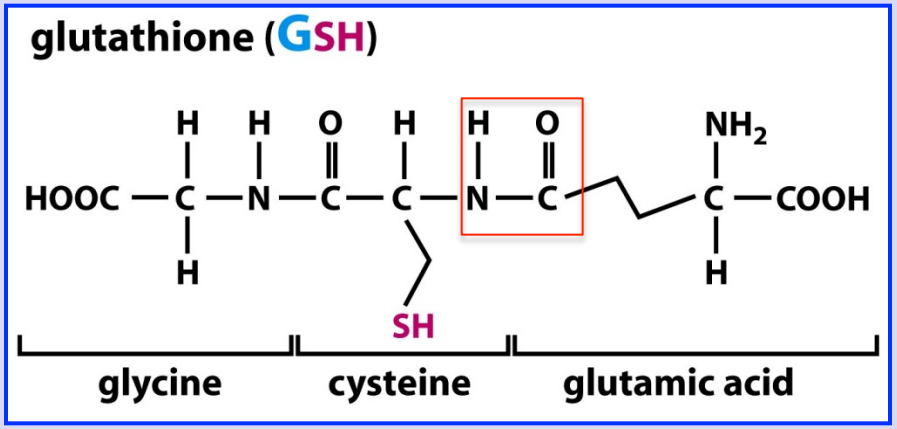
- Normal metabolism of cells releases as side products considerable amount of reactive molecules including mutagens/ cancerogens.
- These (same as environmental cancerogens) are immediately neutralised by **detoxification enzymes**:
  - Smokers with low levels of NAT are in 2,5x higher risk of developing tumors compared to smokers with high NAT
  - Low levels of GSTM1 increases the risk of lung cancer 3x

Function of glutathion-S-transferase

- GSTs are metabolic isozymes: catalyze the conjugation of the reduced form of glutathione (GSH) to xenobiotic substrates for the purpose of detoxification.
- Many tumors have reduced levels/activity of GST (e.g. Prostate adenocarcinomas by 90%)

Glutathion is antioxidant synthesized by cells: tripeptide with unusual peptide bond – protection from peptidases

GST uses SH group of glutathion to modify cancerogens before their interaction with DNA



Cancer predisposition genes

2.-3. Differences in DNA repair system
Mutations of tumor suppressors (and oncogenes)

- p53, *RB*, *WT1*, *BRCA1* and *BRCA2*,…
- *RET*, c-*met*, …

penetrance: percentage of individuals of certain genotype with manifestation of this genotype (with specific phenotype)
(probability of cancer development in specific age)

Hereditary cancer

examples: Li-Fraumeni syndrome, retinoblastoma, medullary thyroid cancer, Wilms tumor, hereditary breast and ovarian cancer, ...

- **5-10 %** of tumors usually represent **hereditary form** (germinal mutations of 1 allele + loss of heterozygosity - LOH), others are **sporadic**

- **hereditary forms** of cancer usually occur earlier in life than sporadic, often bilaterally and multiple foci; may be associated with developmental disorders

- **sporadic** tumors occur at higher age and unilaterally

Indications for genetic screening

1. Tumor in early age.
2. Mulifocal tumors in one organ or bilateral tumors in paired organs.
3. More primary tumors in one individual.
4. Family history of the same type of tumor in close relatives.
5. High incidence of cancer in family.
6. A case of cancer with congenital developmental disorders in family.

Genetic counseling

1. Family health history (at least 3 generations, complete genealogical tree including healthy individuals, verified diagnoses).
2. Identification of affected gene (mutation) (informed consent).
3. Genetic analysis of family, including healthy relatives older than 18y, with informed consent.
4. If mutation confirmed: Personalized medical management and cancer screening recommendations, prophylaxis, chemoprevention, change of the life-style.

- Psychological and ethical aspects of genetic testing!

gatekeepers and caretakers

Gatekeepers – genes that directly control (limit) tumor growth either by inhibition of the proliferation or induction of cell death

Caretakers - their inactivation cause genetic instability and that only indirectly promote tumor growth by increasing mutation frequency

APC, p53 – are both „gatekeepers“ and „caretakers“

gatekeepers and caretakers in hereditary cancer

caretakers

- NER, *xeroderma pigmentosa*, cockayne syndrome, trichothiodystrofie
- ataxia telangiectasia
- Bloom syndrome
- Fanconi anemia
- Hereditary non-polyposis colorectal tumor
- Werner syndrome
- Li-Fraumeni syndrome
- Breast cancer

gatekeepers

- retinoblastoma
- Li-Fraumeni syndrome
- Wilms tumor
- neurofibromatosis type 1 and 2
- Renal carcinomas
- MEN type 1 and 2
- melanoma
- Cowden syndrome
- Gorlin syndrome
- Breast cancer
- Colorectal cancer

Retinoblastoma

- Rare cancer disease in children; malignant tumor in retina; incidence − 1 in 13.500 - 25.000 newborns
- **Prototypical** example of hereditary predisposition to cancer: based on the epidemiologic studies of retinoblastoma the Knudson hypothesis was formed → Knudson model
- *RB* was first known tumor suppressor
- autosomal dominant inheritance: mutations od *RB* are **recessive** (at the cellular level) and **dominant** (at the organismal level) at the same time!!!
- germinal mutation of ***RB*** (**13q14**) cause **increased risk** of **retinoblastoma** and **osteosarcoma** (everyone who inherits mutated allele), that may be passed to the next generation (even if it is not manifested in a parent because of reduced penetrance- 95%) − dominant inheritance
- But second somatic mutation of RB is needed for retinoblastoma development − recessive

Knudson two-hit model

40% cases:

- Average age at diagnosis - 14 months
- Bilaterally in both eyes − in average 3 independent tumors
- If removed surgically at early stages, high incidence of osteosarcomas in adolescence
- Often family history

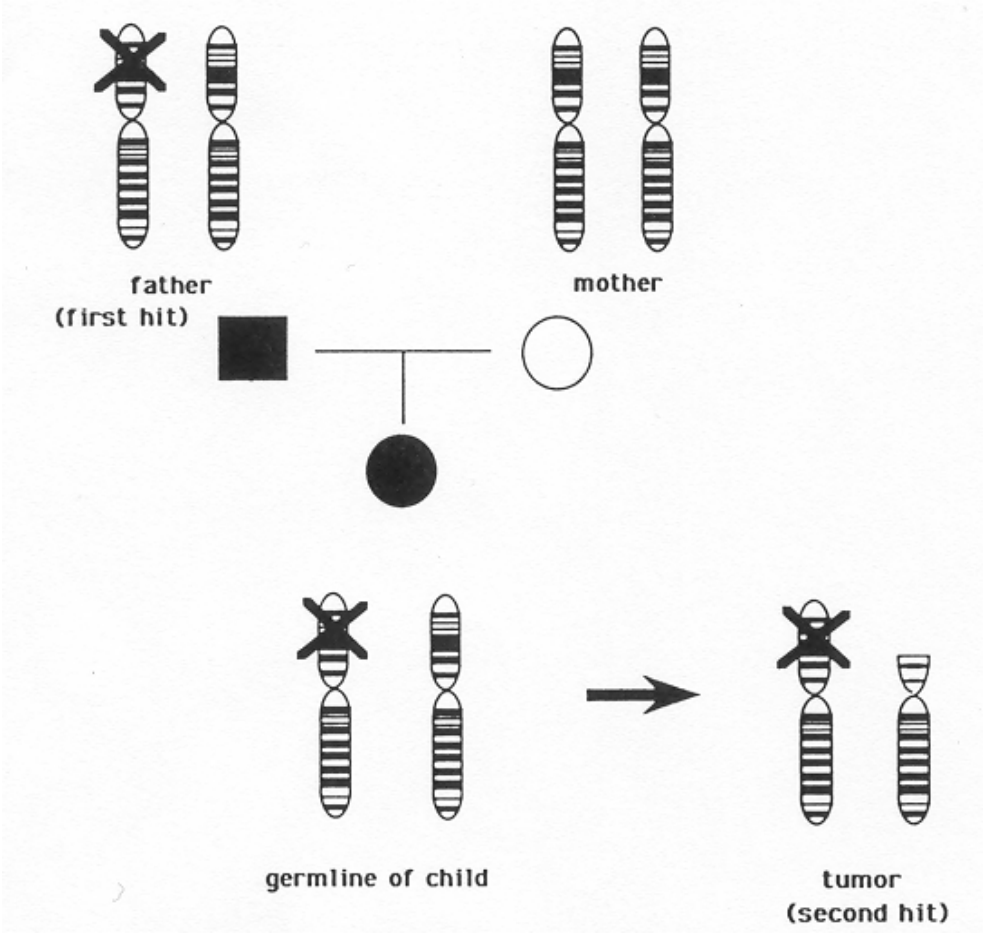
60% cases:

- Without family history
- Average age at diagnosis - 30 months
- unilateral tumors

Knudson (1971):

1. group: 1 mutated allele of *RB* gene is inherited (germinal), second mutation is somatic
2. group: two independent somatic mutations of *RB* are needed ⇒ one hypothesis explained two epidemiologically different variants of disease

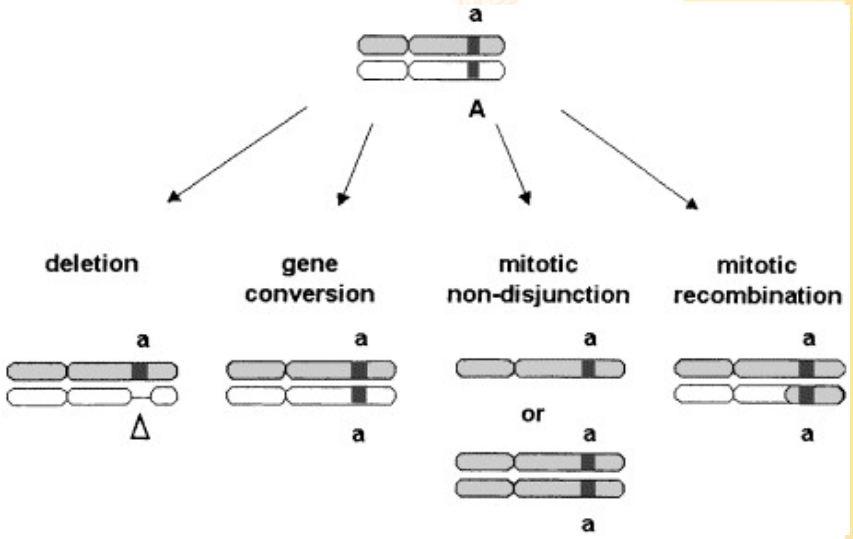
Knudson two-hit model, germinal mutation + LOH



Loss of heterozygosity (LOH)

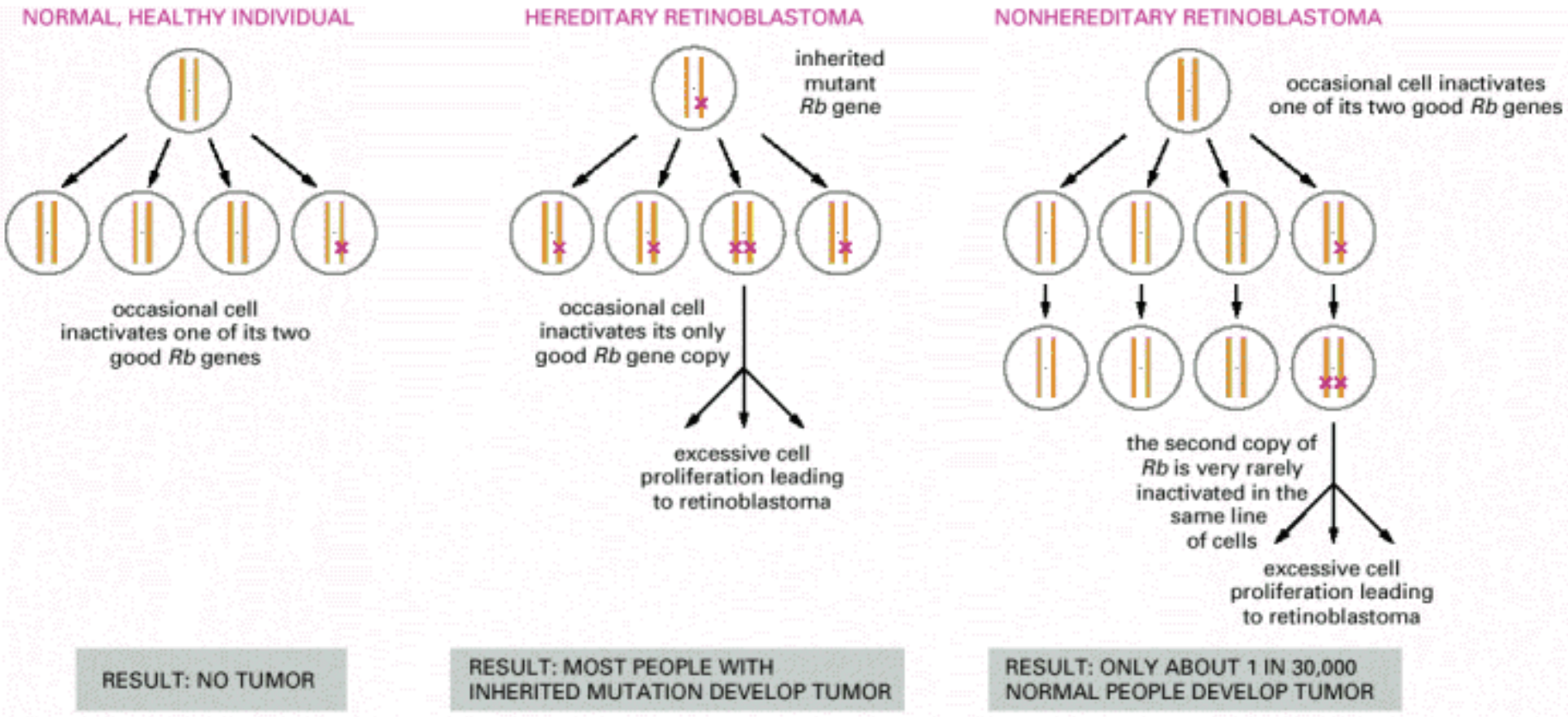
1. LOH with copy number losses : Cells with germline mutation in one allele of a tumor suppressor (heterozygots) lose **part of a chromosome (deletion**) with functional allele. Only 1 allele of gene remains. Detectable by CGH-based gene counting

2. Copy number neutral LOH: Cells with one inherited mutation of tumor suppressor, lose the functional allele by mitotic recombination, gene conversion, point mutation etc. − homozygot for mutated allele



