Molecular and Cell Biology of Tumors

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Molecular and Cell Biology of Tumors

5. Cancer predisposition genes II

Hereditary leukemia

Table 1
Genetic predisposition to MDS/AML.

Gene	Syndrome	Hematologic malignancies
CEBPA	Familial AML with mutated *CEBPA*	–
DDX1	Familial AML with mutated *DDX41*	MDS, CMML
RUNX1	Familial platelet disorder with propensity to myeloid malignancies	MDS, T-ALL
ANKRD26	Thrombocytopenia 2	MDS
ETV6	Thrombocytopenia 5	MDS, CMML, B-ALL, PCM
GATA2	Familial MDS/AML with mutated *GATA2*	MDS, CMML
SRP72	SRP72-associated familial aplasia and myelodysplasia	MDS
SAMD9	MIRAGE syndrome	MDS
SAMD9L	Ataxia-pancytopenia syndrome	MDS

1. **familial cancer syndromes** associated with increased risk of various malignancies including myelodysplasia and acute myeloid leukemia such as Li-Fraumeni syndrome

2. **pediatric inherited bone marrow failure syndromes** such as Fanconi anemia

3. **germline mutation**s conferring a specific increased risk of myelodysplastic syndrome and acute myeloid leukemia

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Hereditary myeloid malignancies

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Colorectal carcinoma - CRC

Sporadic

Hereditary:

- **Non polyposis**

  Lynch syndrome

- **Polyposis**

  Familial adenomatous polyposis (FAP)

  Peutz–Jeghers syndrome

  Juvenile polyposis syndrome

  Hyperplastic polyposis syndrome

Colorectal cancer

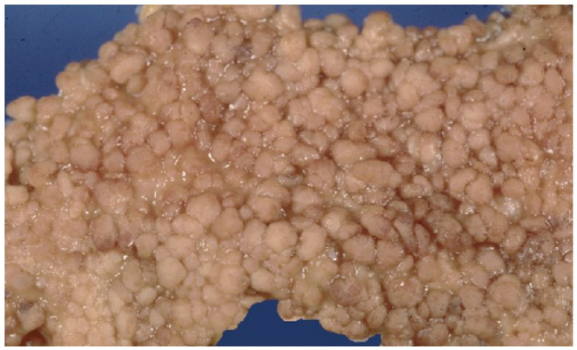
- Only 5-10 % of CRC patients have apparent inherited genetic predisposition for the cancer.

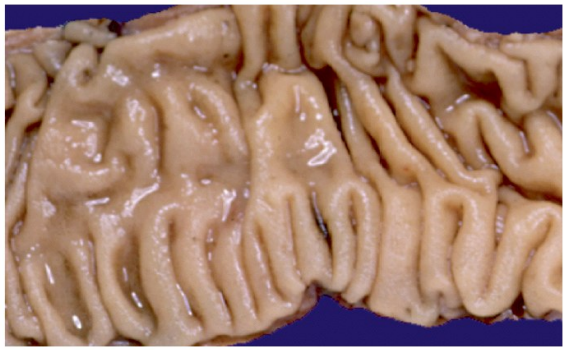
- Significant impact of environmental factors, especially **diet**! (high-fat, high processed meat, low-fibre).

  Historical example: In Japan, there is a low incidence of colorectal cancer, but high incidence of stomach cancer. Japanese immigrants in USA (but not Brazil) had higher CRC risk. Changes in incidence of other cancers too.

Familial adenomatous polyposis - FAP

- autosomal dominant inherited condition
- FAP patients develop numerous adenomatous polyps mainly in the epithelium of the large intestine. These polyps are benign, though malignant transformation may occur. Average age of tumor diagnosis in FAP patients is 40 years..





Polyps formed in FAP patients are histologically similar to sporadic polyps and individual FAP polyps do not have increased risk of malignant transformation compared to sporadic polyps. But their high numbers means that in almost 100 % of FAP patients occur cancer foci.

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

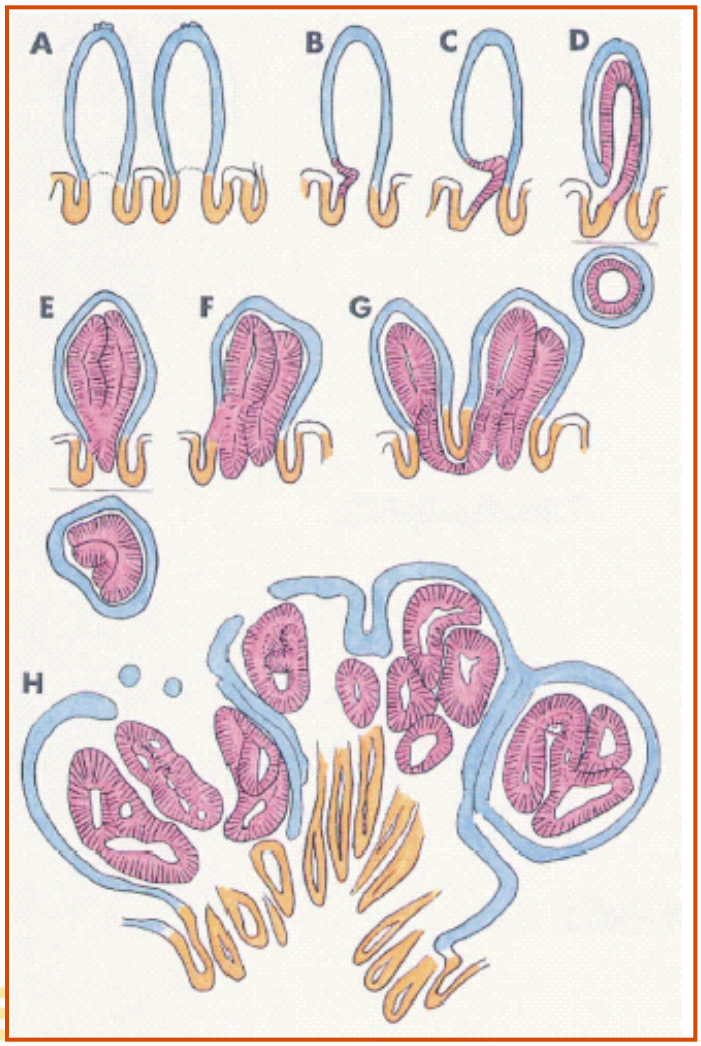
Familial adenomatous polyposis

- Increased risk of other cancers: thyroid, small intestine, stomach and brain
- Often „extra-intestinal“ symptoms, such as retinal defects, osteomas, etc.
- Usually associated with germinal mutation of tumor suppressor *APC* (*Adenomatosis Polyposis Coli*)

Huge variability among FAP patients, subtypes described:

- Gardner syndrome – autosomal dominant form of polyposis, characterized by extra-colonic growths in soft tissues, osteomas, dental anomalies, …
- Attenuated FAP – residual *APC* activity, less polyps (10-100)
- Turcot syndrome – characterized by combination of neuronal tumors (glioblastomas, medulloblastomas) and colon tumors (not all cases of Turcot syndrome is linked with *APC* mutations)

Formation of adenomatous polyps in FAP



A. Normal intestinal epithelium: stem cells, differentiated cells in the villi

B. - C. Formation of polyps (from the cells of proliferative zone): adenomatous tissue

D. - F. Microadenomas

G. Expansion of a microadenoma into a neighboring villus

Bienz M and Clevers H, *Cell* 103 (2000) 311-320

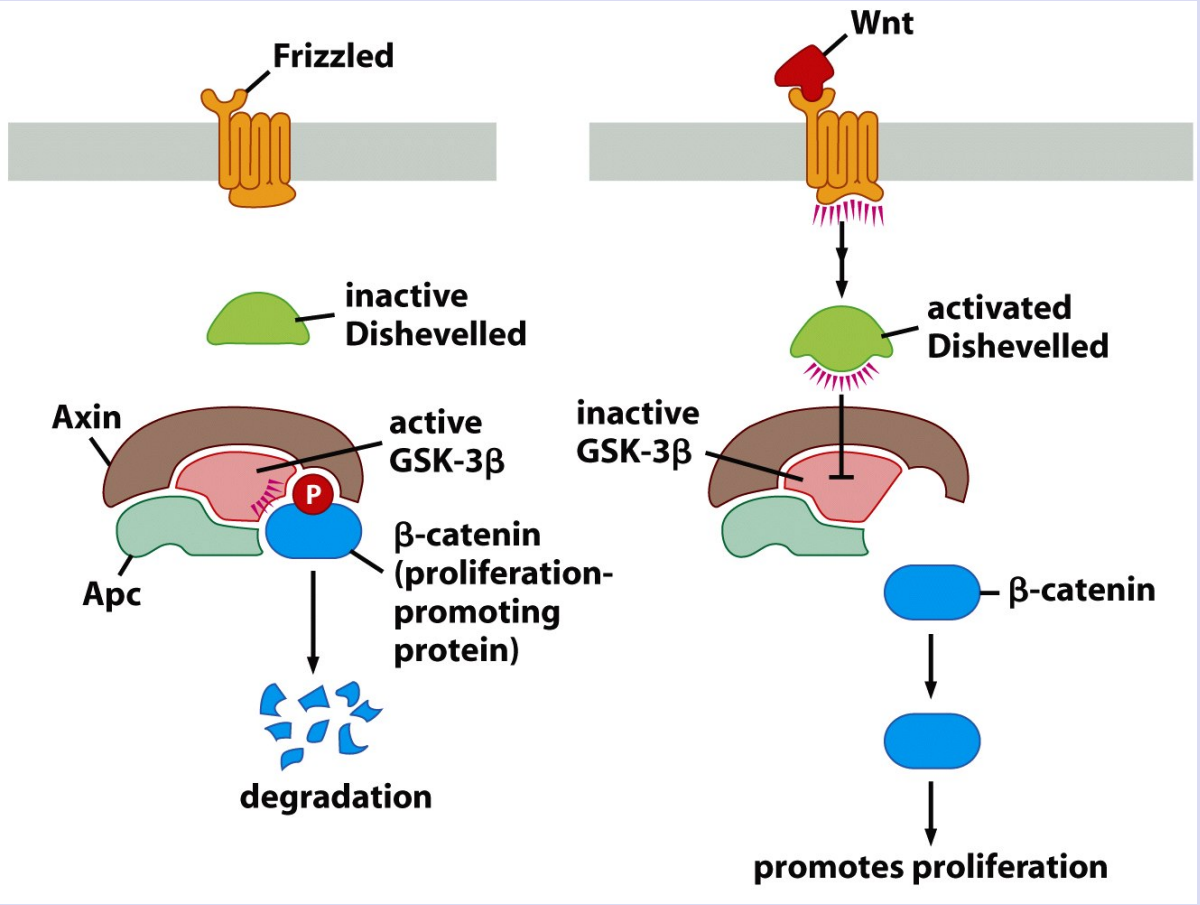
Familial adenomatous polyposis

- ***APC*** gene located at **5q21**
- During cancerogenesis both alleles of *APC* gene have to be inactivated

  (⇔ frequent „LOH“ 5q)
- somatic mutations of *APC* are also key events in development of sporadic CRC, detected in almost 80 % patients
- High portion of mutations that lead to the truncated forms of protein
- Relation between type of mutation and clinical manifestation iof disease (between genotype and phenotype)

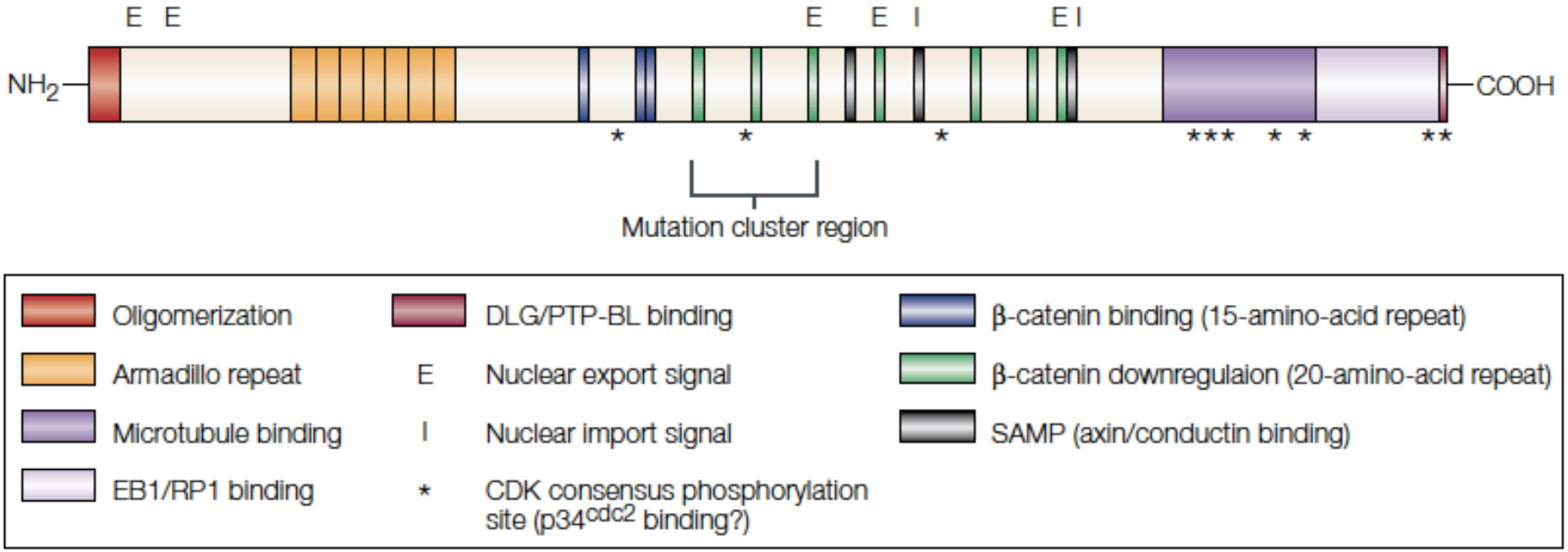
APC and Wnt/β-catenin pathway

- APC is a component of a cytoplasmic protein complex that targets β-catenin for destruction
- Maintains low levels of free β-catenin unless Wnt triggers mitogenic pathway (target genes: c-*myc*, *CCND1*)