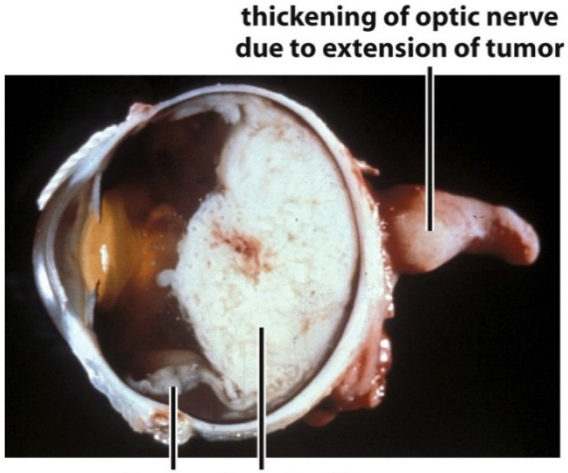
Wijnhoven et al, Mutat Res. 2001 473(1):23-36

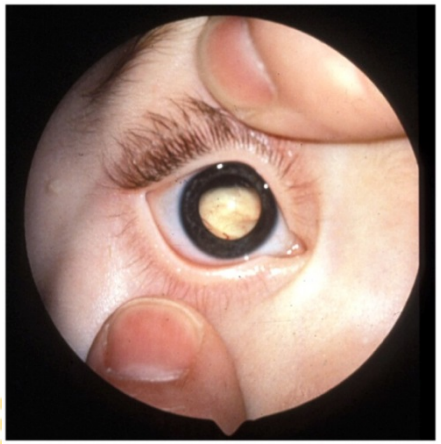
Knudson two-hit model

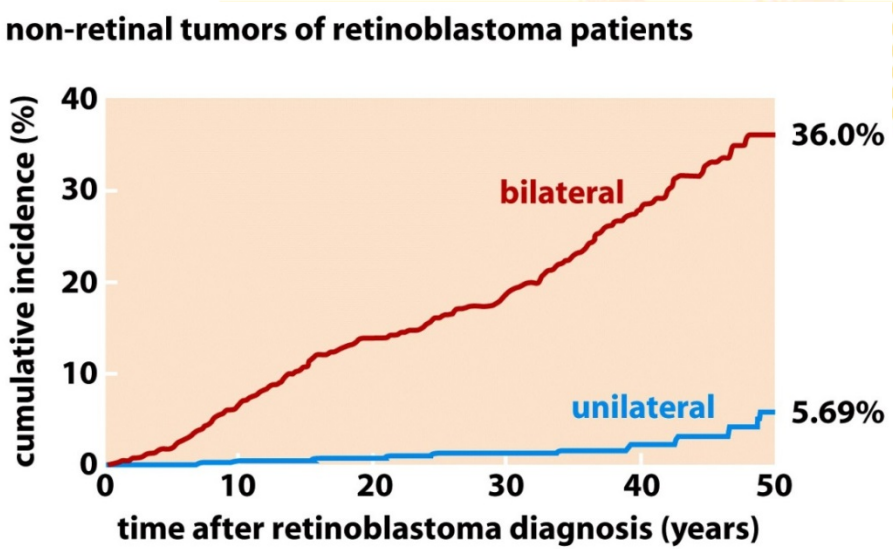


http://dpuadweb.depauw.edu/cfornari_web/DISGEN/retinoblastoma_website/public_html

Retinoblastoma







Risk of other cancers in patients with childhood retinoblastoma :

- for unilateral tumors the increased risk is caused by radioation therapy (secondary- therapy related tumors)
- for bilateral tumors − inherited mutation in all cells

Retinoblastoma

60% - unilateral sporadic

10% - unilateral hereditary (w/o family history)

5% - bilateral with family history

25% - bilateral - new „germ-line“ − also hereditary, but no family history

60% - unilateral sporadic

15% - unilateral hereditary

25% - bilateral hereditary

- According to the prediction in **5%** of persons with inherited *RB* mutation, retinoblastoma **will not occur**!
- Almost complete penetrance (**95%**).
- Many − maybe most - people have one-hit clones in retina

* Project „resilience“

- ***resilient*** – resistant (**filters**?)

- „classical“ studies: search for causal mutations in (cancer) patients
- Complemetary approach: search for a carriers of highly penetrant disease-causing mutations **without** manifestation of the disease

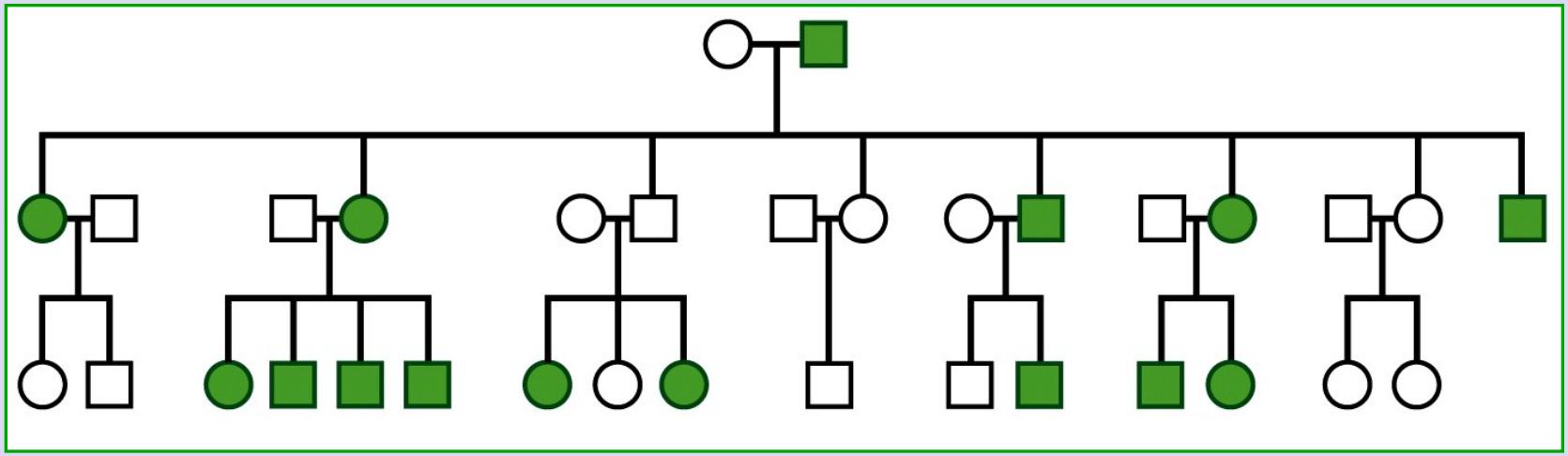
➢ Screen of **874** genes (causing 584 severe childhood diseases) in **589 306** genomes: identification of 13 adults with a mutation (causing 8 diseases) – without clinical symptoms! 13 resilient candidates

First step to identify „protecting“ genetic variants

Chen R. et al: Analysis of 589 306 genomes identifies individuals resilient to severe mendelian childhood diseases. *Nat Biotechnology*, 34 (5) 531-538, **2016**

Retinoblastoma risk in families

- Sporadic unilateral: siblings 1%; children 2-6%
- Sporadic bilateral: siblings 2%; children 50%
- Hereditary bilateral: siblings almost 50%; children 50%
- Hereditary, reduced penetrance: siblings < 40%; children < 40%



RB: gene and protein

- nuclear (85%) protein $p110^{RB}$
- In non-dividing cells is pRB bound in complex with TF **E2F**; E2F transactivates a set of genes required for S-phase of cell cycle; pRB inhibits E2F and thus blocks cell cycle progression („cell cycle brake“)
- Levels of RB are constant during cell cycle, but RB is differently **phosphorylated** throughout cell cycle; there is more than 10 phosphorylation sites in RB molecules (Thr, Ser); hypophosphorylated RB is active and binds E2F, phosphorylated pRB is inactive

➢ pRB is **integrator of signals**

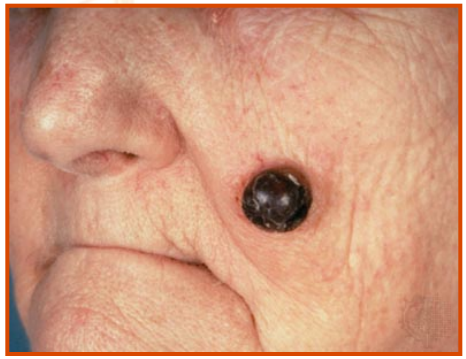
pRB pathway inactivaton in cancer

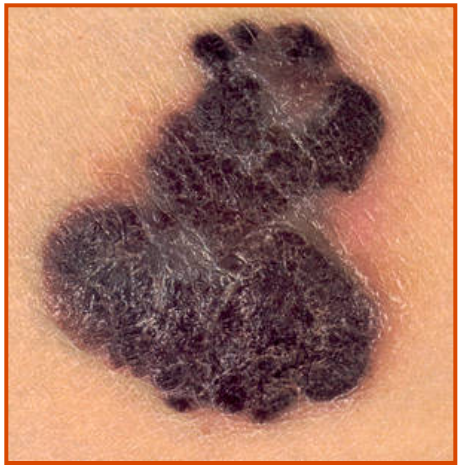
- *RB* gene may be mutated, deleted, its promoter may be methylated (retinoblastomas, small cell lung carcinomas, sarcomas, bladder carcinomas, breast carcinomas)
- Inhibited by viral proteins E1A, LT SV40, E7 (cervical carcinoma)

- Amplification of cyclin D1 (esophageas cancer, breast cancer) or overexpression of cyclin D1 as a result of chromosomal translocation (MCL); potentially oncogenic virus *Herpesvirus saimiri* encodes its own cyclin D
- Amplification of Cdk4 (glioblastomas, gliomas)
- Deletion of p15, p16 or both (esophageal, lung, bladder, pancreatic cancer and glioblastomas)

Malignant melanoma

- Incidence of MM (9th most frequent cancer in USA) in western countries increases at the highest rate compared to other tumors. Every 10 years the number of patients doubles.
- Melanomas arise from transformation of **melanocytes** − skin cells producing melanin. Melanin is involved in protection of skin against UV.
- Number of melanocytes is the same in people with fair and dark complexion, there is difference in amount of melanin produced. People of fair complexion have 10x higher risk of melanomas.
- Risk is increased by tanning, especially in persons likely to get sunburn.





Malignant melanoma

- 5-10 % melanomas is **familial**, penetration is around 50 % at the age of 80.

- Genetic predisposition − some hereditary cancer syndromes:

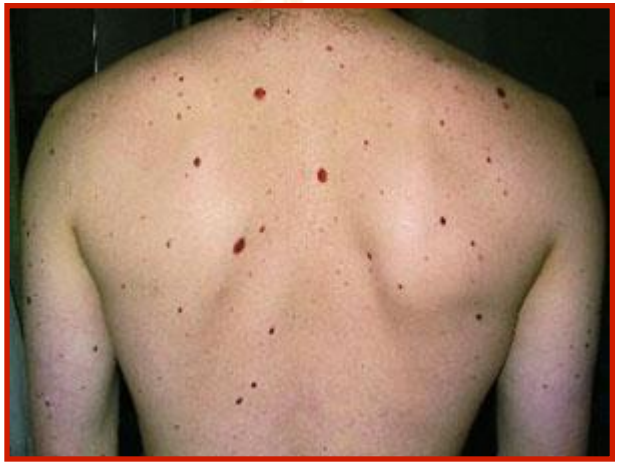
- **FAMMM** (*Familial Atypical Multiple Mole and Melanoma*)
- **Dysplastic Nevus Syndrome** (DNS) and **Atypical Mole Syndrome** (AMS)

- (xeroderma pigmentosum)

Familial Atypical Multiple Mole and Melanoma / FAMMM

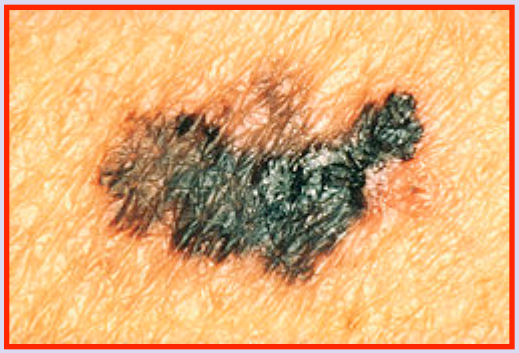
- High number of atypical moles → high risk of melanoma
- autosomal dominant inheritance

- melanocytes may form pigmented moles on skin: high numbers of nevi is associated with increased risk of melanoma development



Pigmented nevi

- Benign skin lesions caused by accumulation of melanocytes (aggregation of melanocytes in so called nest), that have different color than surrounding skin
- Melanocytes are normally dispersed in skin (occur as single cells distant from each other)



malignant melanoma

Malignant melanoma

- for MM typical high frequency of complex cytological rearrangements ⇒ difficult to identify causal genes involved in melanoma development
- Predicted region: **9p21-22** (altered in 50 % of affected families)
- Contain gene ***CDKN2A*** encoding **p16^{INK4A}**: mutations of *p16* cause **20-40 %** of hereditary melanomas → there are other causal genes
- Mutation of *CDKN2A* (p16) in 0,2– 2% of sporadic melanomas
- protein p16^{INK4A}: 158 AA − forms binary complexes wih **Cdk4** and **Cdk6**, and inhibits their activity (and thus phosphorylation of RB) (INK4A - **in**hibitor of Cd**k4**)

- In families with hereditary melanomas there are other cancers as well: pancreatic, rarely cervical, bladder, breast cancer, gliomas and some hematologic cancers

Malignant melanoma

Locus 9p21 contains other genes:

- ***p15*^{INK4B}** - homology with p16, also inactivates Cdk4 and Cdk6: inherited mutations have **not** been detected!

- ***p14*^{ARF}** - binds MDM2 and prevents ubiquitinylation and degradation of p53; $p14^{ARF}$ is encoded by same DNA sequence, only in **a**lternative **r**eading **f**rame − possible to turn off two pathways critical for cancerogenesis (p53 and RB); but **not** involved in hereditary predisposition to MM!!

Malignant melanoma

•At around **1%** of hereditary melanomas there is germinal mutation of ***CDK4 gene*** encoding cyclin dependent kinase 4 (12q14.1)

•Exchange of cystein 24 for arginin: interferes with binding of p16 and p15, but not with binding of cyclin D!!

⇒ **Rare** example of inherited mutation of protooncogene!

Hereditary papillary renal cancer (c-*met*)

some **RASopathies**
(*ras*, *raf*, *MAPK*,..)

Multiple endocrine neoplasia type 2 (*RET*)

Familial
medullary thyroid carcinoma (*RET*)

Familial gastrointestinal stromal tumors (c-*kit*)

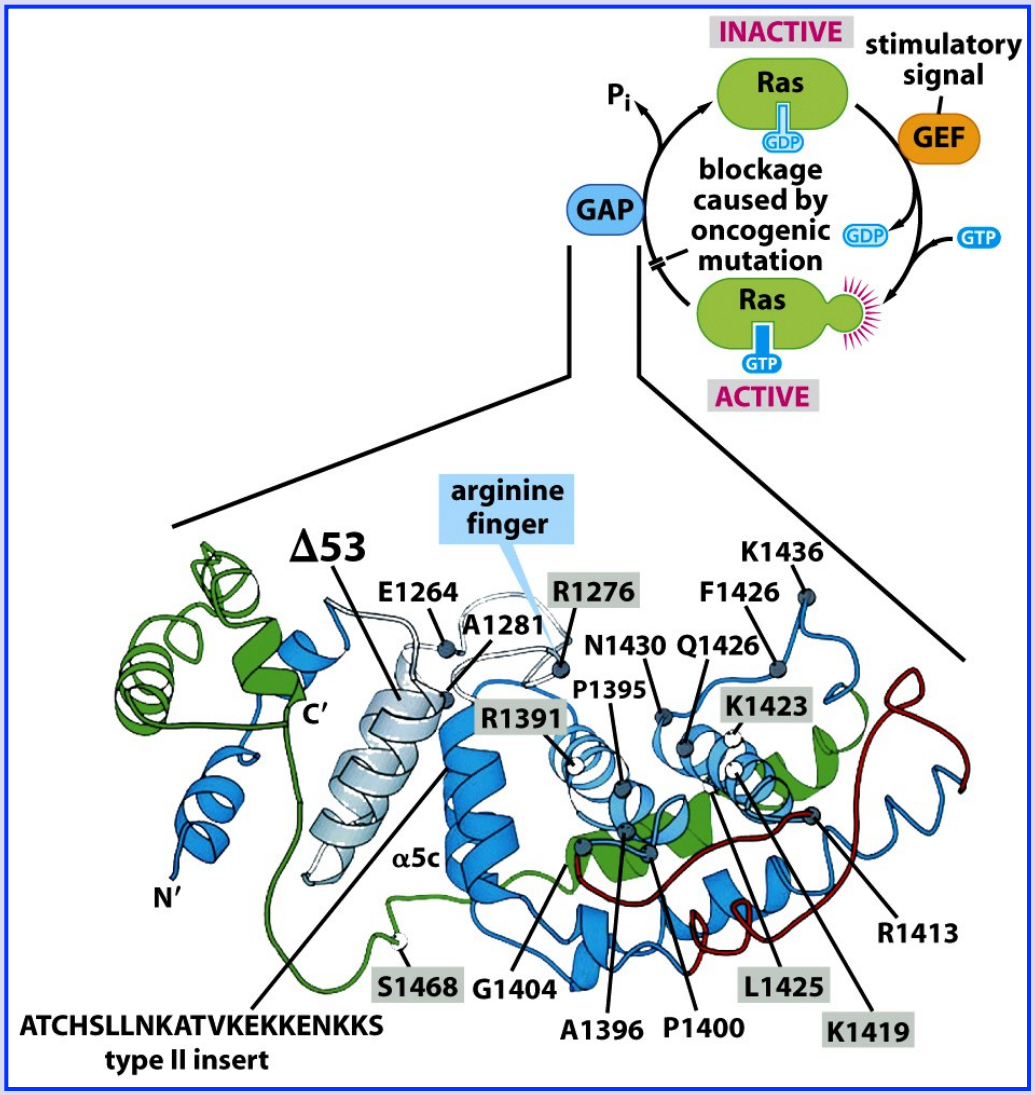
Malignant melanoma (*CDK4*)

Neurofibromatosis type 1 - NF1

- autosomal dominant inheritance
- Patients have multiple **benign neurofibromas**, some of them may progress into **neurofibrosarcomas**, increased risk of **AML**.
- ***NF1*** gene is localized at chromosome **17q11.2**
- Inactivation of both alleles of *NF-1* described also in melanomas and neuroblastomas

- It encodes protein **neurofibromin**: GTPase activating protein (GAPs; negative regulation of Ras)
- Belongs among RASopathies

Neurofibromin



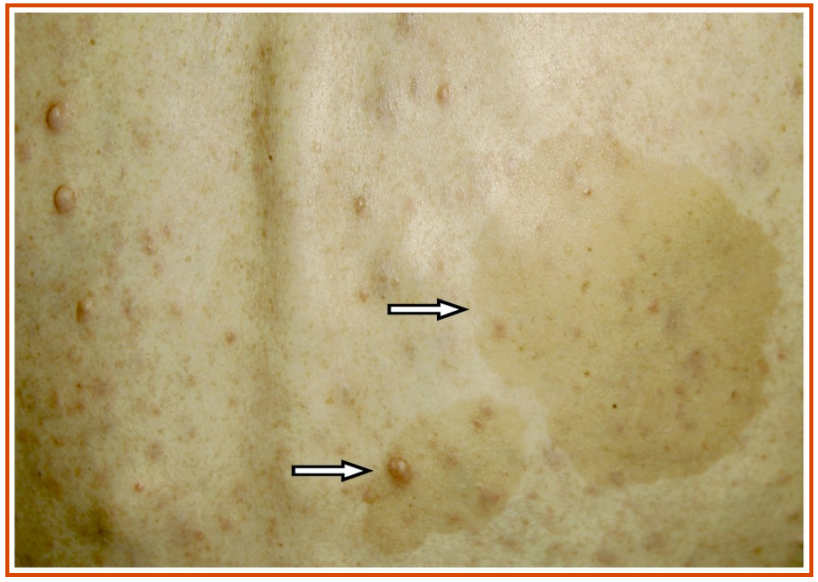
NF1 is **GAP** (GTPase activating protein) of **Ras** protein.

Interaction of Ras and NF1 may increase GTPase activity of Ras (more than 1000x).

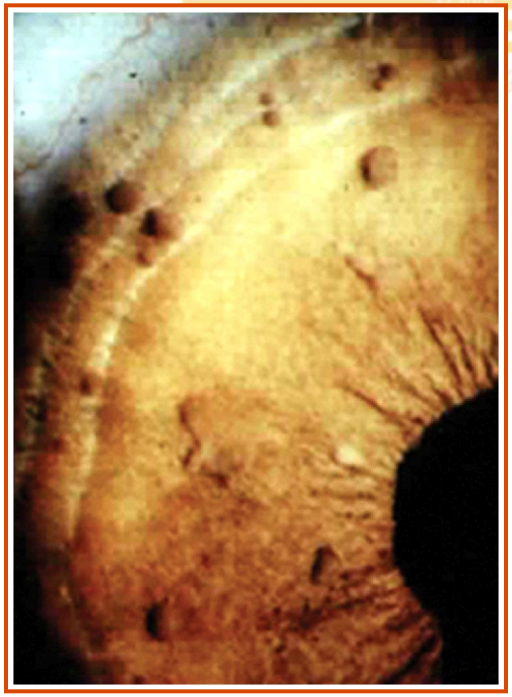
For NF1 function is crutial arginin R1276 that interacts with Ras and contributes to GTP hydrolysis.

In grey marked identified mutations of NF1.

Neurofibromatosis type 1



Café-au-lait spots on skin



pigmented iris hamartomas, i.e. Lisch nodules

Hamartomas

- Benign nodules, different histological structure compared to adenomas: cells are fully differentiated but disorganized
- „right piece at the right place but with wrong orientation“ –
- Normal differentiated tissue but not physiologically integrated
- They grow at the same rate as the organ from whose tissue they are made, not invasive

Neurofibromatosis type 2 - NF2

- autosomal dominant inheritance
- Genetic condition characterised development of **bilateral vestibular schwannomas,** also known as **acoustic neuromas**: benign tumors of the acoustic nerves for balance and hearing
- Associated with **multiple other benign intracranial and spinal tumors,** or tumors of **periferal nerves**, only rarely malignant
- Clinical symptoms starting at age 21y, average survival time is 36 years; high variability
- Incidence 1:40.000
- Causally connected with germinal mutation of ***NF2*** gene (**22q12.2**): almost ½ of mutations arise *de novo*
- Gene encodes protein **merlin** (schwanomin)

Neurofibromatosis type 2

- **merlin** belongs to a large family of cytoskletetal proteins (merlin: moesin, ezrin, radixin-like protein) that interacts with membrane proteins: mostly located in zonula adherens
- Merlin interacts with **actin** and transmebrane molecule **CD44**; expressed mostly by neuronal tissues
- Absence of functional merlin leads to changes in intracellular signaling and increases proliferation of Schwann cells

Cowden syndrome - CS

- *multiple hamartoma syndrome*
- autosomal dominant inheritance
- Characterised by **multiple benign hamartomas** in many organs (skin, breast, thyroid, GI) and an increased lifetime risk of breast, thyroid, uterine, and other cancers
- First symptoms of CS appear no later than 3rd decade of life
- Most of the tumors are benign, but around **10%** of patients with Cowden syndrome develop non-medullary **thyroid carcinoma** and around **30 to 50%** affected women malignant **breast carcinoma**

Cowden syndrome

- Genetic condition is associated with germinal mutation of ***PTEN*** gene (localized at chromosome **10q23.3**)
- *PTEN* mutation found in 80 % patients with CS
- Phenotypic variability may be explained by different *PTEN* mutations

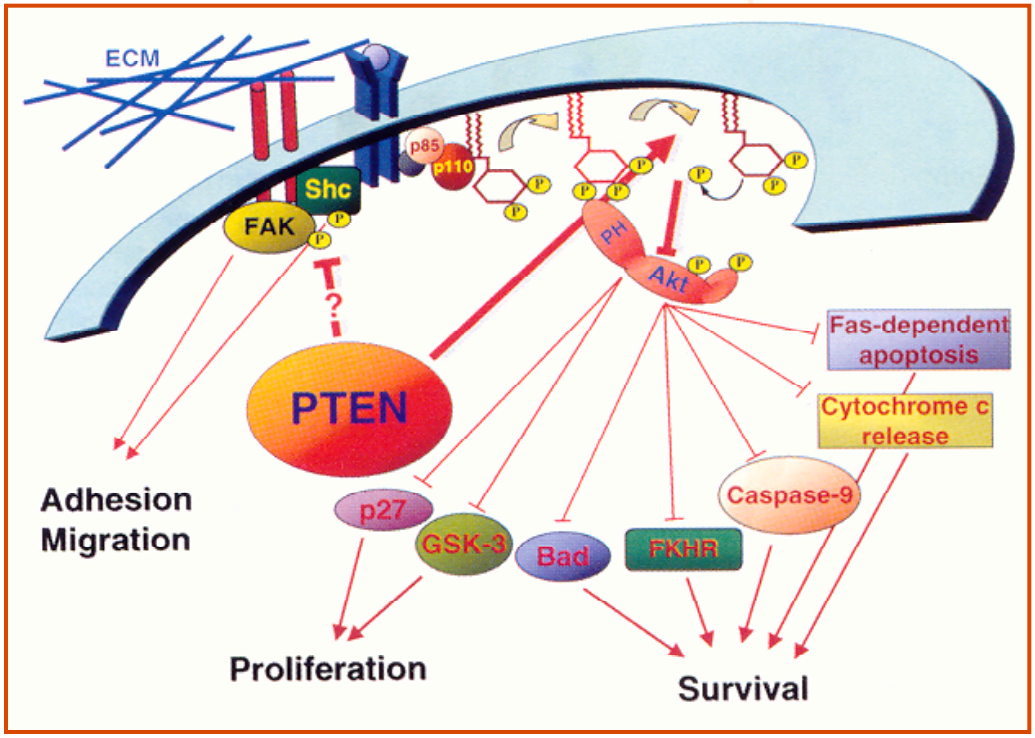
PTEN function in cells

- PTEN is protein/lipid phospatase (***P****hosphatase and* ***T****ensin homolog deleted on chromosome* ***TEN***) alias: MMAC1 (*mutated in multiple advanced cancers*)
- Dephosphorylates both Thr/Ser, and Tyr, but for his tumor suppressor function is crucial its ability to dephosphorylate **phosphatidylinositol-(3,4,5)-triphosphate** (**PIP-3**), a product of **PI3K** kinase (upon activation by mitogenic signaling); (PIP-3 activates **Akt** that blocks apoptosis and enable cell survival)
- PTEN keeps levels of PIP-3 low: inactivation of PTEN results in elevated PIP-3 levels and Akt hyperactivation

Role of PTEN in regulation of cell death, proliferation and adhesion

1. PTEN acts as a tumor suppressor primarily via cell cycle arrest at G1 phase, evetually by enabling apoptosis (through suppression of survival signaling induced by growth factors).

2. Another PTEN substrate is kinase **FAK** („focal adhesion kinase“): via dephosphorylation of FAK PTEN inhibits cell migration mediated by integrins



Di Cristofano A and Pandolfi PP, *Cell* 100 (2000) 387-390

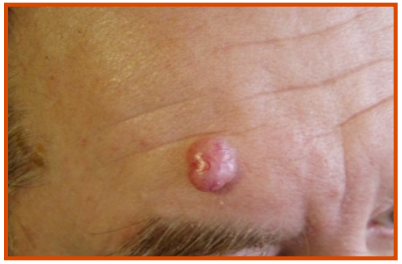
Model of PTEN function during cancerogenesis

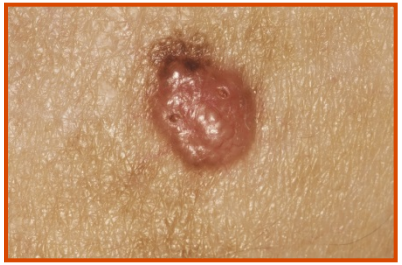
- „LOH“ 10q23 is frequent in tumors − 25 to 50%, but complete loss of function of PTEN is quite rare in early stages of cancerogenesis (except for endometrial and ovarian cancer).
- In other cancer types complete PTEN deficiency is associated with later stages of tumor progression, i.e. metastatic dissemination

➢ In early stages of cancerogenesis there is haploinsufficiency of PTEN (only one functional allele is insufficient to produce phenotype of wt homozygot − tumor suppressor function). Complete loss of function (LOH) occurs later in tumor progression.