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

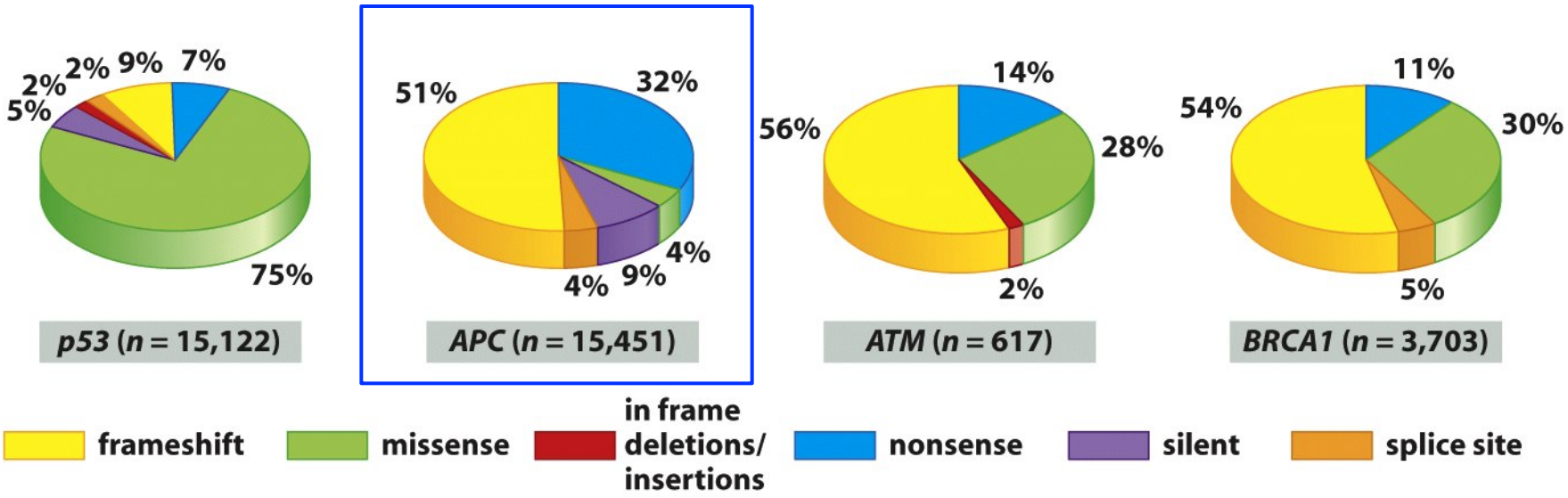
Structure of APC protein



Fodde R et al, *Nat Rev Cancer* 1 (2001) 55-66

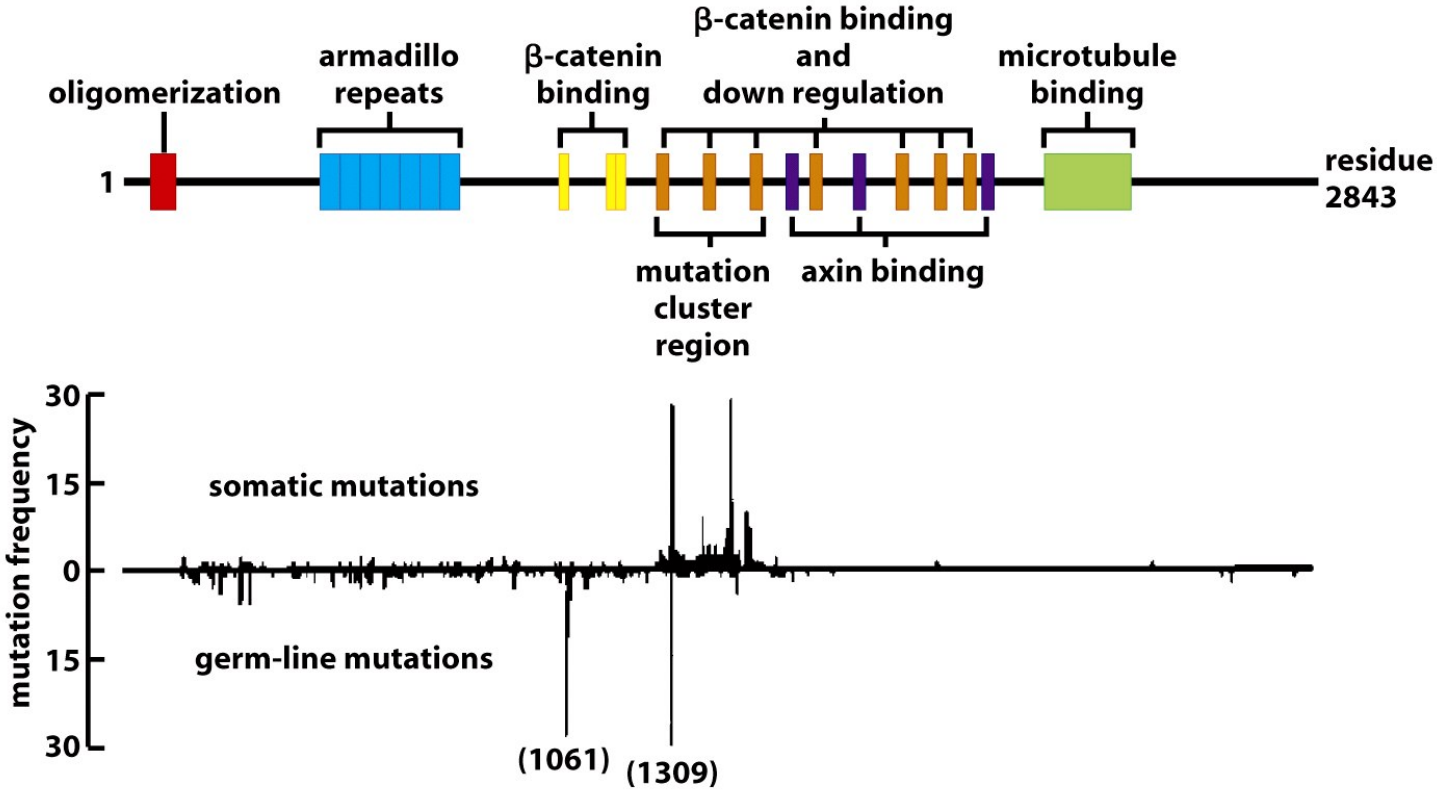
APC mutations

- Most *APC* mutations cause production of truncated proteins that lack axin or β-catenin binding motifs
- Germ-line mutations are scattered in 5‘ half of APC gene, somatic mutations are clustered mainly between codons 1286 and 1513.



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

Structure of APC and mutation frequency

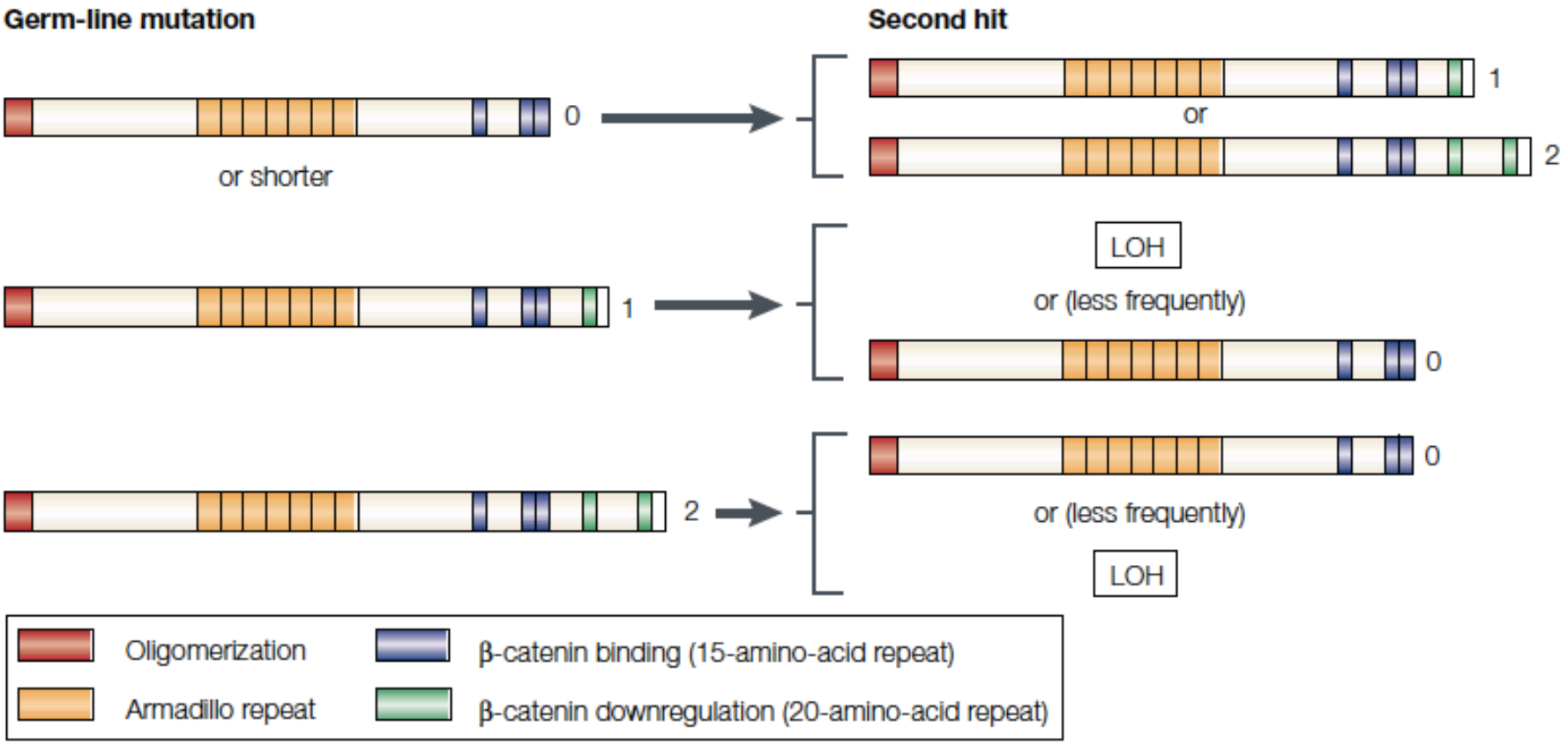


APC mutations

- **Both position and type of second-hit somatic mutation depend on position of first germ-line mutation!**
- Concrete genotypes are selected during early phases of cancerogenesis in order to secure „optimal“ levels of free β-catenin, maximal levels are not desirable because of apoptosis induction!

⇒ Either germinal or somatic mutation preserve at least one specific (2AA) sequence required for β-catenin down-regulation.

Interdepedence of germ-line and somatic mutations of *APC*



Fodde R et al, *Nat Rev Cancer* 1 (2001) 55-66

Dual function of APC in cell: dual role in CRC development

APC contributes both to the tumor initiation (conferring the growth advantage via activation of Wnt-1/β-catenin pathway), and tumor progression at the later phases via induction of chromosomal instability.

APC is both „caretaker“ and „gatekeeper“.

Next stages of CRC development

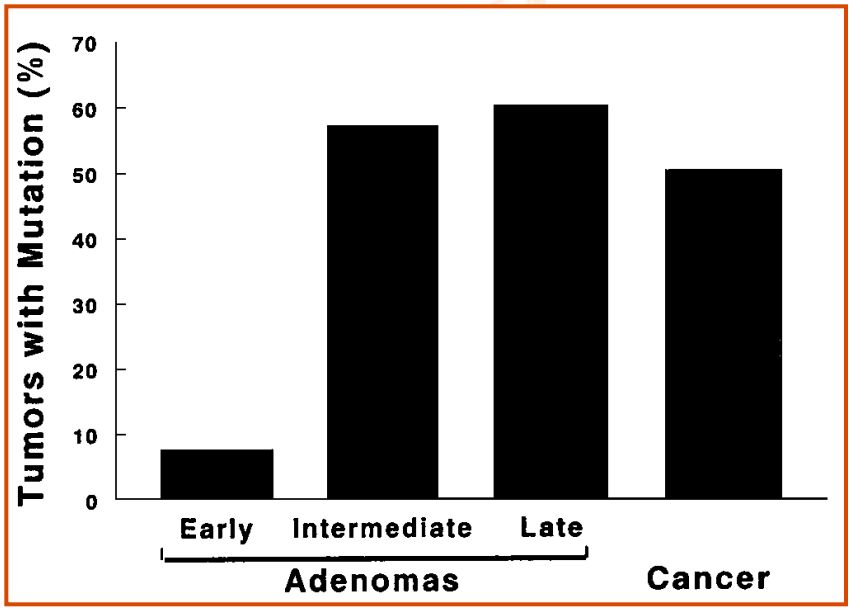
Colorectal carcinomas represent a good **model to study cancerogenesis**:

- Relatively steady and predictable course from small adenomas (up to 1 cm) to big adenomas and malignant carcinomas
- Projection of molecular changes into anatomical and histological features of each stage
- model of co-operation between tumor suppressors and oncogenes

Next stages of CRC development – activation of K-*ras*

- Specific point mutations of proto-oncogene **K-*ras*** or N-*ras* are present in 50 % of colorectal adenomas bigger than 1 cm and in 50 % of late adenomas/carcinomas
- Mutations of *ras* are very rarely (up to 10 %) found in smaller adenomas (< 1 cm)

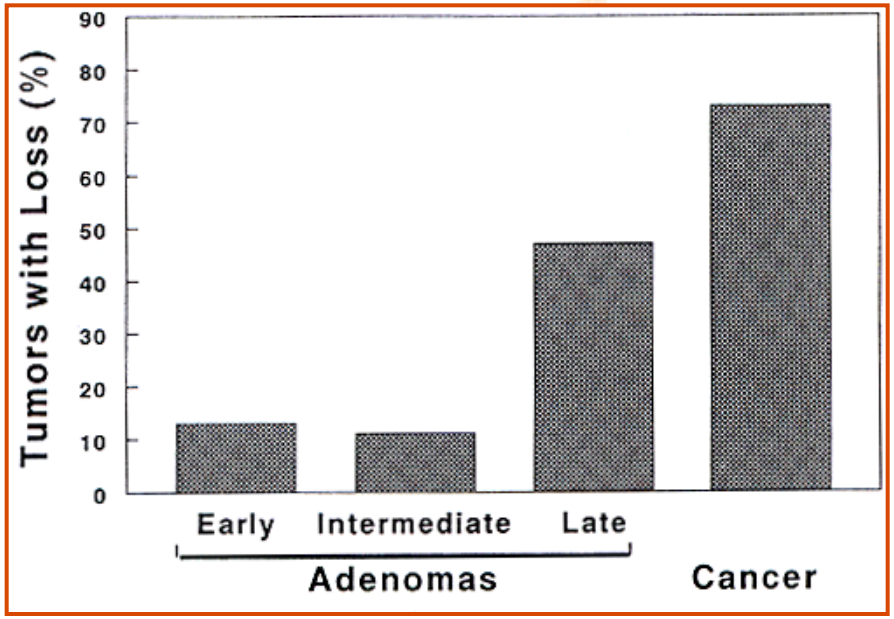
⇒ Mutations of *ras* occur during progression of adenomas (transition between early and intermediate adenomas)



Kinzler KW and Vogelstein B, *Nature Rev Cancer* 2 (2002) 673-680

Next stages of CRC development - „LOH“ 18q

- Deletions of 18q found in 50 % of late adenomas and 70 % of carcinomas
- 18q contains gene ***SMAD4***, alias *DPC4* (*deleted in pancreatic cancer*)
- SMAD4 is a component of TGF-β signaling