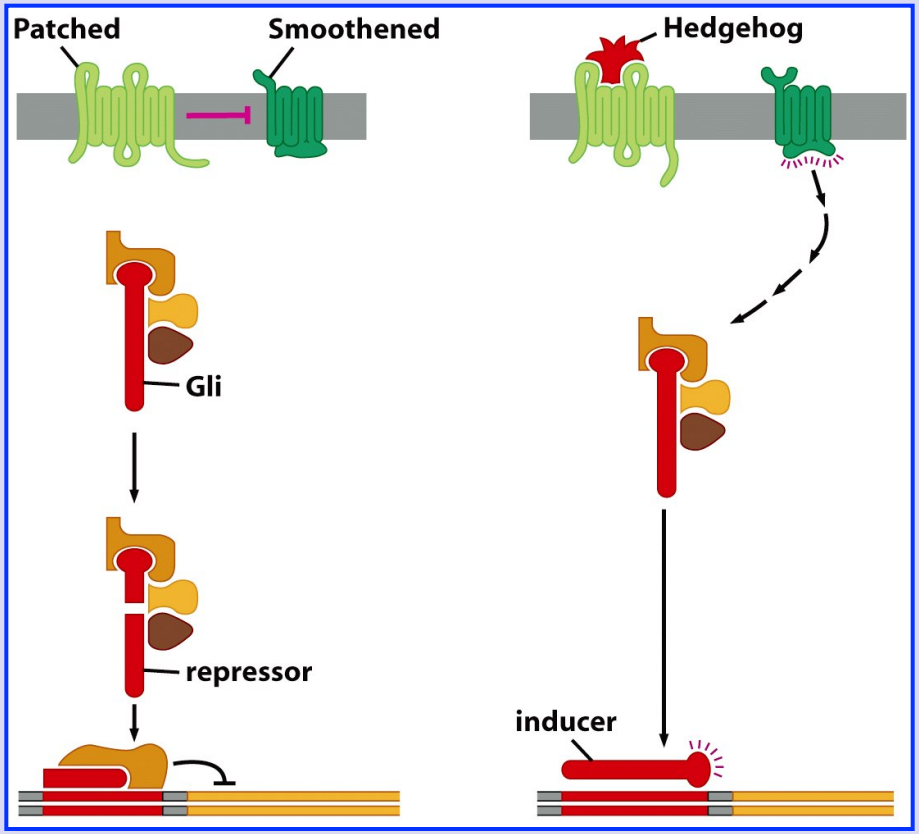
Gorlin syndrome - Nevoid basal-cell carcinoma syndrome

- autosomal dominant cancer syndrome, rare (1: 50-100 000; in patients with basal cell carcinoma 1:200).
- Functionally linked with germinal mutations of ***PTCH*** that mostly (80%) leads to the truncated form of PTCH and thus enhanced expression and activation Gli1; gene located **9q22.3**
- Developmental malformations: skeletal defects, craniofacial defects, polydactyly, syndactyly
- **Skin basal cell carcinomas** (almost all patients with Gorlin syndrome have skin tumors, often early in life), other neoplasms: **medulloblastoma** (from cerebellum), ovarian fibromas, meningiomas, fibrosarcomas, rhabdomyosarcomas
- almost 1/3 of germinal mutations appears *de novo*





Patched signaling pathway



Active Smoothened (released from Patched) protects Gli from proteolytic cleavage. Full-length Gli is transported to the nucleus and function as a transcription activator.

Hereditary endocrine cancer syndromes

- Multiple endocrine neoplasia type 1 - **MEN1**: prone to developing multiple endocrine and nonendocrine tumors (lesions in pituitary, parathyroid gland and pancreas)

- Multiple endocrine neoplasia type 2 −
- **MEN2A**: medullary thyroid carcinoma, parathyroid adenoma, pheochromocytoma

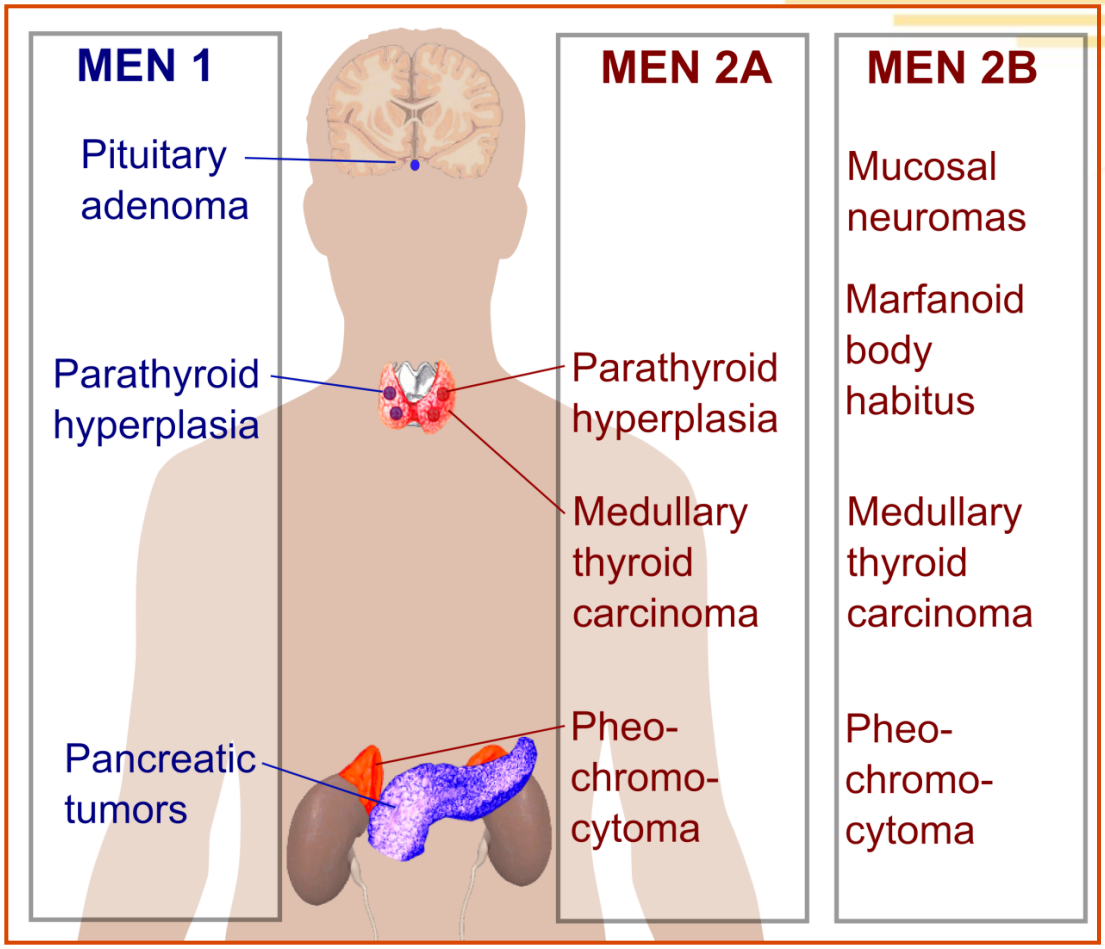
  MEN2B: medullary thyroid carcinoma, pheochromocytoma, mucosal neuromas

- Familial medullary thyroid carcinoma **FMTC**

Multiple endocrine neoplasia type 1 - MEN1

- autosomal dominant genetic disease associated with development of tumors of parathyroid gland (90 - 97 % of patients), islet cells of the pancreas (50 - 65 % of patients), and pituitary gland (40 - 50 % of patients), sometimes non-endocrine tumors
- almost 100 % penetrance; around 10 % of mutations arise *de novo*
- First symptom is usually hyperactivity of endocrine system
- Syndrome is causally linked with germinal mutations of ***MEN1*** (**11q13**), in tumors associated with LOH („2nd hit“)
- *MEN1* gene encodes **menin**, mainly nuclear protein, function unknown, interacts with some TFs, probably negative regulator of proliferation

MEN1 and MEN2



Wilms tumor - nephroblastoma

- Malignant renal cancer in children; one of the most frequent (1:10.000)
- First described by Max Wilms in 1899
- Formation and histological features of Wilms tumor result from aberant embryonic development
- Both hereditary and sporadic form
- Sporadic Wilms tumor occur typically around ages **4 and 5 years** more often in girls; hereditary form (very rare) manifests by the age **2 years**, common **bilateral** formation
- autosomal dominant inheritence with reduced penetrance

Wilms tumor

- 10% of tumors occur bilaterally. If present without family history, usually linked to germinal mutation *de novo*.
- incidence of other neoplasms in cured children (90%) is rather low, and linked more likely with therapy than germinal mutation
- Very rarely occurs in adults, 20 % of that at age 15-20 years

Tumor suppressor WT1

- Wilms tumor associated with mutations of several genes
- First identified (1990) was tumor suppressor ***WT1***, localized at **11p13**; WT1 mutations are associated with Wilms tumor but also with developmental malformations of urogenital tract

- 1 % of patients suffer from WAGR syndrome (**W**ilms tumor, **A**niridia, **G**enital defects, mental **R**etardation).

  Wilms Tumor (WT) in 1 child of 10.000, aniridia in 1 child of 70.000. Aniridia occurs in 1 of 70 children with WT, WT develop 1 of 3 children with aniridia.
- Aniridia is linked with *Pax6* mutation

  „Genes for“ aniridia and WT (*Pax6* and *WT1*) are distinct, but located in close proximity at chromosome 11

  WAGR syndrome is associated with larger cytogenetic deletions of 11p13 (microdeletion syndrome).

Tumor suppressor WT1

- Expressed in kidneys, tightly controlled during development both temporarily and spatially
- Tissue specific transcription factor, plus plays role in post-transcriptional modifications − interacts with RNA
- has anti-proliferative function and probably stimulates specific differentiation of renal cells
- Presumably involved in haematopoiesis: the second most frequent neoplasm conneted with WT1 mutations is **leukemia**; 20 % of AML/ ALL patients have (somatic) mutations of *WT1*
- Other genes causally linked with Wilms tumor: *WT2-WT5*, microdeletion 11p13

Li-Fraumeni syndrome

- Rare autosomal dominant genetic disease (again: dominant at the level of organism and recessive at the level of cell).
- Familial syndrome with characteristic clustering of early neoplasms of different types, including **sarcoma**, **breast and brain carcinomas, leukemias, adrenocortical tumors**
- Tumors in LFS appear in children and young adults, often as a multiple primary foci.
- Syndrome is mostly caused by mutation of tumor suppressor gene ***p53*** (**17p13.1**).

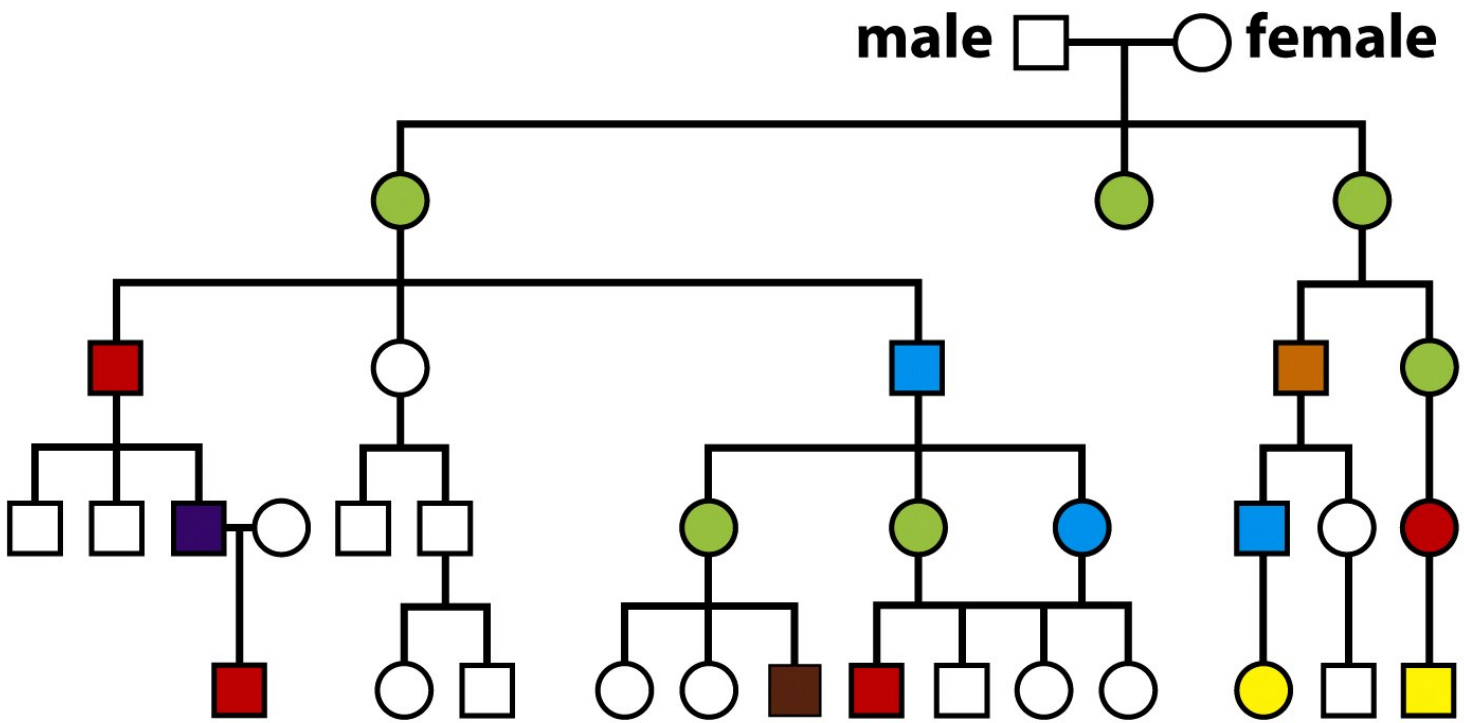
Li-Fraumeni syndrome

LFS is diagnosed according to the following criteria:

- Person with sarcoma by the age of 45 years, 1st degree relative with tumor by the age 45 years, 1st or 2nd degree relative with a tumor by the age 45 years or with sarcoma at any age.

- Around **80%** cases caused by germinal mutation of ***p53***.

Tumors in family with germinal *p53* mutation



Breast cancer
glioblastoma
lung cancer
pancreatic carcinoma
sarcoma
Wilms tumor
leukemia

History of LFS

1969: Li and Fraumeni performed retrospective analysis of children that died from sarcoma. They found several families where siblings and/or cousins were affected and found a parent lineage with high incidence of tumors of various types, especially sarcomas and breast carcinomas at early age.

SBLA syndrom: **S**arcoma, **B**reast and **B**rain cancer, **L**eukemia, **L**ung and **L**aryngeal cancer, **A**drenocortical carcinoma (1978).

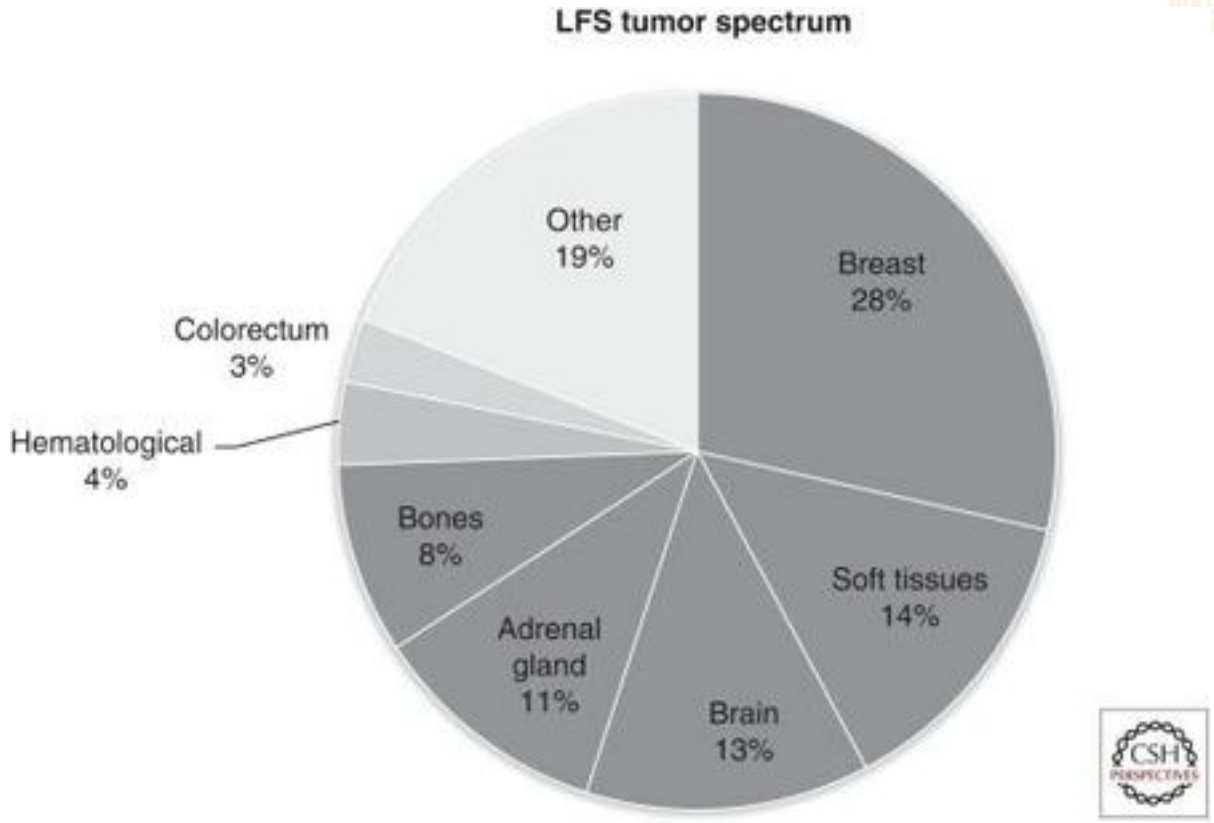
1988: first draft of LFS definition

1995: besides „classical“ LFS also LFLS (LFS-L) defined

Epidemiology of LFS

- occur in 1-9 in 100,000 people worldwide.
- Penetrance: 50% at the age 30 years, 90% at 60 years
  Penetrance higher in women (~100%) − associated with breast cancer.
- *p53* mutations are among the most frequent somatic mutations identified in solid tumors **X** extremely rare LFS syndrome
- **(Genetic) anticipation** – the increased incidence, earlier onset, or increased severity of a disease in successive generations.
  (in a number of neurodegenerative disorders has been shown to be attributable to trinucleotide repeat instability, but mechanism of anticipation in LFS families not known)

LFS tumor spectrum



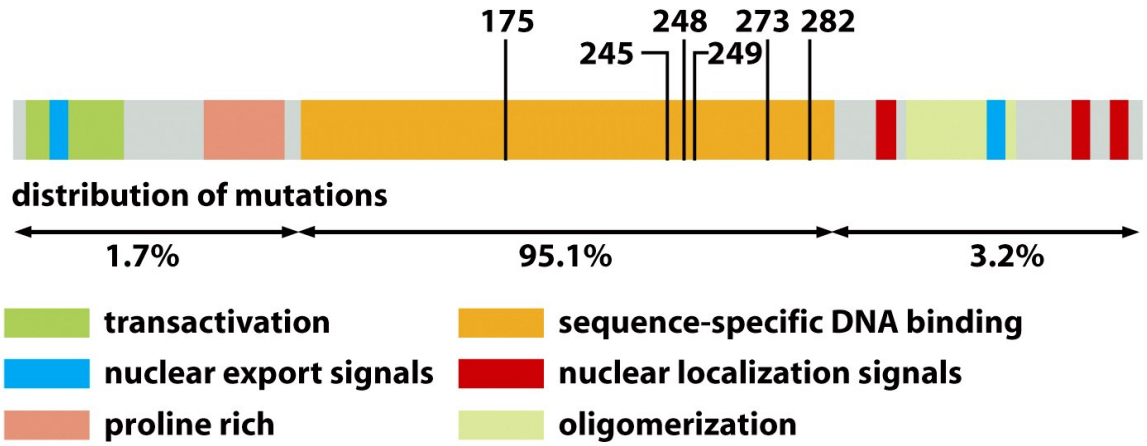
P53 gene and protein

Gene (*TP53*) localized at chromosome 17 (17p13)

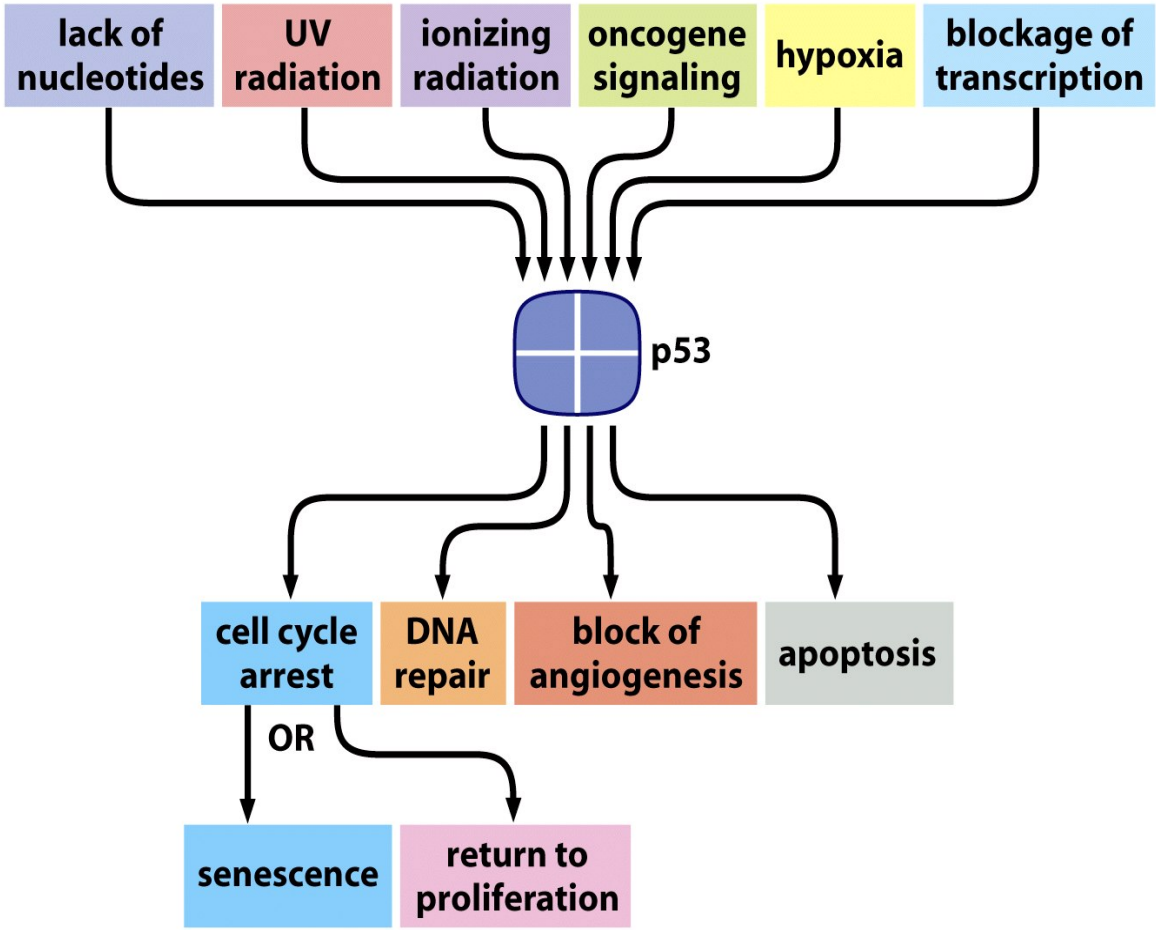
Protein − first described as an interaction partner of LT SV40

- transcription factor
- functions as tetramer

Structure of protein: DNA binding domain, transactivation domain, oligomerization domain. NLS. C-terminal regulatory domain

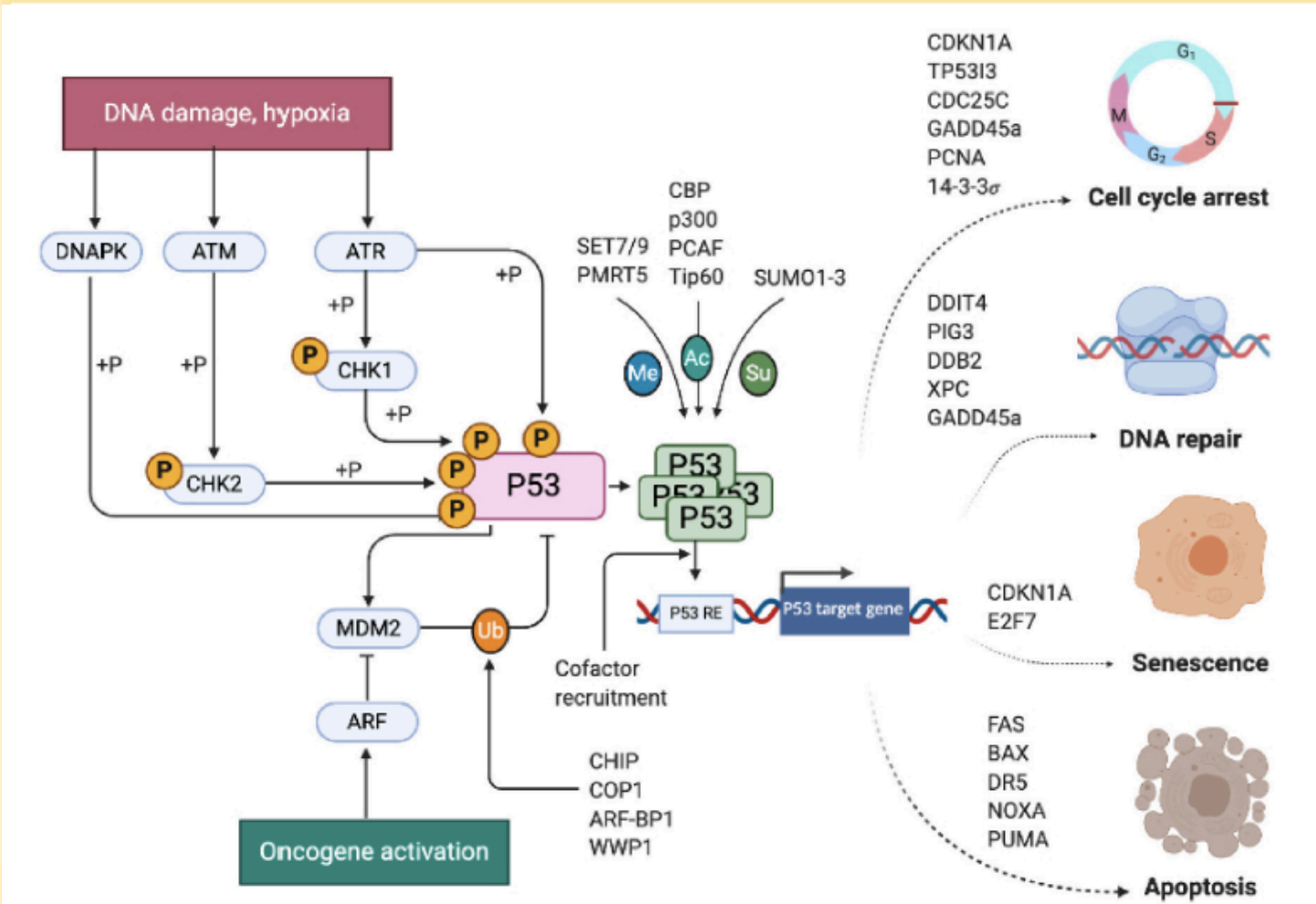


Functional network of p53



Weinberg RA. The Biology of Cancer. *Garland Science* 2007

P53 signaling pathway



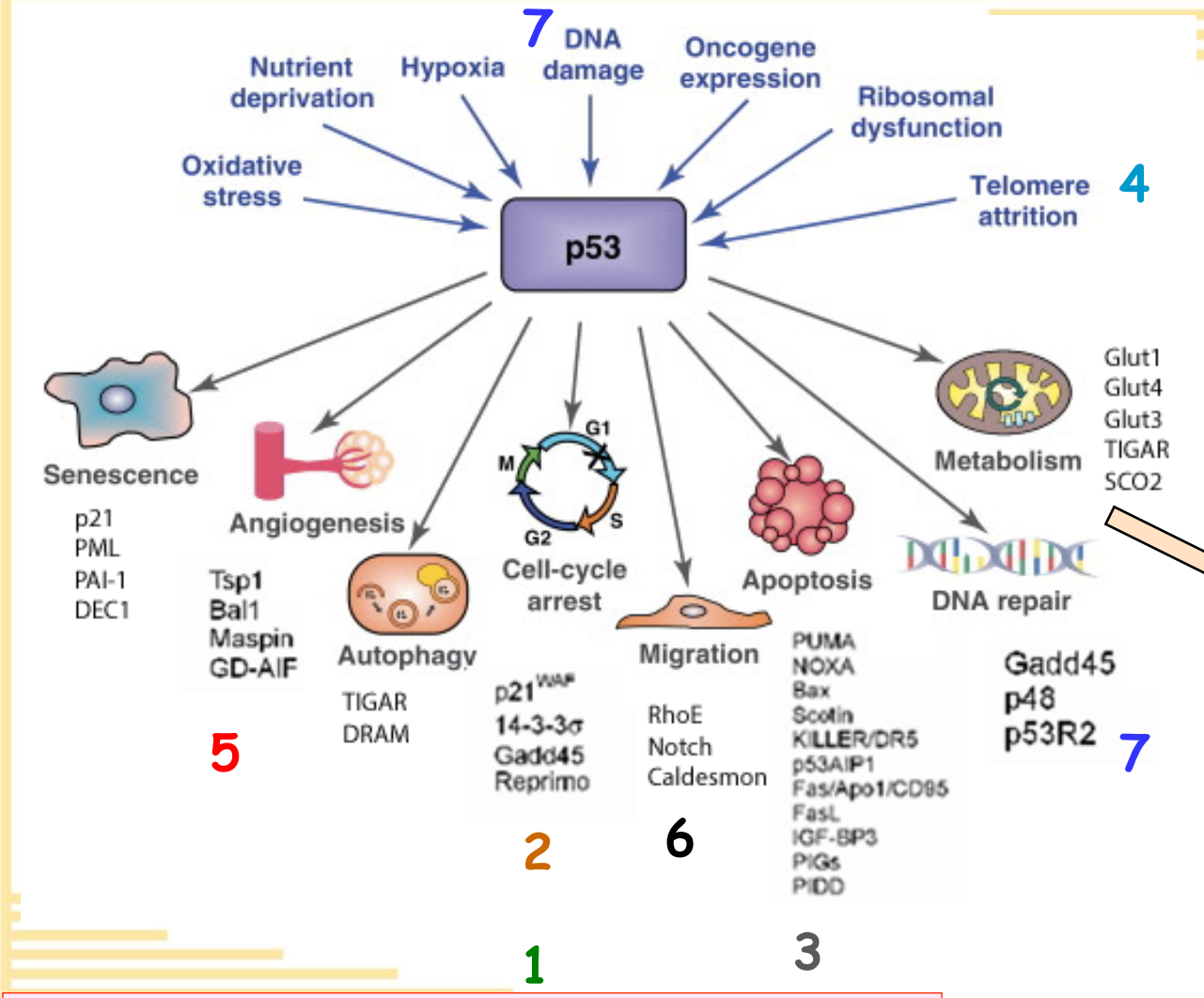
Borrero LJH et al. *Biochim Biophys Acta Rev Cancer*. 2021;1876(1):188556.