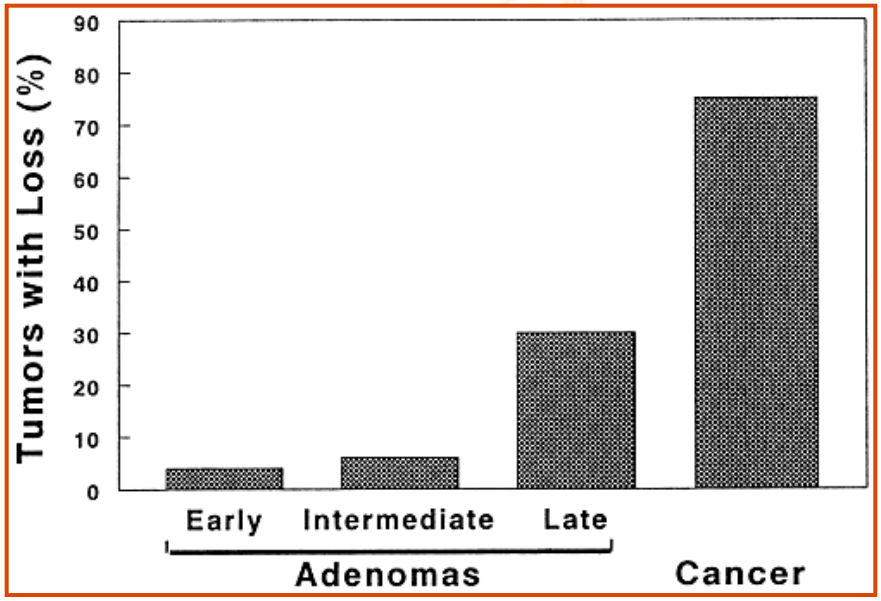


Kinzler KW and Vogelstein B, *Nature Rev Cancer* 2 (2002) 673-680

Next stages of CRC development – inactivation of p53

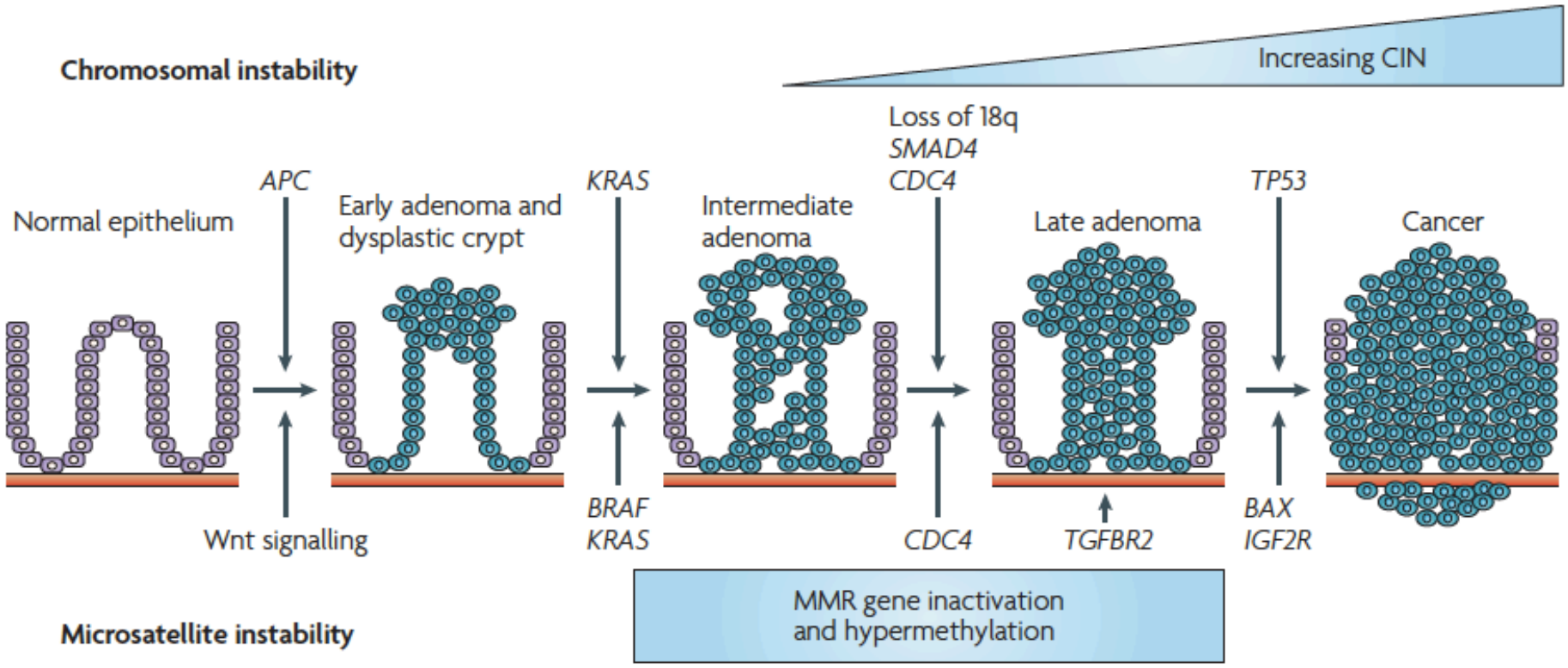
- Loss of **17p** („LOH“) is observed in 75 % of colorectal carcinomas
- Loss of 17p rarely detected in adenomas

⇒ Inactivation of tumor suppressor p53 is a late event during development of CRCs



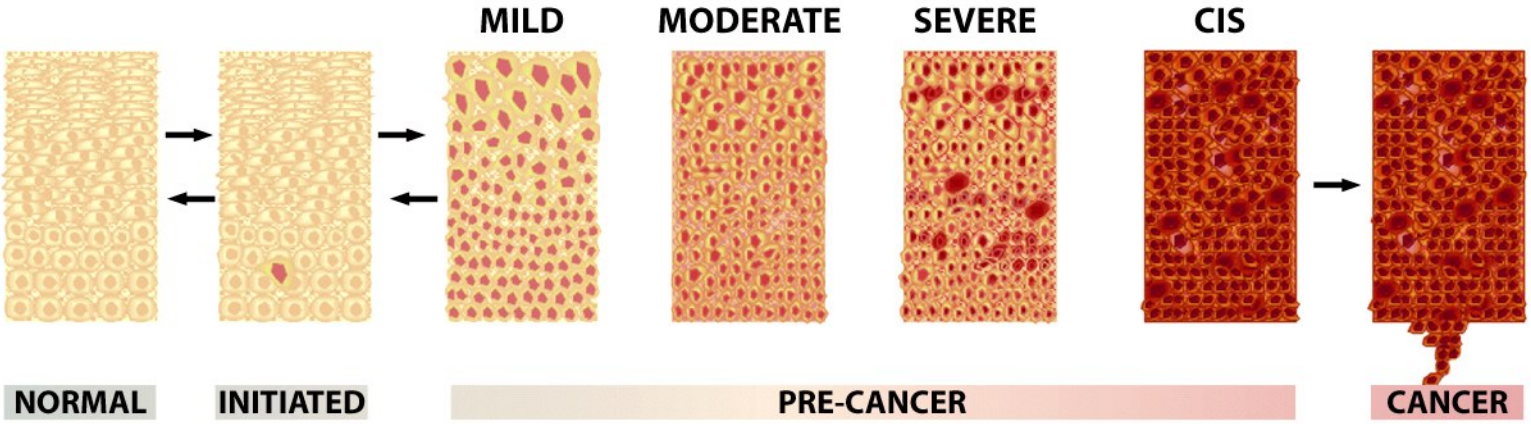
Kinzler KW and Vogelstein B, *Nature Rev Cancer* 2 (2002) 673-680

Genetic model of CRC development



Walther A et al. Nat Rev Cancer. 2009;9(7):489-99.

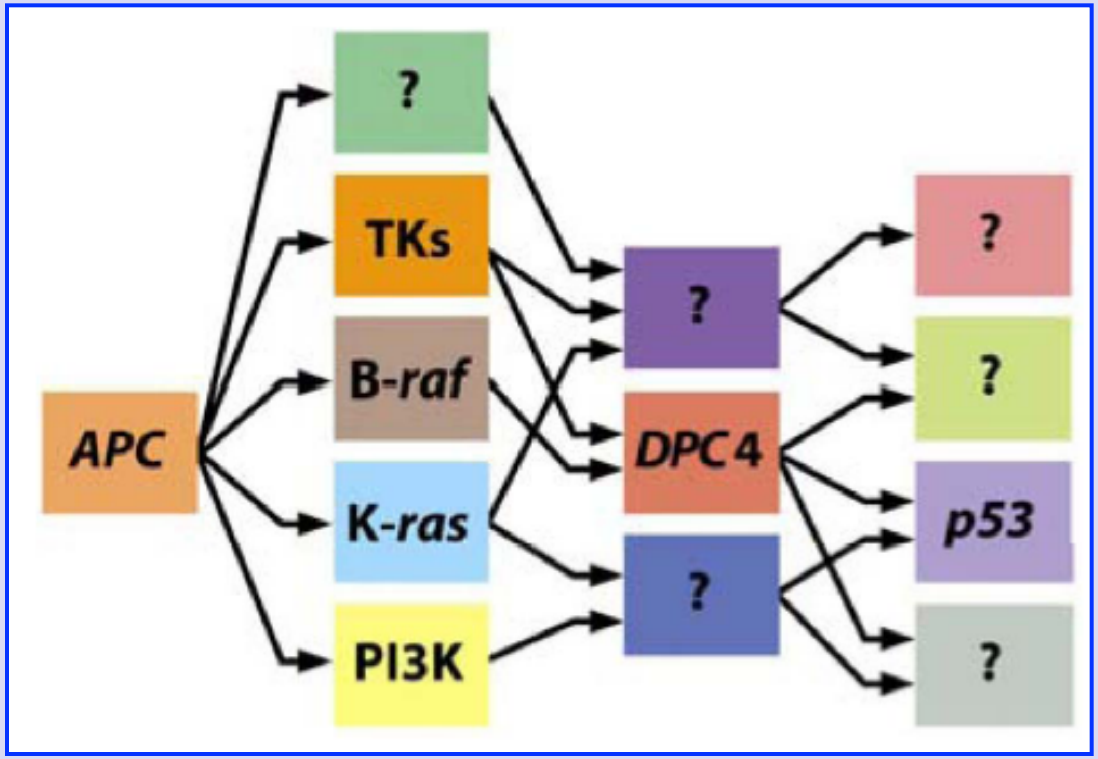
It takes years for cancer to develop



colon	5–20 years	adenoma	5–15 years			
head and neck	tobacco use 4–10 years	dysplastic oral leukoplakia	6–8 years			
cervix	CIN 1	9–13 years	CIN 3/CIS	10–20 years		
lung (smokers)	20–40 pack-years					
breast	atypical hyperplasia		DCIS	6–10 years		
prostate	20 years	PIN	≥10 years	latent cancer	3–15 years	

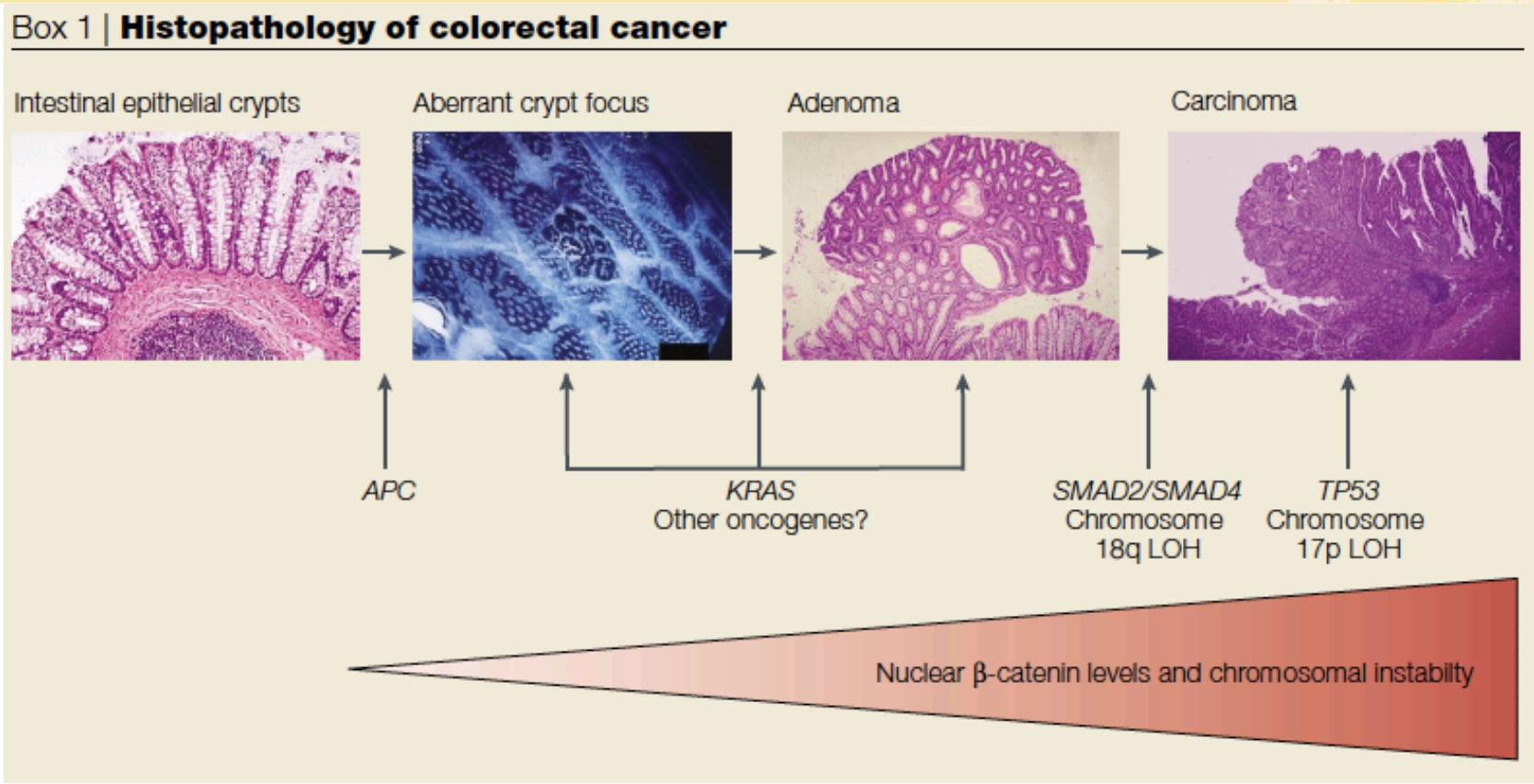
Weinberg RA. The Biology of Cancer. *Garland Science* 2007