Hallmarks of cancer

(1) Sustaining proliferative signaling
(2) Evading growth suppressors
(3) Resisting cell death
(4) Enabling replicative immortality
(5) Inducing angiogenesis
(6) Activating invasion and metastasis
(7) Genome instability and mutation
(8) Tumor-promoting inflammation
(9) Deregulating cellular energetics
(10) Avoiding immune destruction

Hanahan D. and Weinberg R.A., *Cell* 144 (2011) 646-674

p53 functions



Chronic inflammation 8

CXCL1
(NF-κB)

Gudkov 2011
Cooks et al. 2014

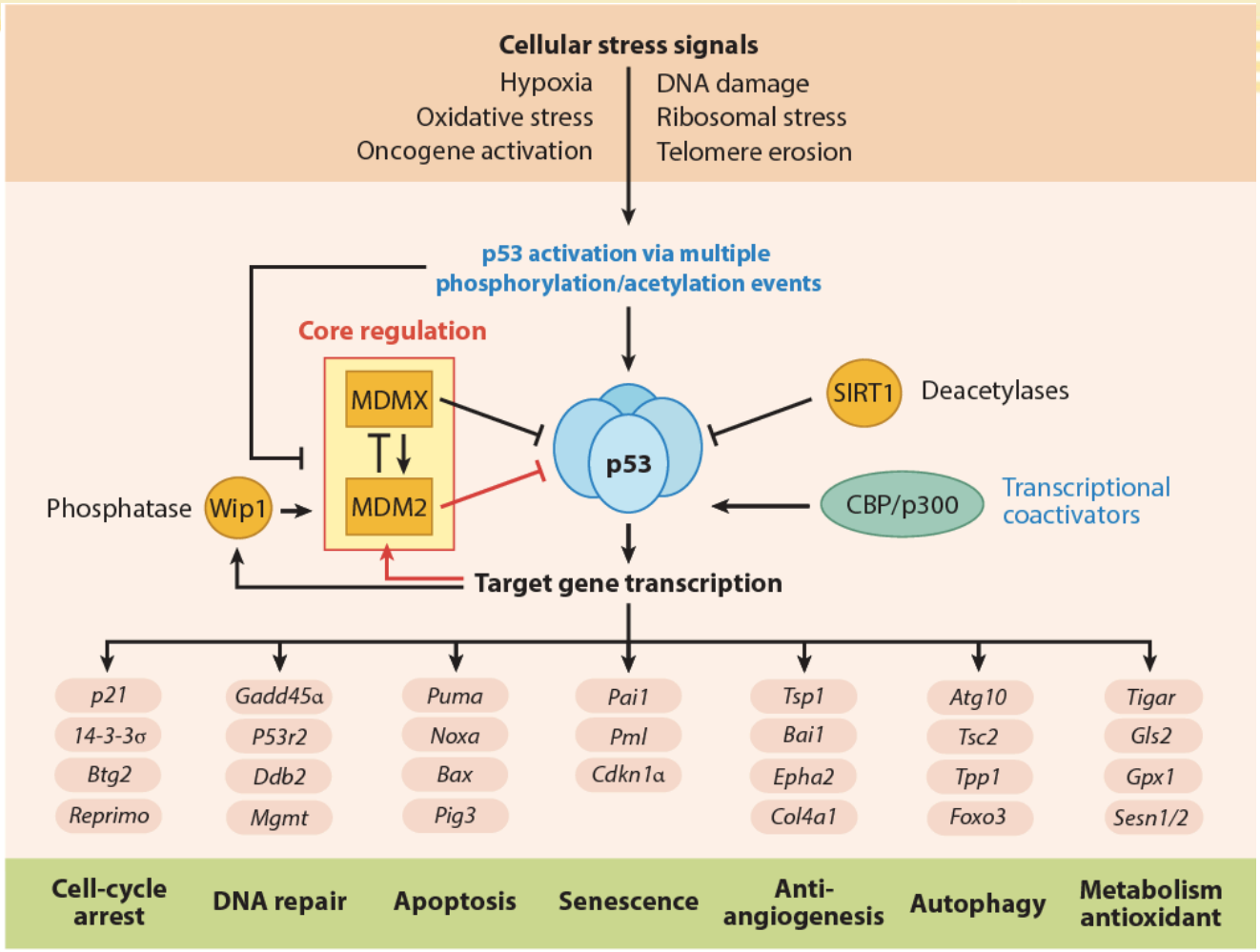
Immune system modulation 10

CD43, ICAM1
Fracktalkine
CSF1, ISG15
IRF5, IRF9
MCP1, PKR

Lowe et al. 2013
Menendez et al. 2013

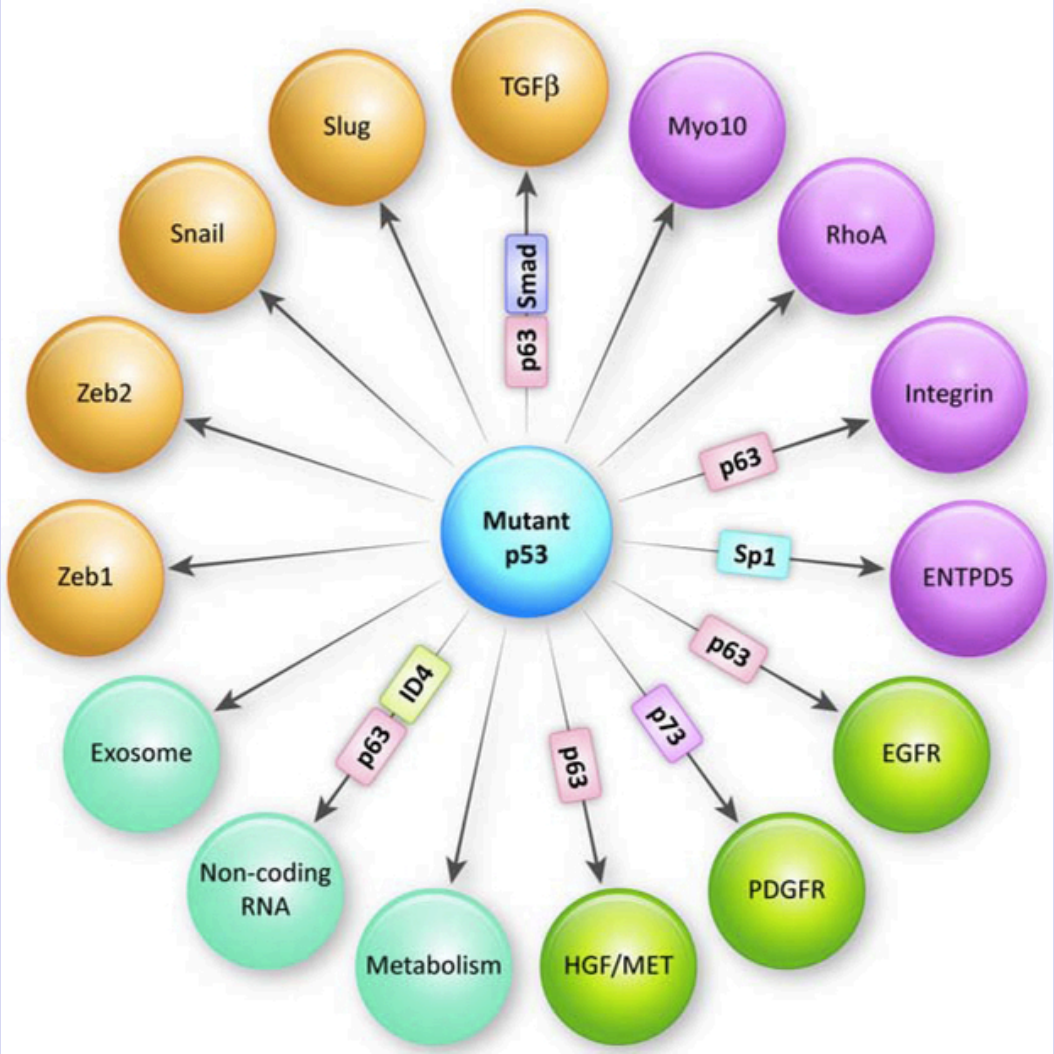
Bieging KT., Trends Cell Biol. 2012;22(2):97-106.

p53 functions

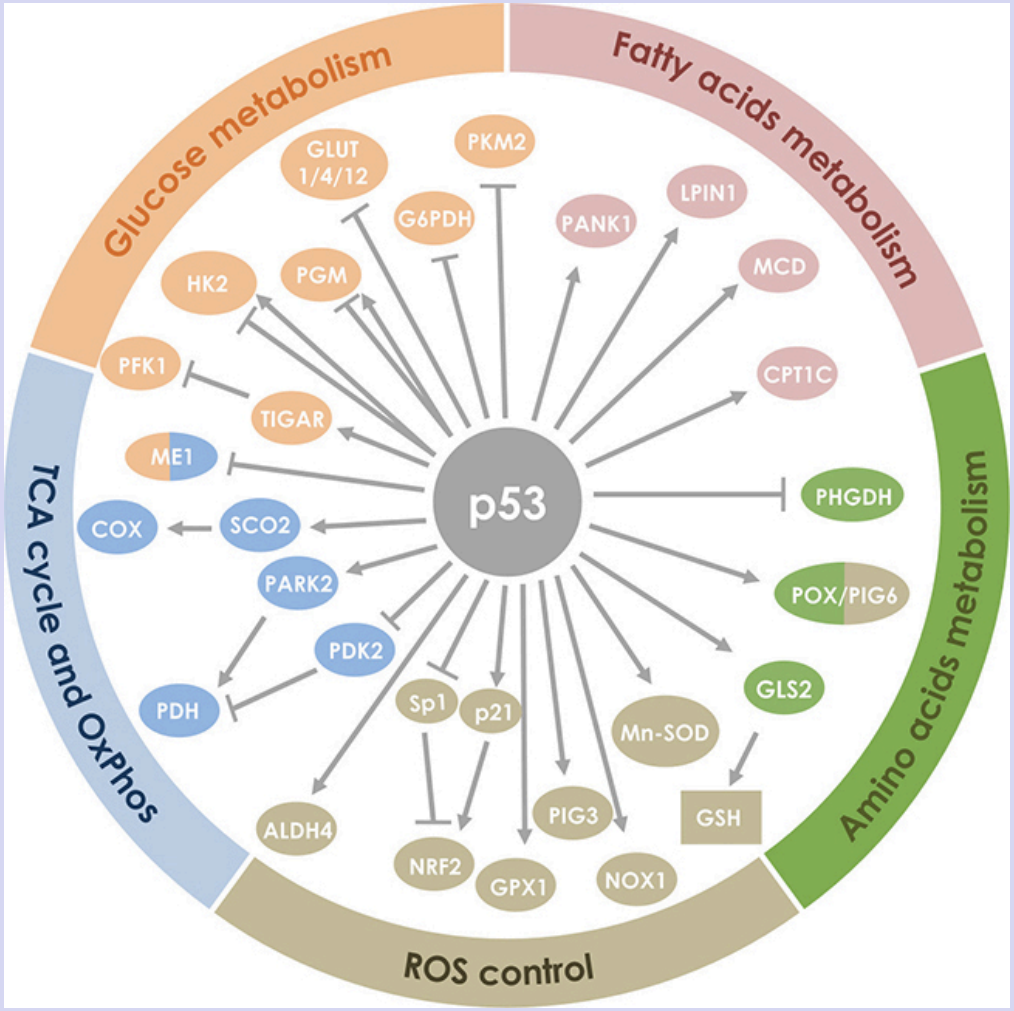


Joerger AC et al. *Annu Rev Biochem*. 2016;85:375-404.

P53 and metastasis



P53 and metabolism



Simabuco FM, et al. Oncotarget. 2018;9(34):23780-23823.

P53 function

- Mediates appropriate cellular response to stress.

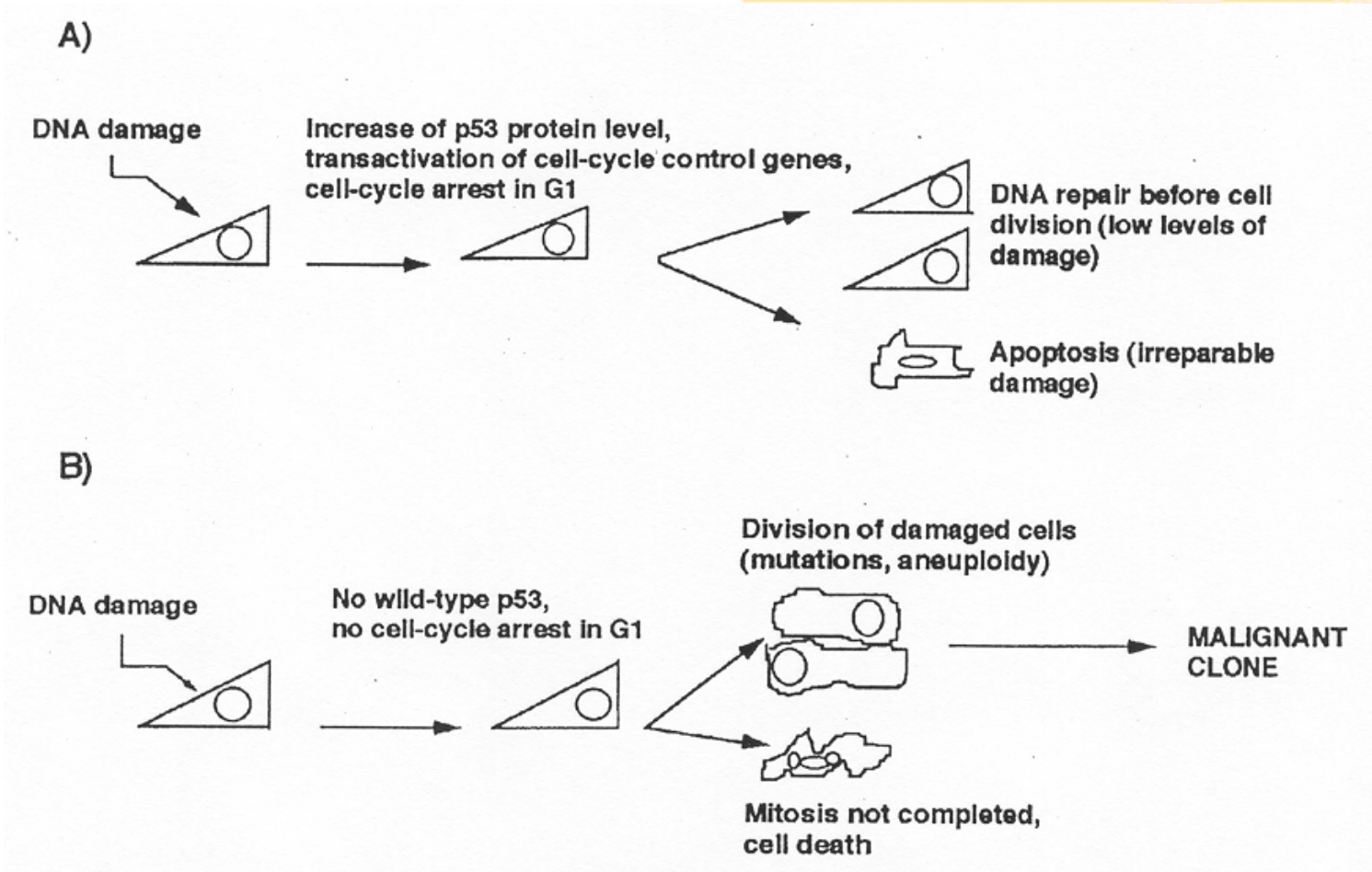
Target genes: ***mdm2*** − negative feedback loop

***p21*$^{CIP1/WAF1}$** - G1 cell cycle arrest

bax - apoptosis

GADD45 − DNA repair

p53 – guardian of genome



Modes of p53 regulation

- Regulation of *TP53* transcription and translation
- Regulation of p53 protein levels/**stability**
- Regulation of p53 subcellullar localization
- Regulation of p53 activity

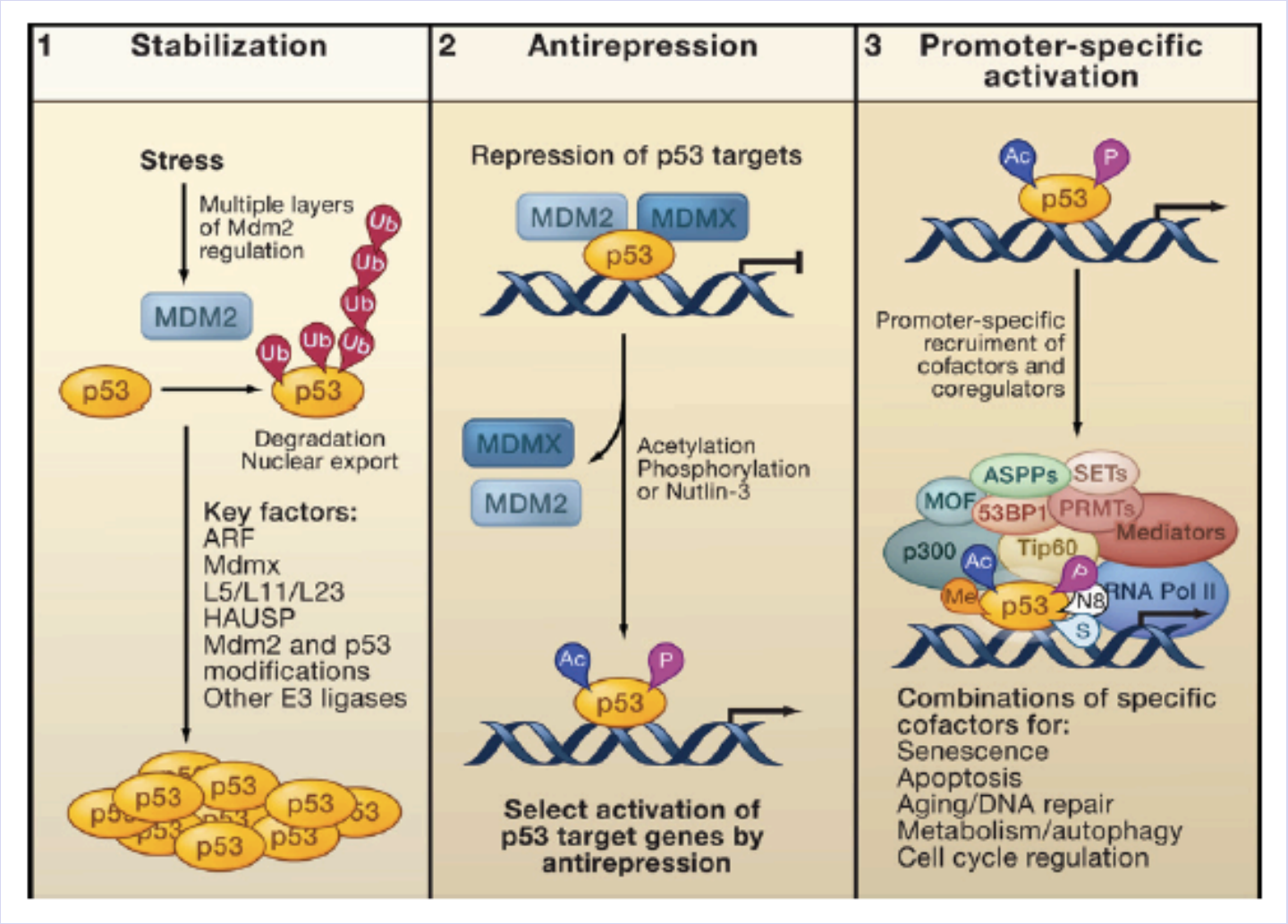
Regulation of p53 stability

- In normal proliferating cells the levels of p53 are kept low by fast proteosome-mediated degradation

- Critical role in regulation of p53 stability plays **MDM2**.
- MDM2 acts as a ubiqutin-ligase E3: after interaction with p53 catalyzes ubiquitinylation of p53 and thereby its proteosomal degradation.
- *mdm2* is p53 target gene (→ feedback loop)

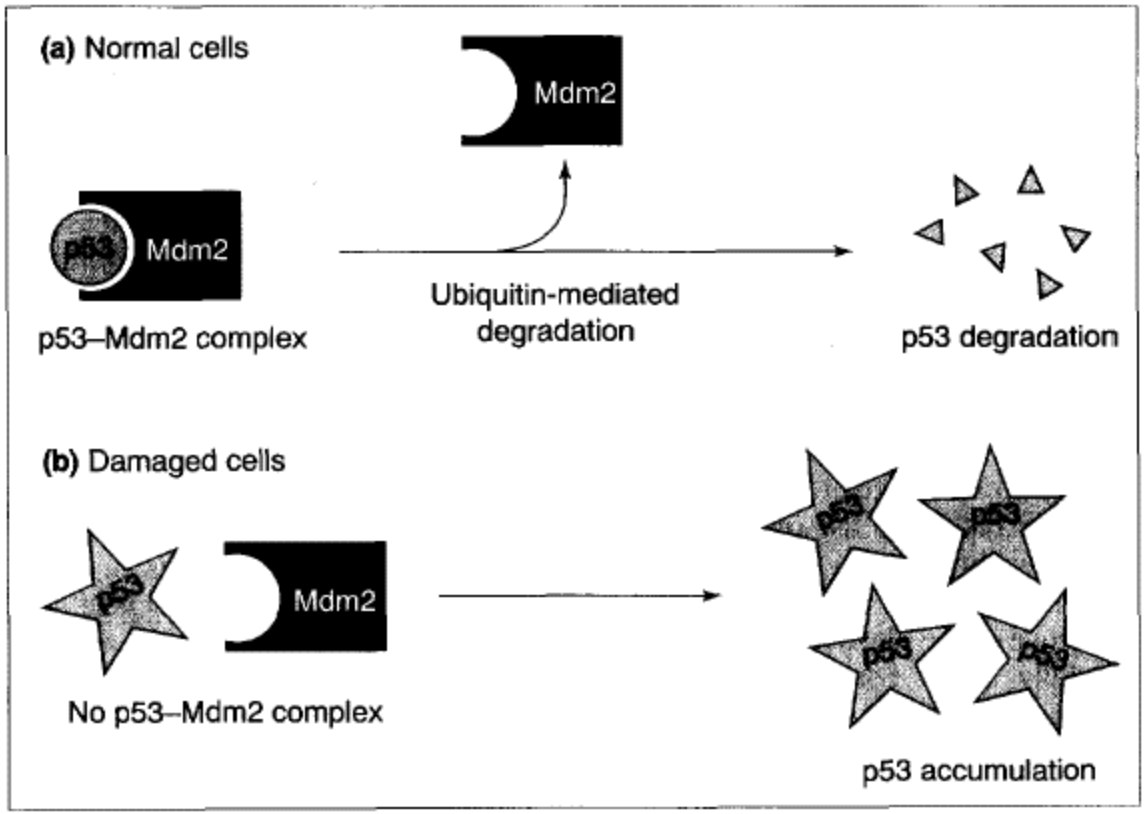
- **MDMX** (MDM4) − another regulator of p53: by interaction with p53 prevent its transcription-activation function (does not affect its protein levels)

Regulation of p53 stability



Kruse JP, Gu W. *Cell*. 2009;137(4):609-22.

MDM2 function in p53 regulation: in normal and damaged cells



Upon cellular damage p53 is released from interaction with MDM2 and stabilized.

Regulation of interaction p53 - MDM2

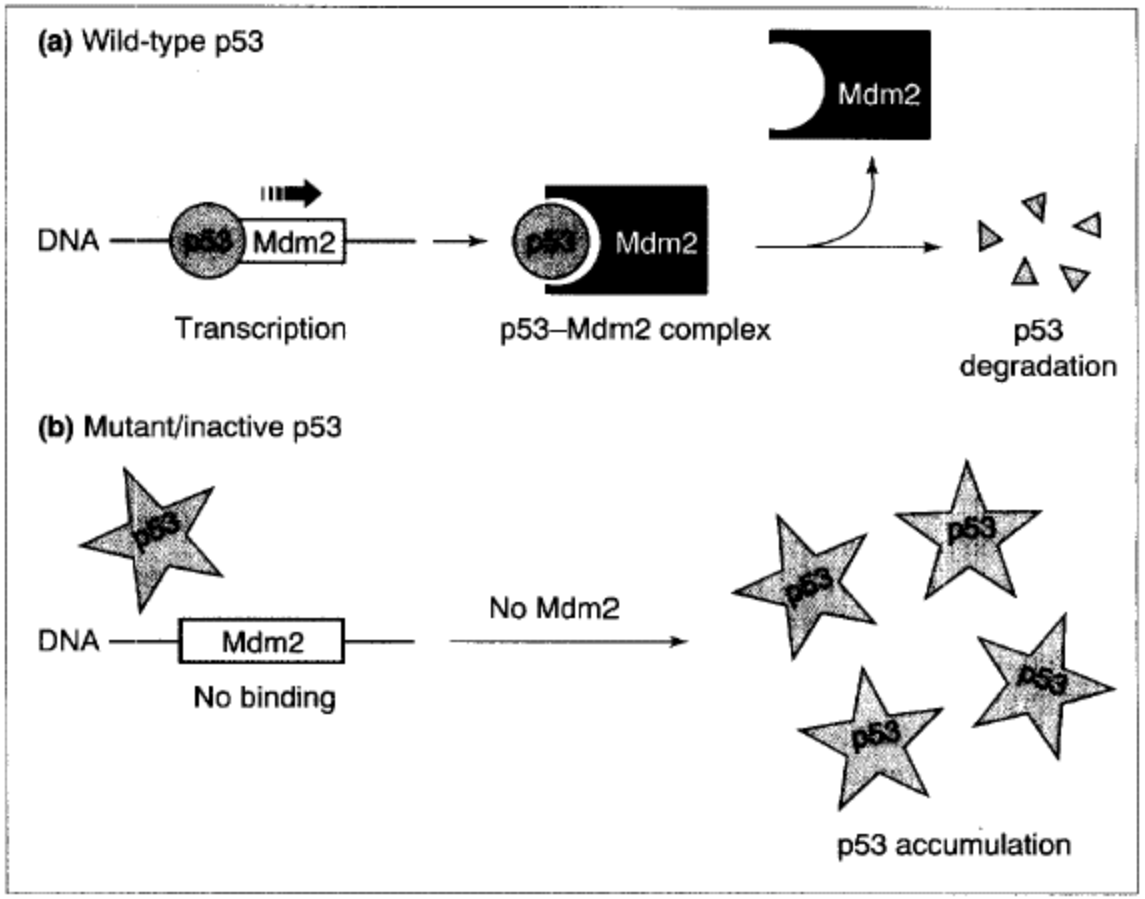
- Interaction of p53 with MDM2 is blocked by **phosphorylation**
- P53 is phosphorylated in response to cellular stress (DNA damage, hypoxia, …) by a range of kinases: **Chk1**, **Chk2**, **ATM** and **ATR**, JNK, Polo-like kinase,...