Alternative genetic changes during CRC development



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

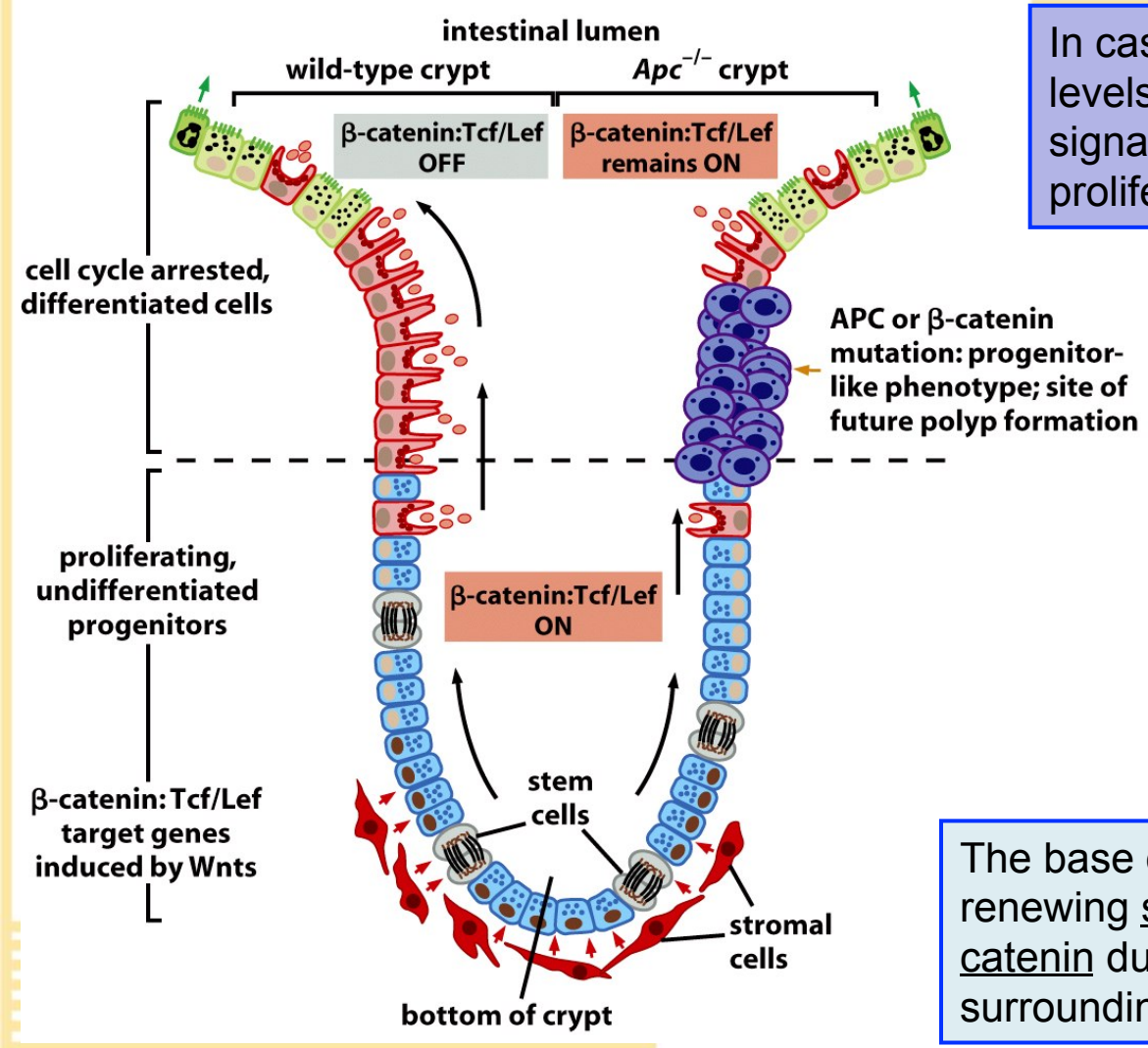
Genetic model of CRC development



Increasing tolerance to high amounts of β-catenin

Fodde R et al, *Nat Rev Cancer* 1 (2001) 55-66

β-catenin and colonic crypts



In case of **mutated *APC*** there are high levels of β-catenin even without Wnt signal and undifferentiated cells keep proliferating and from polyp.

Proliferating undifferentiated progenitor cells migrate upwards, Wnt stimulation weakens, β-catenin is degraded. Proliferation decreases and cells achieve differentiated state. Differentiated cells die after 3-4 days.

The base of colonic crypts contains self-renewing stem cells that have high levels of β-catenin due to the Wnt signals produced by surrounding stromal cells

Gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS)

GAPPS is a rare familial gastric cancer syndrome with an autosomal dominant pattern of inheritance

characterized by:

- fundic gland polyps (FGPs) in proximal part of stomach, some may be dysplastic, associated with a significant risk of gastric adenocarcinoma
- Intestines and distal stomach (pylorus) are without polyps (unlike FAP)

- In 12-84 % of **FAP** patients develop FGPs

- **sporadic** FGPs are less frequent, only rarely dysplastic

Li J. et al., *Am.J.Hum.Genet*. 98: 830-842, **2016**

GAPPS

- autosomal dominant inheritance
- Causal gene located at 5q22 (frequent LOH)
- Whole-exome nad whole-genome sequencing have not found associated mutations
- Li et al. 2016: analysis of 6 GAPPS families (27 affected individuals) revealed 3 point **mutations in promoter 1B of *APC* gene**, that consegregated with disease phenotype
- Mutations decreased binding of TF YY1 to APC promoter, leading to the decreased transcription
- Such mutations are not in FAP!
- promotor 1A is methylated in GAPPS and sporadic FGPs and also in normal gastric musosis ⇒ transkripts 1B are more abundant in gastric mucosa!! Colonic cells are „protected“ by expression of isoform 1A

Li J. et al., *Am.J.Hum.Genet*. 98: 830-842, **2016**

GAPPS

- *APC* haploinsufficiency is responsible for growth of fundic gland polyps (FGPs), and loss of the second allele leads to dysplasia

- isoforms 1A and 1B are *in frame*, but have different N terminus (1B isoform has one extra exon 1B and lacks exons 2 and 7)

- **Specific point mutations in binding site for TF YY1 in promoter 1B of *APC* gene causes GAPPS, new and potentially severe variant of FAP**

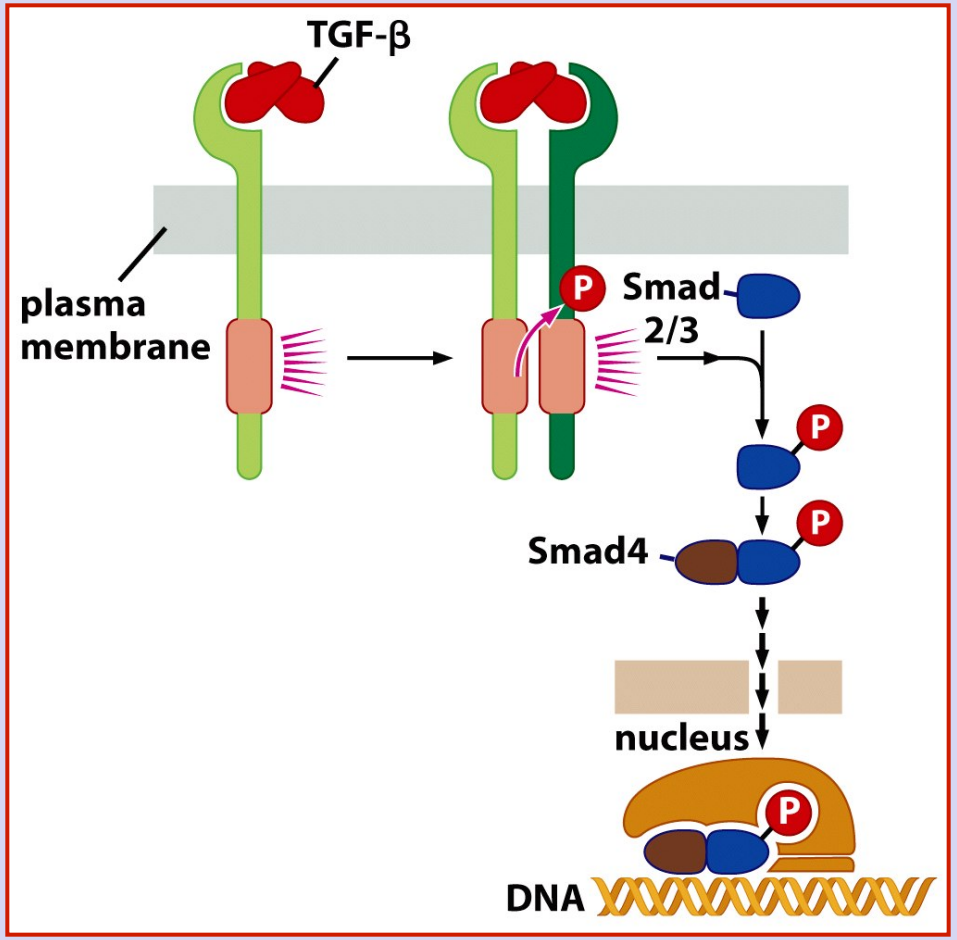
Li J. et al., *Am.J.Hum.Genet*. 98: 830-842, **2016**

Juvenile polyposis syndrome

- autosomal dominant pattern of inheritance
- The term “juvenile” refers to the type of polyp (juvenile polyp), not age of manifestation
- 34x higher risk of CRC development; life-long risk of CRC is around 40%; average age at diagnosis of CRC in JPS patients is 44 years
- Some cases of JPS (approx 20%) are associated with germ-line mutations of ***SMAD4*** (*DPC4*) (**18q21.1**)

TGF-β signaling pathway

- SMAD proteins are signal transducers of TGF-β pathway; TGF-β inhibits growth of many cell types, resistance to TGF-β is associated with colorectal tumors.
- Binding of TGF-β to receptor leads to phosphorylation of **SMAD2** and/or **SMAD3**, and these bind **SMAD4** and together translocate to nucleus. In complex with other TFs transactivate target genes (e.g. **p21^{CIP1}**, **p15^{INK4B}**, …).



Heteditary non-polyposis colorectal carcinoma - HNPCC = Lynch syndrome

- HNPCC represent 2-4 % of all **colorectal tumors** in western countries
- Average age of tumor diagnosis in HNPCC patients is 40 years
- Patients **do not have** increased numbers of precursor adenomas
- Lifetime risk of **CRC** is 50 % for women with HNPCC, for men 80 % and lifetime risk of **uterine** cancer is 60 %
- HNPCC patients are further predisposed to ovarian, stomach, pancreatic and bladder cancer
- Cancer risk is associated with mutations of genes involved in MMR (DNA mismatch repair), tumors are genetically instable and progress fast
- **somatic** mutation in MMR genes (most frequent je methylation of *MLH1*) are common in CRC, uterine and stomach cancer, rare in other cancer types

Hetediatary non-polyposis colorectal carcinoma

- autosomal dominant inheritance
- Syndrome is linked with germ-line mutations of genes involved in *mismatch repair*:
- ***MSH2*** (first identified; **2p15-16**, 106 kDa)
- ***MLH1*** (**3p21**; 85 kDa)

  - these two account for more than **90 %** of HNPCC
- ***PMS2*** (**7p22**; 96 kDa)
- ***MSH6*** (**2p16**) – cause different phenotype

  - these two only minor HNPCC
- ***PMS1*** (**2q31**)
- ***MSH3*** (**5q11-q12**)

  - only rarely present as germ-line mutations
- ***MLH3*** (**14q24.3**)

DNA mismatch repair

- MMR is in focus of cancer research after discovery of sporadic CRC tumors with large changes in poly(A) regions of their genomes; later identified changes in poly(CA) repetitive sequences and this phenomenon was termed **microsatellite instability (MSI)**

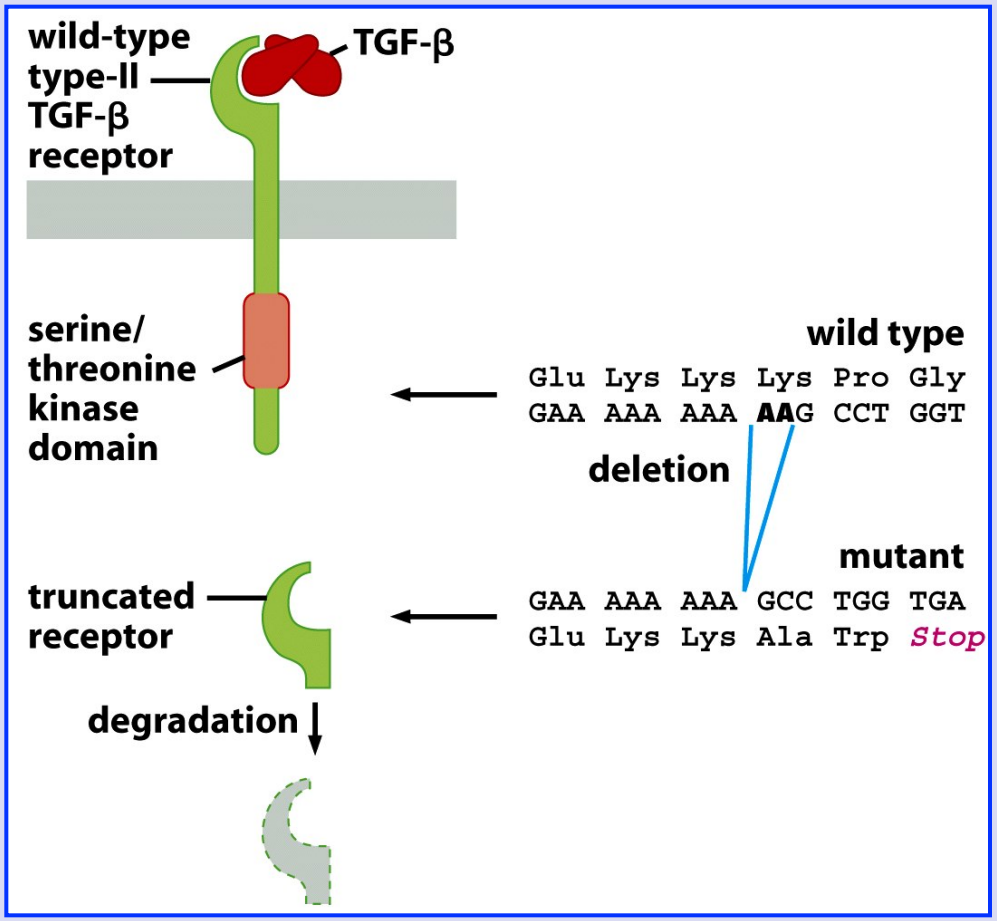
- microsatellite DNA is prone to mismatch errors during replication, because during synthesis of short tandem repetitions the newly synthesized chain may slide and shift against DNA template :

  thus e.g. A7 → A6 or A8

Impact of mutations in MMR genes

„oncogenic“ mutations that are secondary to defects in MMR (microsatellite instability - MSI) may occur in any gene; described e.g. in ***APC*** gene, gene encoding **receptor II for TGFß** (**TGFßRII**; 10A DNA sequence encoding 3 lysines), ***BAX*** (gene contains DNA sequence of 8G - codons 38-41), in MMR^+ tumors there are frequent insertions and deletions of 1 nt – typically associated with wt p53

Example of impact of MMR defects: mutation of TGF-βRII



TGF-βRII is frequently mutated in CRC with MSI

There is a microsatellite of 10A. Often deletion of 1 or 2 A → non-sense mutation → premature stop codon → absence of C-terminal kinase domain ⇒ resistance to TGFβ signaling.