- Cells with mutated *ATM* and *Chk2* are unable to activate p53

- germinal mutation in ***chk2*** (*G2 checkpoint kinase*) was associated with LFS

  (germinal mutations of *chk2* are linked with higher risk of breast cancer − occur in 5% of patients with breast carcinoma)

MDM2 in p53 regulation: functional vs. non-functional p53



Regulation of subcellular localization of p53

- p53 has several nuclear localization signals (**NLS**) at the C-terminus, upon synthesis it is transported to the **nucleus**

- p53 has 2 sequences for nuclear export signals - **NES**: 1st in oligomerization domain (masked in functional tetramer) and 2nd at the N terminus in MDM2 binding domain. Nuclear export is facilitated by MDM2 interaction (but not required for export) and modulated by phopsphorylation − stress-induced phosphorylation prevent nuclear export (and prevent interaction with MDM2)

- Kinase **Parc** withold p53 in cytoplasm

Regulation of p53 activity

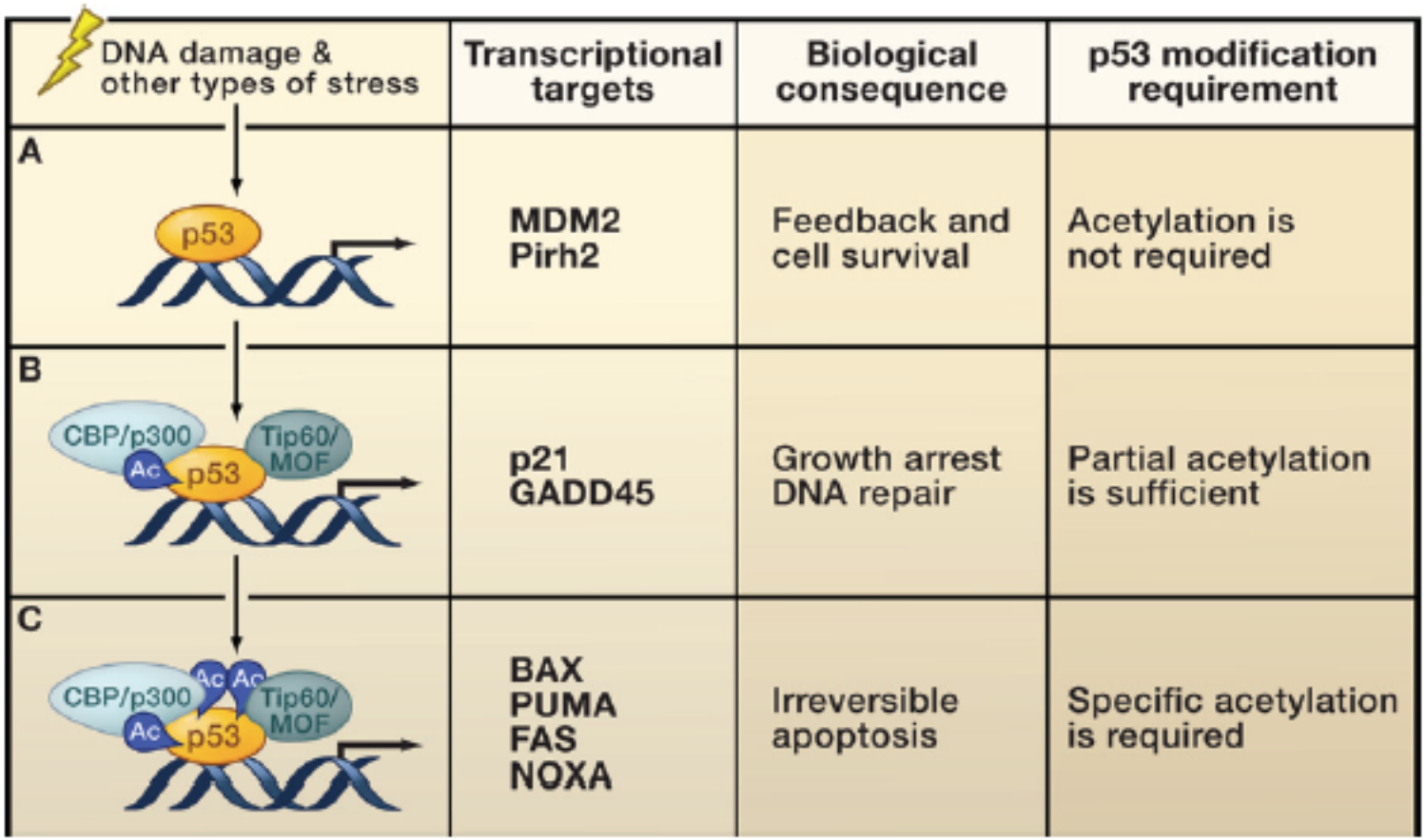
Ability of p53 to interact with DNA and other co-factors is regulated by **posttranslational modifications**.

C-terminus: different covalent and non-covalent modifications (phosphorylation, sumoylation, glycosylation, acetylation, *ubiquitination*, *neddylation*) affect the ability of p53 to bind DNA: e.g. p53 is activated by ser392 phosphorylation (induces by UV), sumoylation of Lys386,

N-terminus: Stress-induced phosphorylation of N-terminus facilitates interactions with acetyltransferases − these acetylate C-terminus and also histones in nucleosomes of target genes, which affect transactivation by p53)

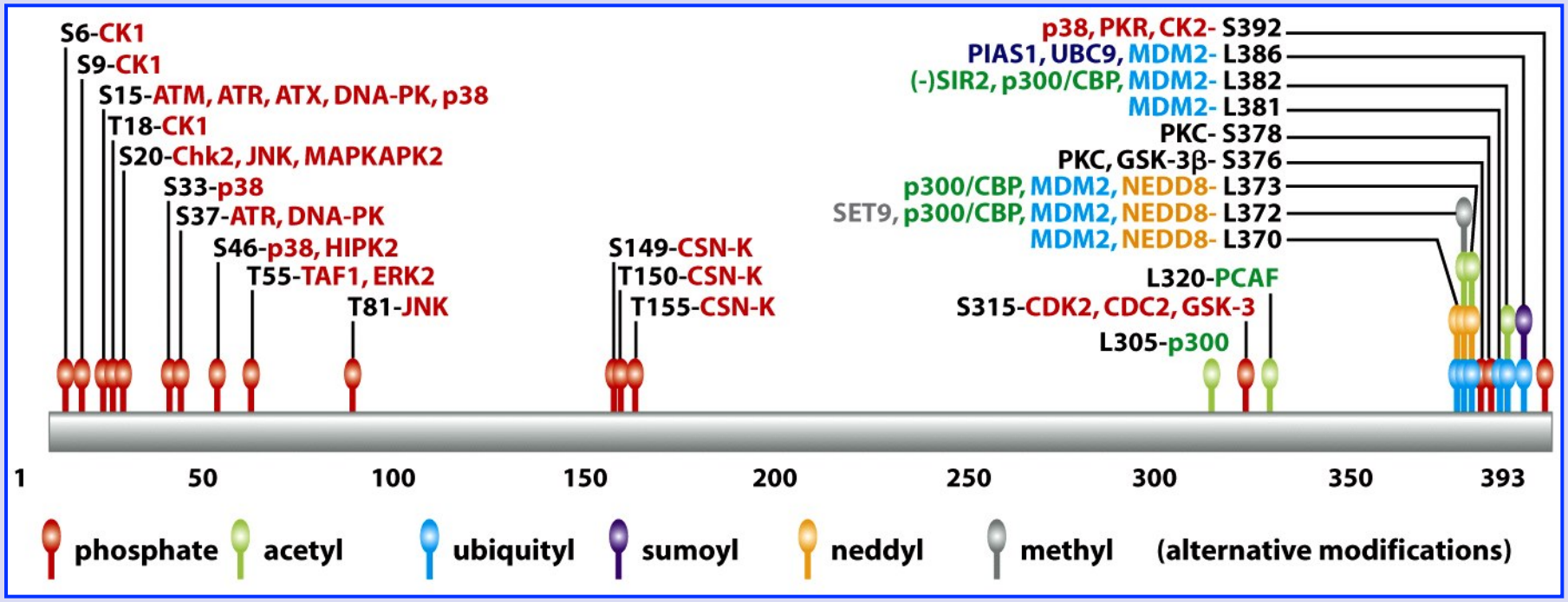
Regulation of p53 activity

Upon stress-induced p53 activation, different sets of p53 target genes have different requirements for p53 posttranslational modifications.



Kruse JP, Gu W. *Cell*. 2009;137(4):609-22.

Posttranslational modification of p53

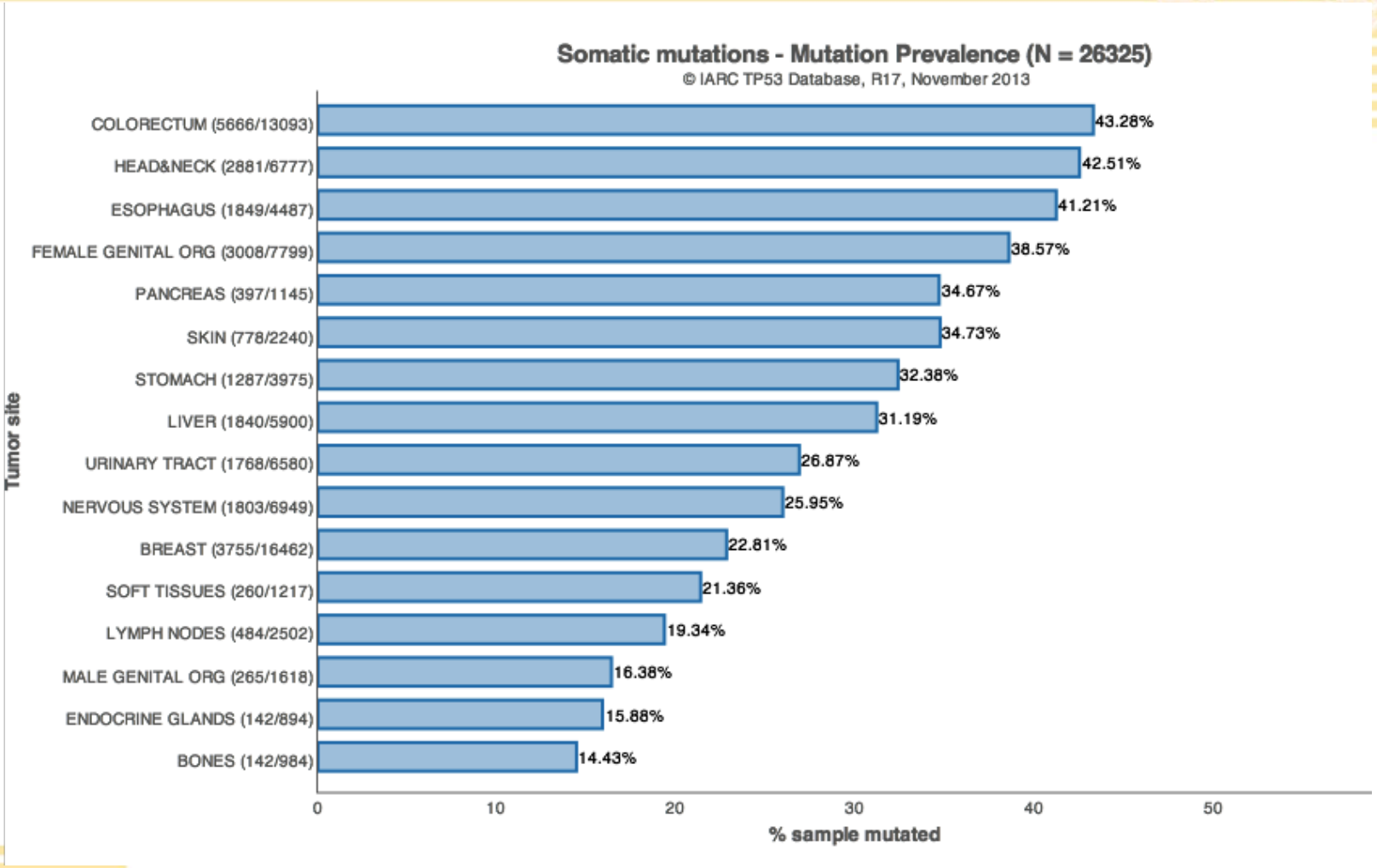


Posttranslational modifications affect p53 activity and stability.

Inactivation of p53 in cancer

- **Mutations**
- **Nuclear exclusion** (37 % of inflammatory breast cancer, more than 90 % of poorly differentiated neuroblastomas)
- **Interaction with viral oncoproteins** (LT SV40, E1B, E6)
- **Amplification of *mdm2*** (neuroblastomas), sometimes ***mdmX*** (gliomas)

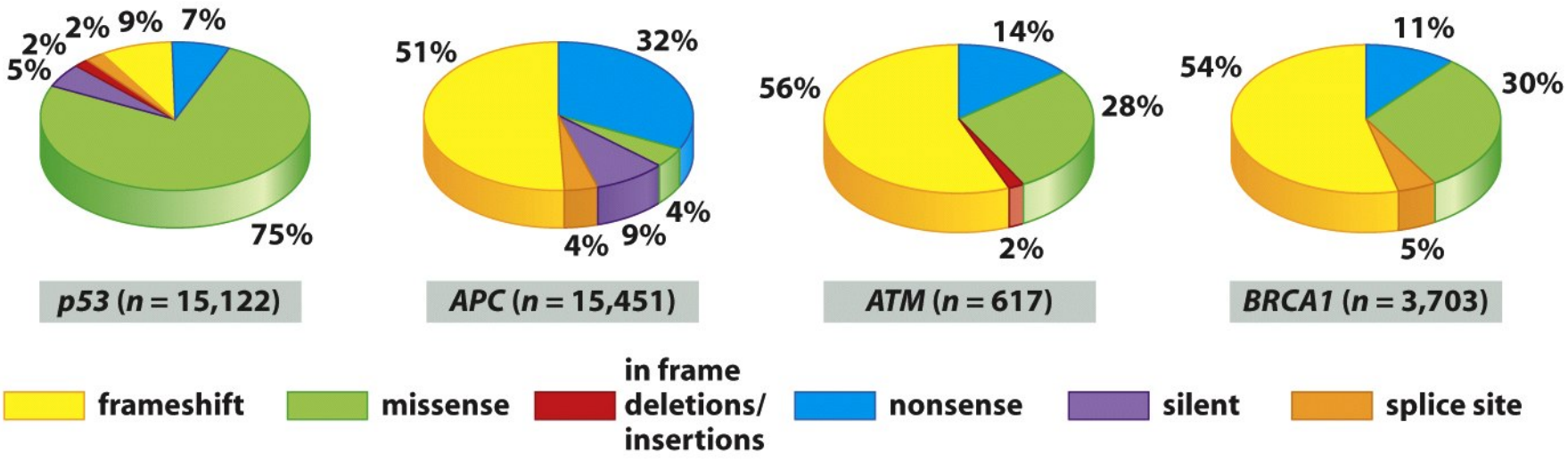
Frequency of p53 mutations in cancer types



https://www.mark53.com/en/for-physicians/tp53-wachter-des-genoms/

Types of p53 mutations

- Mostly point missense mutations
- short deletions and insertions (more in terminal regions of the gene)



Weinberg RA. The Biology of Cancer. *Garland Science* 2007

Thank you for attention!