(mutated protein has 129 or 161AA instead of 565 AA)

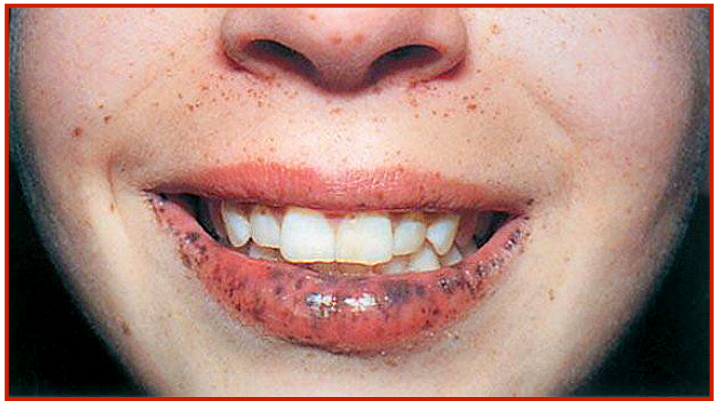
Table 12.2 Genes and proteins that have been inactivated in human cancer cell genomes because of mismatch repair defects

Gene	Function of encoded protein	Wild-type coding sequence	Colon	Stomach	Endometrium
ACTRII	GF receptor	A_8	X		
AIM2	interferon-inducible	A_{10}	X		
APAF1	pro-apoptotic factor	A_8	X	X	
AXIN-2	Wnt signaling	A_6, G_7, C_6	X		
BAX	pro-apoptotic factor	G_8	X	X	X
BCL-10	pro-apoptotic factor	A_8	X	X	X
BLM	DNA damage response	A_9	X	X	X
Caspase-5	pro-apoptotic factor	A_{10}	X	X	X
CDX2	homeobox TF	G_7	X		
CHK1	DNA damage response	A_9	X		X
FAS	pro-apoptotic factor	T_7	X		X
GRB-14	signal transduction	A_9	X	X	
hG4-1	cell cycle	A_8	X		
IFRIIR	decoy GF receptor	G_8	X	X	X
KIAA097	unknown	T_9	X		
MLH3	MMR	A_9	X		X
MSH3	MMR	A_8	X	X	X
MSH6	MMR	C_8	X	X	X
NADH-UO8	electron transport	T_9	X		
OGT	glycosylation	T_{10}	X		
PTEN	pro-apoptotic	A_6	X		X
RAD50	DNA damage response	A_9	X	X	
RHAMM	cell motility	A_9	X		
RIZ	pro-apoptotic factor	A_8, A_9	X	X	X
SEC63	protein translocation into endoplasmic reticulum	A_{10}, A_9	X		
SLC23A1	transporter	C_9	X		
TCF-4	transcription factor	A_{10}	X	X	X
TGF-βRII	*TGF-β receptor*	A_{10}	X	X	X
WISP-3	growth factor	A_9	X		

From A. Duval and R. Hamelin, *Cancer Res.* 62:2447–2454, 2002.

Peutz-Jeghers syndrome - PJS

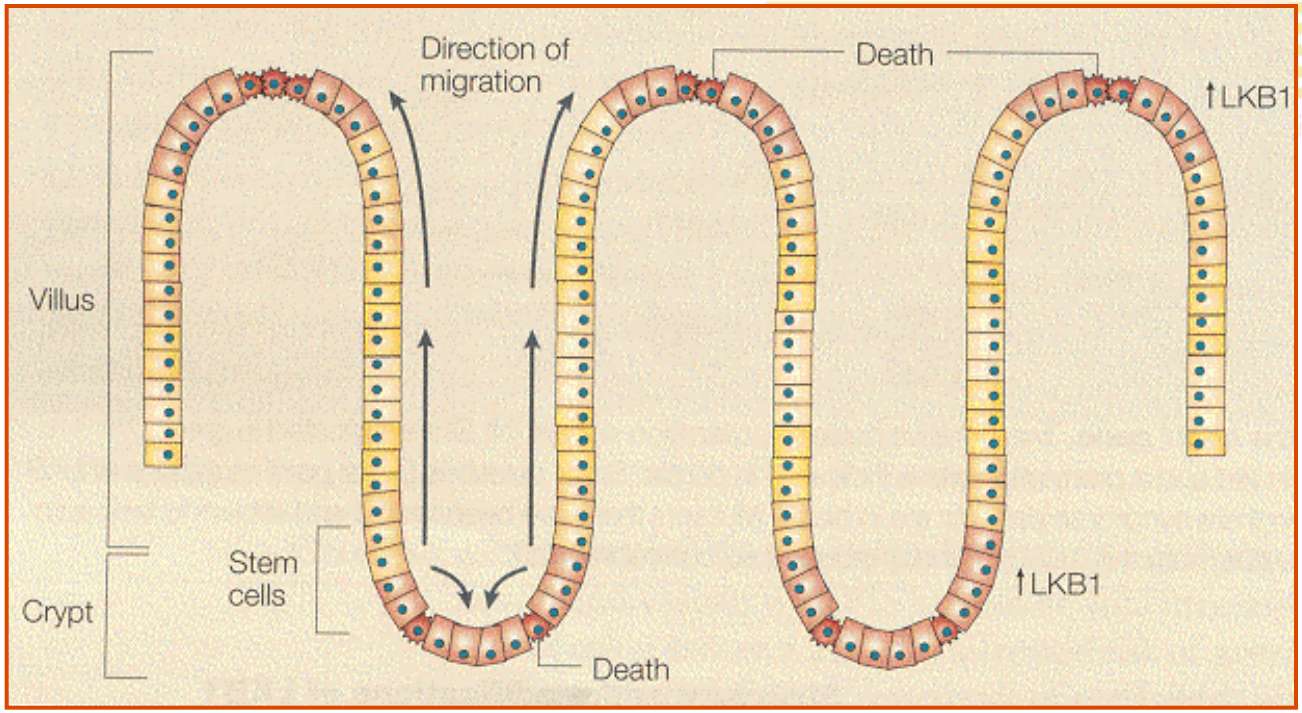
- autosomal dominant syndrome (Peutz 1921; Jeghers 1949)
- Characterized by dark blue or dark brown **pigmented freckling**, especially around the mouth and on the lips, fingers, or toes (fade with age) and growth of **hamartomatous polyps** in small instestine, colon, stomach
- Polyps may progress into malignant tumors
- Predisposed for other cancers - **pancreatic**, **gastrointestinal**, bilateral **breast** carcinomas



Peutz-Jeghers syndrome

- PJS causally associated with mutations of ***LKB1*** (*STK11,* Liver Kinase B1) gene (**19p13.3)**
- LOH present in smaller fraction of hamartomatous polyps and in 70 % of malignant tumors of PJS patients → haploinsufficiency of *LKB1* triggers growth of hamartomas
- *LKB1* mutations are rare in sporadic tumors
- Germ-line mutations of *LKB1* are in 70 % of affected families, thus existence of other gene causing PJS is suspected
- Penetrance is high (93%); average age of detection of malignant tumor is 43 years
- LKB1 is serine-threonine protein kinase present in nucleus and cytosol
- Most mutations result in complete loss of LKB1 protein, point mutations inactivate kinase activity

Expression of LKB in small intestine



- Stem cells are at the base of colonic crypts, they produce progenitor cells and these migrate upward (a smaller fraction also in opposite direction to the base), differentiate and die by apoptosis after 3-5 days
- **High expression of *LKB1*** is at the top of the vili and base of crypts – in cells undergoing apoptosis

Yoo LI et al, *Nature Rev Cancer* 2 (2002) 673-680

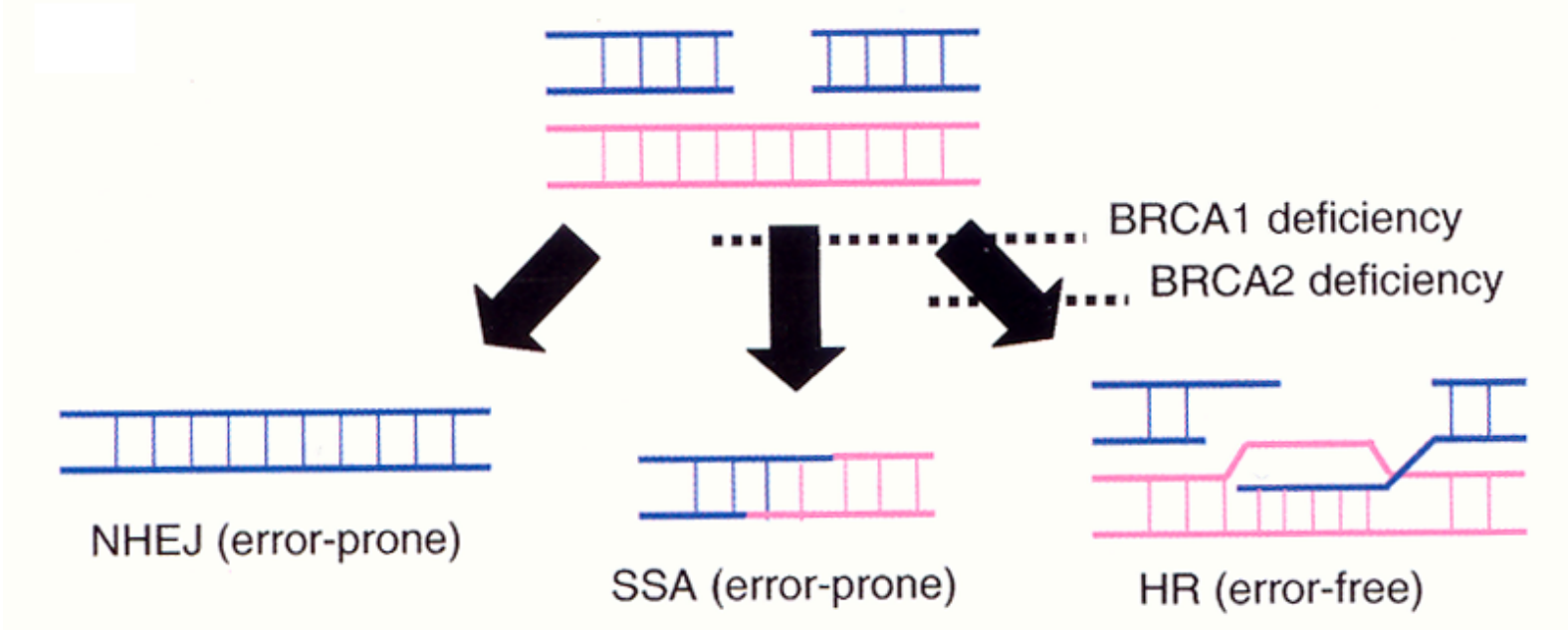
LKB1 function

- *LKB1* is expressed in cells undergoing apoptosis;
- In apoptotic cells LKB1 is translocated to mitochondria

→ protein LKB1 probably involved in apoptosis regulation

- Some studies demonstrated absence (low frequency) of TP53 mutations in tumors harbouring LKB1 mutations – indicates functional crosstalk between LKB1 and **p53** (apoptosis?)
- LKB1 also induces cell cycle arrest, probably via **$p21^{WAF1/Cip1}$**

Result of defects in repair of DSBs for genetic stability



NHEJ - nonhomologous end joining

SSA - single-strand annealing

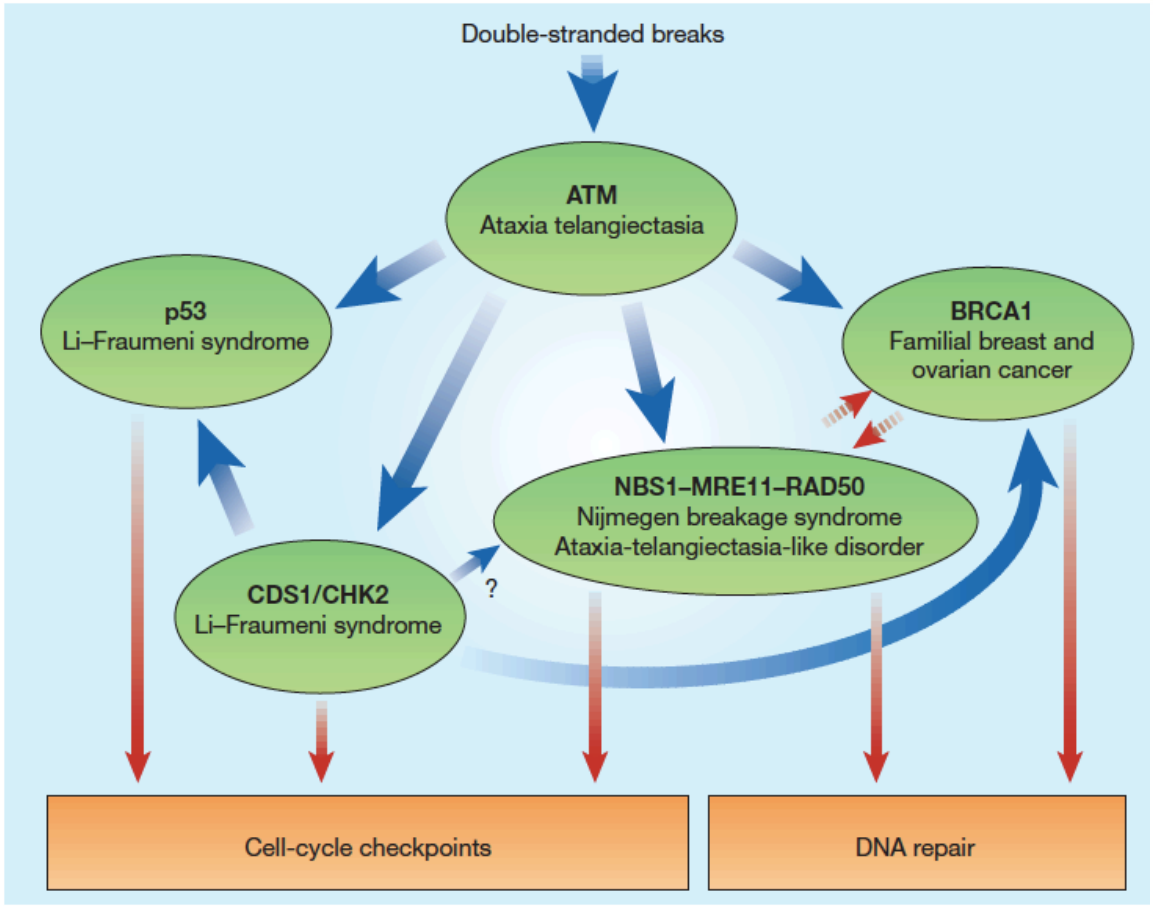
HR - homologous recombination

Venkitaraman AR, *Cell* 108: 171-182, 2002

Physiological DSBs

- meiotic recombination
- Gene (gene segments) rearrangements in immunoglobulin and T- and B-cell receptor genes (V(D)J recombination)

Protein network involved in DSBs repair by homologous recombination



- these proteins control genome integrity
- Their germ-line mutations predispose to cancer

Blue arrows: phosphorylation

Wang JYJ, *Nature* 405: 404-405, 2000

Ataxia – Telangiectasia (A-T)

- autosomal recessive pattern of inheritance
- Prevalence around 1:40.000 (USA); frekvention of carriers (heterozygots) around 1%
- progressive neurodegenerative disease: impairs certain areas of the brain (cerebellum), causing difficulty with movement and coordination (ataxia)
- Endocrine dysfunctions
- Dysfunctions of immune system
- Sterility, premature aging
- Sensitivity to ionizing radiation (X-rays and gamma rays), topoisomerase inhibitors
- Defects in repair of DSBs = increased risk of cancer

Ataxia telangiectasia

Predisposition to cancer development:

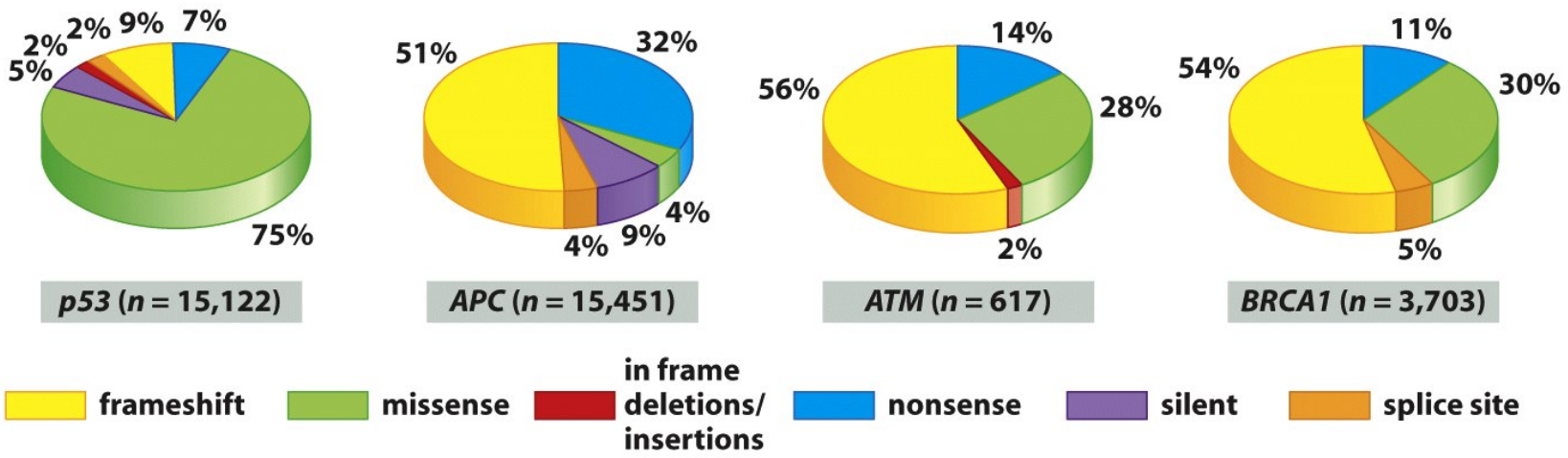
- Approx 38 (25) % of AT patients develop tumor during lifetime - mostly (85 %) **T-lymphomas** and **CLL** - chronic lymphocytic leukemia, but also higher risk of **stomach cancer, medulloblastomas, gliomas, tumors of urinary trackt,**

Tumor type	Approximate incidence in A-T syndrome
Non-Hodgkin's lymphoma	13%
Leukemia	2%–8%
Hodgkin's lymphoma	4%
Breast cancer	2%–3%
Adenocarcinoma of stomach	<1%
Dysgerminoma	<1%
Gonadoblastoma	<1%
Medulloblastoma	<1%
Pancreatic carcinoma	<1%
Thyroid carcinoma	<1%
Total (All types)	25%

Choi M et al. 2016, DOI: 10.1158/1535-7163.MCT-15-0945

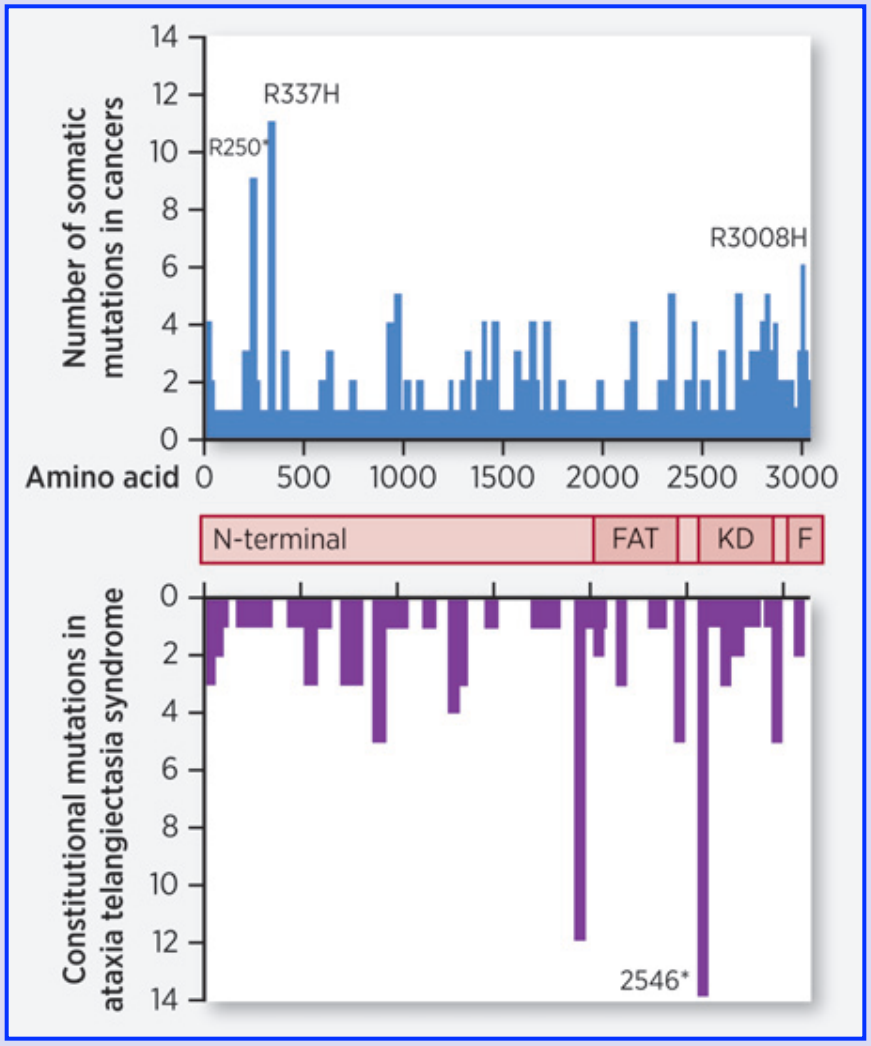
Ataxia telangiectasia: gene

- Located at chromosome 11 (**11q22.3**), 66 exons
- Expressed in all tissues
- More than 250 (167) different **mutations of *ATM*** described, common are truncated forms
- Mutation type affects phenotype



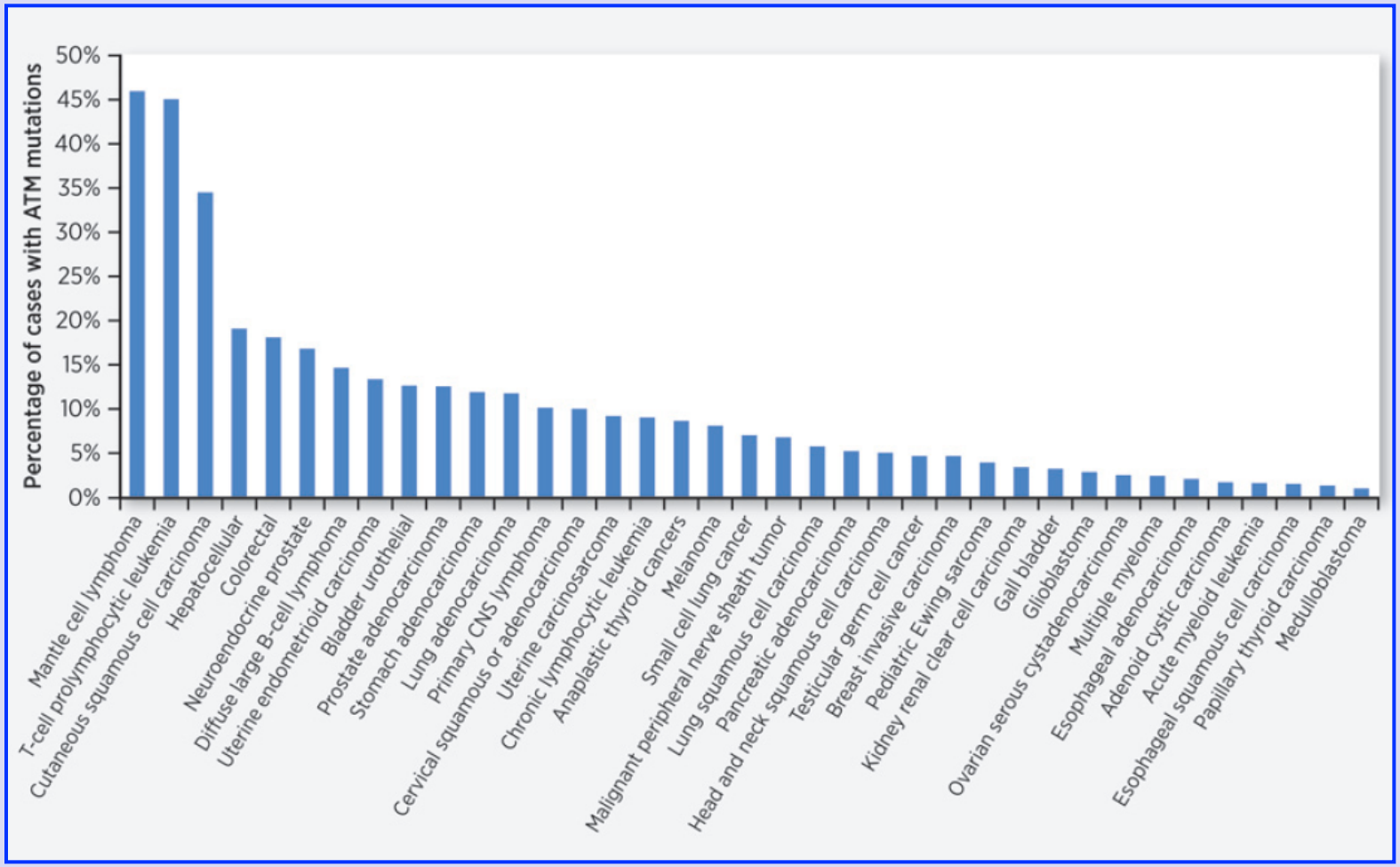
Weinberg RA: The Biology of Cancer. *GS*, 2007

ATM mutation spectra



Choi M et al. 2016, DOI: 10.1158/1535-7163.MCT-15-0945

Frekvention of somatic mutations of ATM in different cancers



Choi M et al. 2016, DOI: 10.1158/1535-7163.MCT-15-0945

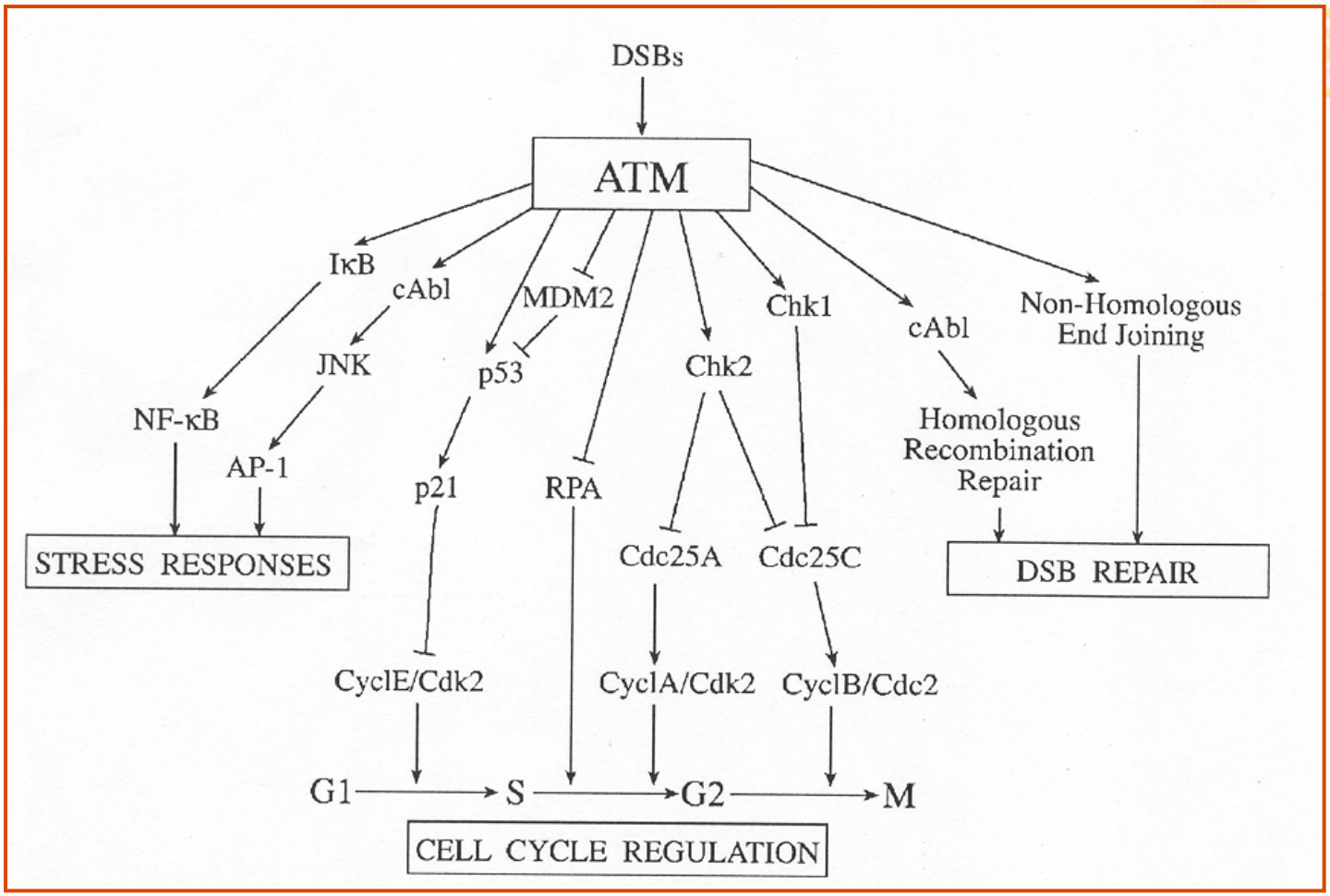
Ataxia telangiectasia: protein

- 3506 AA, 370 kDa
- serine/threonine protein kinase, C-terminus high homology with PI3K kinase (**PIKK** family „PI3K-like protein kinases“: PIRK, ATM, ATR, DNA-PKs, TRRAP,..)
- Activated by double stranded breaks in DNA (DSBs) induced e.g. by ionizing radiation (not UV), topoisomerase inhibitors
- Activation of ATM is **primary and direct** reaction to DSBs (but not the only one); later other ATM-independent pathways are triggered

Substrates

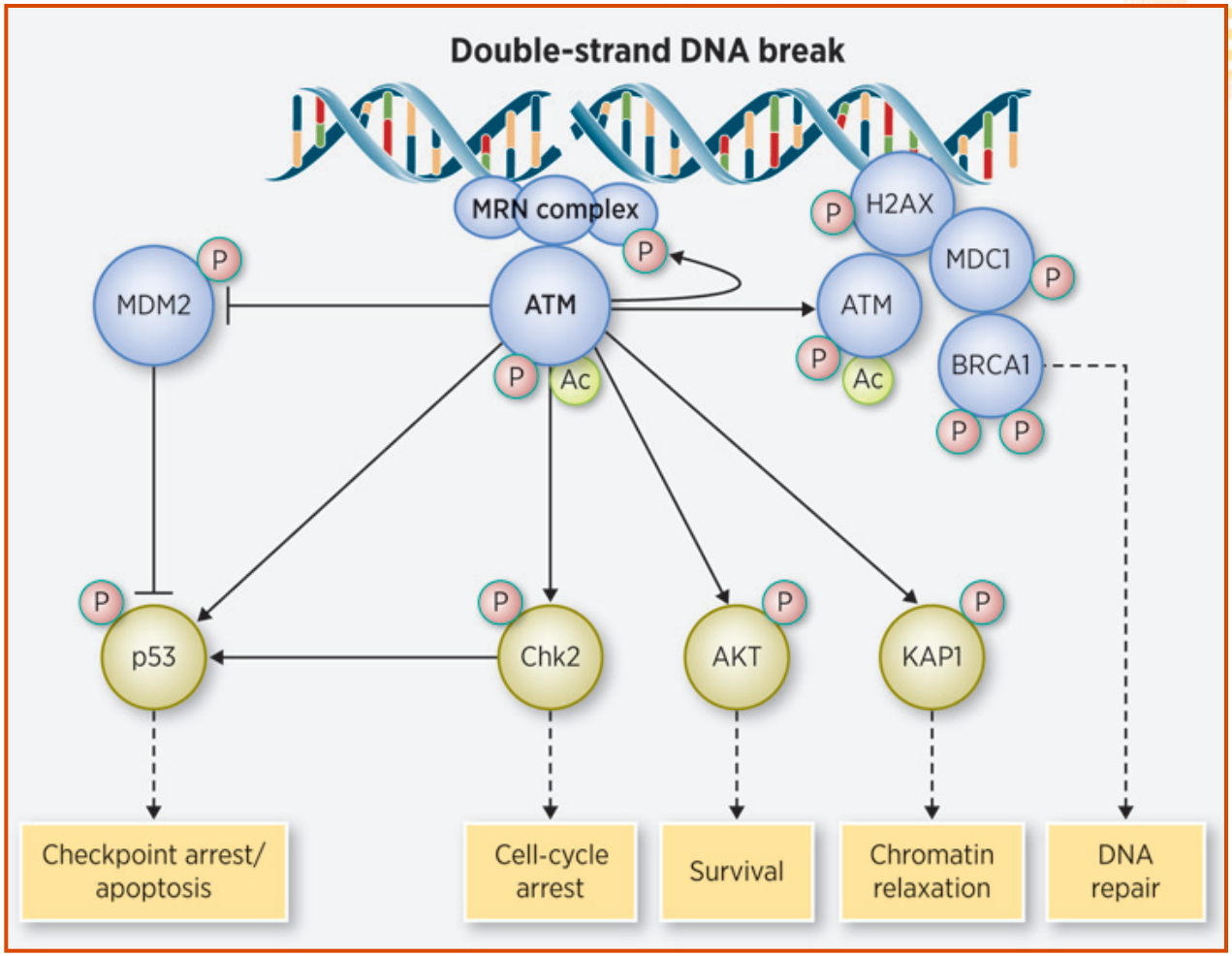
- phosphorylates **p53** at ser15 → stabilization and activation
- phosphorylates **MDM2** at min 2 sites → release of p53 from MDM2
- Other substrates, e.g. **Chk2**, **c-Abl**, **BRCA1**, **NBS1**, **FANCD2** and others

ATM-dependent pathways



Rotman G and Shiloh Y, *Oncogene* 18 (1999) 6135-6144

ATM function in repair of DSBs



Choi M et al. 2016, DOI: 10.1158/1535-7163.MCT-15-0945

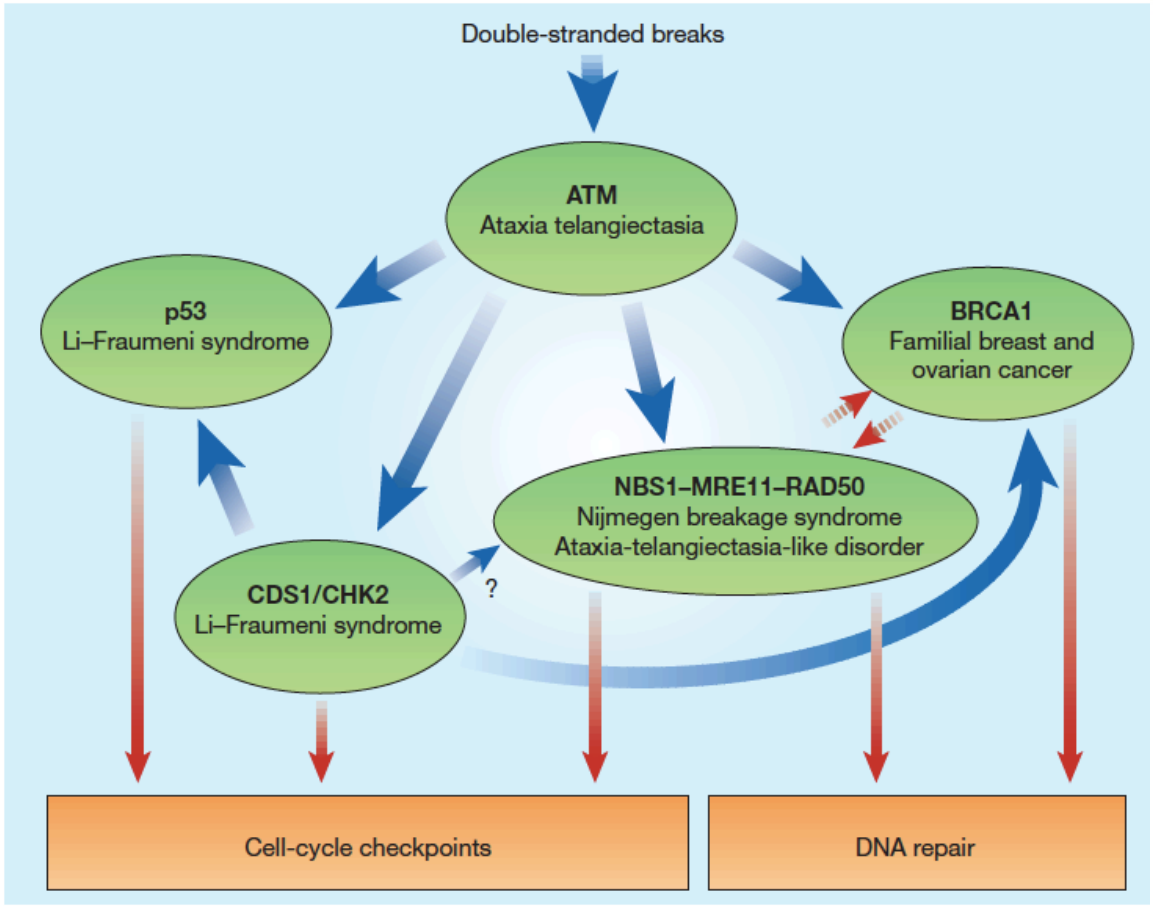
ATM function

In response to ds breaks in DNA:

- Induce cell cycle arrest (prevent replication of damaged DNA)
- Induce repair of DSB: besides other mechanisms also by direct inetraction with **RAD51** (RAD51 interacts with **BRCA1** and **BRCA2**)

Its function is reflected in clinical symptoms of A-T: germ cell that undrego meiosis (and meiotic recombination) and maturing lymphocytes undergoing V(D)J recombination are more sensitive to the ATM mutations ($\Rightarrow$ sterility, immunodefficiency,..)

Protein network involved in DSBs repair by homologous recombination



- these proteins control genome integrity
- Their germ-line mutations predispose to cancer

Blue arrows: phosphorylation

Wang JYJ, *Nature* 405: 404-405, 2000

Nijmegen breakage syndrome - NBS

- similar symptoms as for A-T: growth defects, endocrine dysfunction, immunedeficiency, sensitivity to radiation, predisposition to cancer, mainly **B-lymphomas** (in 40 % of patients by the age 20
- Distinct from A-T: microcephaly, mental retardation
- Also similar phenotypes at cellular levels: associated with defects in DSB repair.
- Cause by mutation of ***NBS1*** (nibrin, **8q21**)
- autosomal recessive inheritance

Nijmegen breakage syndrome - NBS

- **protein** NBS1 (nibrin) forms trimeric complex with (**MRE11** and **RAD50**) that interacts with damaged DNA (DSBs)
- NBS1 function as a „**sensor**“ – if absent, MRE11-RAD50 complex is not recruited to DSBs – and also as **effector** – contributes to the repair

- **MRE11** is mutated in rare genetic disorder called **ATLD** (*AT-like-disorder*)
- MRE11 is nuclease, at DSBs it degrades nt of one strand leaving single strand overhangs

Hereditary Breast and Ovarian Cancer syndrome (HBOC)

- around 10 % breast cancer is hereditary, out of that 50 % is associated with mutations of ***BRCA1*** and ***BRCA2***

- Breast cancer genetic predisposition is caused by other germ-line mutations: ***TP53***, ***PTEN***, ***LKB1*** (5-10%) and possibly other genes (40%)

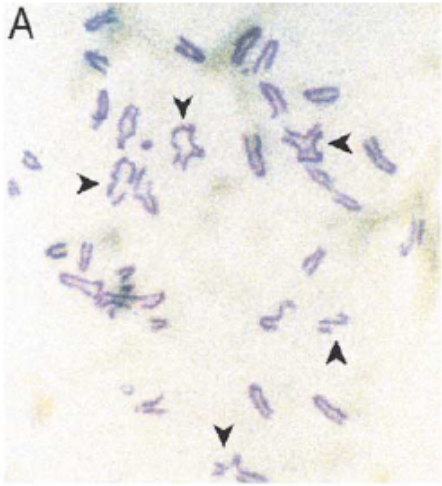
➢ **somatic mutations** of *BRCA* genes are less frequent in sporadic tumors.

BRCA1, BRCA2

- No homologues in yeast and Drosophila – „late“ in evolution

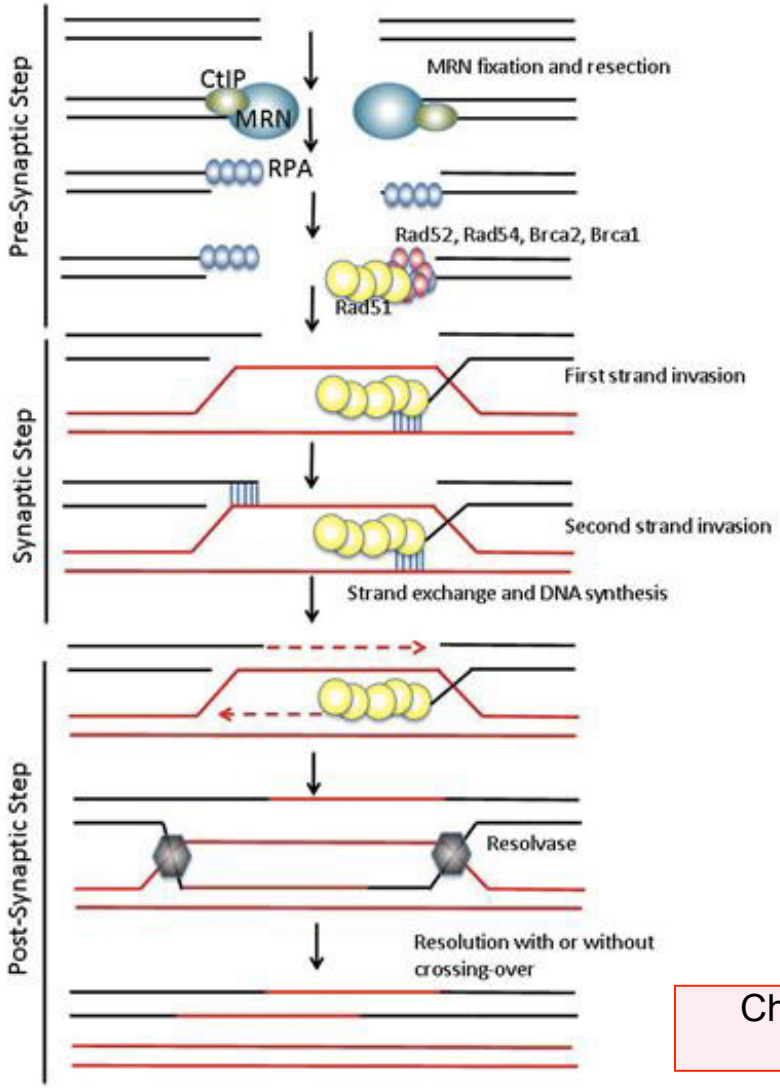
- *BRCA1*: discovered in 1994; locus **17q21.31**; 22 exons
- *BRCA2*: discovered in 1994/1995; locus **13q12.3**; 27 exons

- Encode large proteins that are expressed in many tissues and localized mostly in nucleus
- Significant function in protection of genome integrity, but their functions are not identical!!
- „caretakers“, preserve genome integrity



Venkitamaran, *Cell*. 2002;108(2):171-82.

BRCA1, BRCA2 in DNA damage response



Homologous recombination

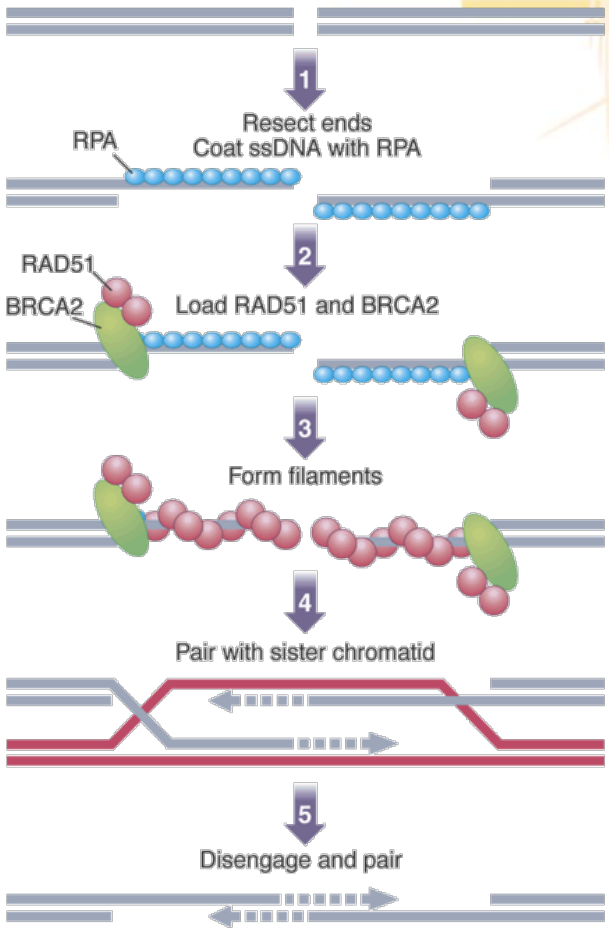
RAD51: a recombinase that catalyzes the homology search and the strand exchange with a homologous sequence and thus ensures the accurate repair of the DSB.

Chen C. editor,. New Research Directions in DNA Repair. 2013; Available from: http://dx.doi.org/10.5772/46014

Model of BRCA2 function

Interacts with **RAD51** and regulate its level and activity.

- DSBs activate ATM or ATR
- ATM/ATR phosphorylate thus activate complex BRCA2-RAD51
- activated RAD51 loads onto damaged ss DNA and activates homologous recombination; release from repaired DNA strands is associated with dephosphorylation of the complex



Wilson JH, *Science*. 2002;297(5588):1822-3.

BRCA1 function

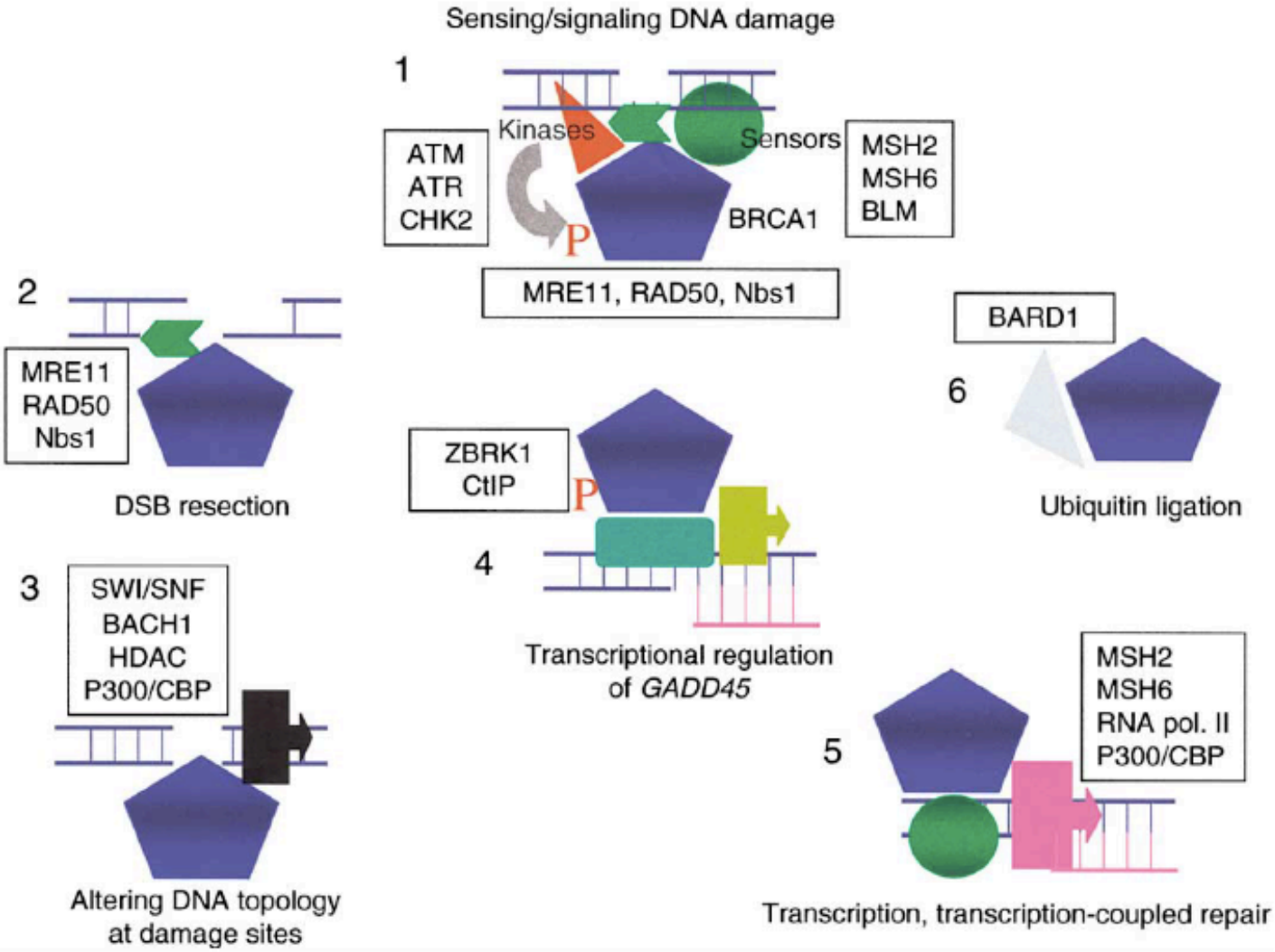
- More difficult to identify exact function of BRCA1:
- In DNA regions with DSBs high turnover of chromatin modifications and protein composition
- Appears fast at DSB sites, substrate of kinases ATM, ATR, ChK2, interacts with SWI/SNF, HDAC/HATs,...
- Interacts with MRE11/RAD50/NBS1 complex (MRN), inhibits nuclease MRE11, and thus regulates lenght of ss overhangs
- Interacts with DNA helicase BLM
- Interacts with proteins MSH2 and MSH6 that participate in DNA mismatch repair
- BRCA1 colocalizes with protein encoded by ***FANCD2*** (mutated in Fanconi anemia)

BRCA1 function

- Difficult to define the **exact** function of BRCA1 (based on interaction partners)

- Responsible for correct assembly of repair complexes at the sites of DSBs – it functions as a **scaffold**

Model of BRCA1 function



Venkitamaran, *Cell*. 2002;108(2):171-82.

Link between *BRCA1* mutations and mutations of other genes

- Mutations of both alleles of *BRCA1* or *BRCA2* is **lethal** for cells, their survival is secured by mutations of some checkpoint genes, e.g. ***P53***
- Timeline: germ-line mutation of *BRCA* → inactivation of *p53* → mutation of second allele of *BRCA*.

  Frequency of *p53* mutations is higher in tumors with BRCA mutations (and other mutation spectra).

- Similar effects as *p53* mutations may have mutations of genes associated with spindle checkpoint (metaphase-to-anaphase transition): **Bub1**, **BubR1**.

- Mutations of *BRCA1* and *2* survive only mammary and ovarian cells due to the anti-apoptotic effect of **estrogens**.

Hereditary Breast and Ovarian Cancer syndrome

- About 1 in 300-800 women has mutation in either *BRCA1* or *BRCA2* gene
- prophylactic mastectomy (a surgery to remove one or both breasts) reduces breast cancer risk from 85% to 1-5% (remaining risk may be connected with residual mammary tissue after surgery)
- prophylactic adnexectomy (removal of ovaries and fallopian tubes) reduces risk of ovarian cancer from 60% (*BRCA1* mutation carriers) or 20% (*BRCA2* mutation carriers) to 1-5%
- Surgery is recommended at age 35-40 years
- Removal of ovaries may diminish the risk of breast cancer by 50 % thanks to the estrogen depletion

Foretová L.: Dědičný syndrom nádorů prsu a ovaria, *Medical Tribune XI*, 24.3.**2015**

Breast carcinoma in men

- Mutations of ***RAD51B*** (SNP 17q24.1) enhance risk of breast cancer in men by 50%
- around 10 % of patients have ***BRCA2*** **mutation**, mutations of ***BRCA1*** are not present in men with breast carcinoma
- Breast carcinoma in men and women is presumably quite different clinical entity

Orr N. **2012**, *Nat Genet* 44: 1182-1184

RecQ helicases

- Family of RecQ helicases (5 in human) contribute to the control of genome stability: they catalyze separation of complementary strains of DNA using energy from ATP hydrolysis

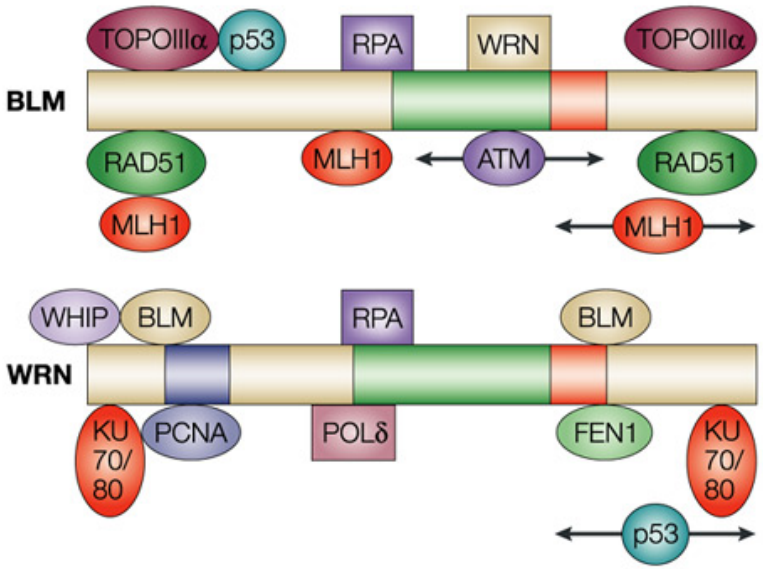
- Family RecQ helicases comprises:

  BLM (RECQ2) (Bloom syndrome)

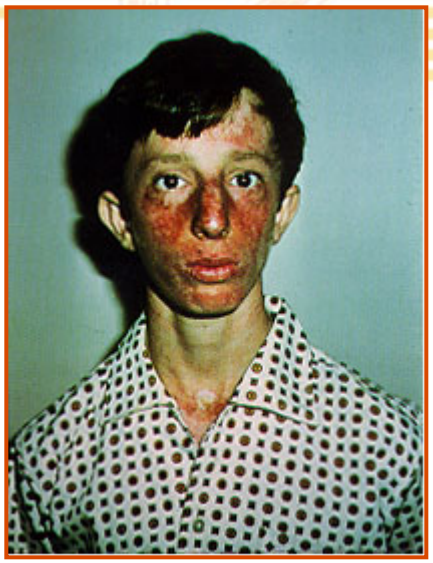
  WRN (RECQ3) (Werner syndrome)

  RECQ4 (Rothmund-Thompson syndrome - RTS)

  - all syndromes are associated with enhanced cancer risk



Bloom syndrome - BS



- Very rare autosomal recessive disease
- **Predisposition to cancer development**
- typical facial feature and thin voice
- Small stature
- Sensitivity to sunlight
- common *Diabetes mellitus*
- Sterility (reduced fertility)
- Immunodeficiency

Bloom syndrome

- Caused by mutation in ***BLM*** gene (**15q26.1**)
- Autosomal recessive pattern of inheritance, heterozygots are without any symptoms

- *BLM* mutations are generally very rare in population, more common amongst people of Ashkenazi Jewish background (carrier is 1 in 110 people, specifically mutation **blm^{Ash}**: 6-bp deletion and 7-bp insetion in exon 10)
- In other populations not specific mutations, may be of many types: missense, frameshift, nonsense, splice-site
- Tumors in BLM patients have high chromosomal instability

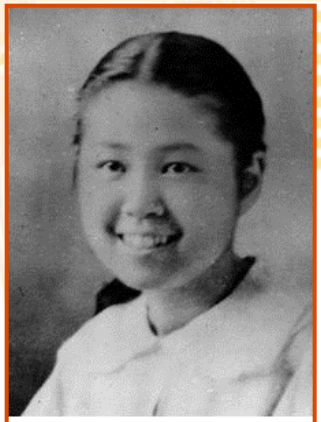
Tumors in BS patients

- High frequency (benign and malignant)
- Wide range of tumor types (acute leukemias, lymphomas, lung, colorectal, skin, breast, cervical cancer, etc)
- Very early onset (average age at diagnosis is 24.7 years)
- high frequency of multiple primary tumors

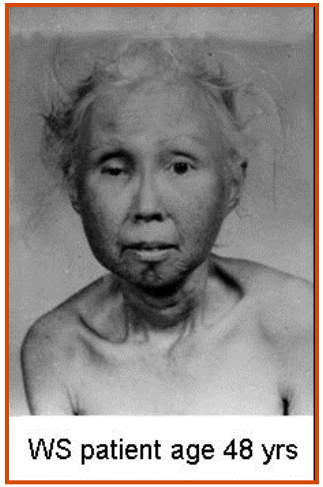
➢ Cancer is the most frequent cause of death

Werner syndrome - WS

- rare autosomal recessive disease, global incidence rate 1:220.000 - 1.000.000 (incidence in Japan and Sardinia is higher)
- Till adulthood normal phenotype. Then onset of rapid **premature aging**: premature graying of hair, hair loss, wrinkling, prematurely aged faces with beaked noses, skin atrophy, loss of fat tissues
- **Increased risk cancer** (30x): common non-epithelial tumors: soft tissue sarkomas, osteosarcomas, melanomas, thyroid cancer...
- Caused by mutation of ***WRN*** gene, located **8p12**
- *WRN* mutations are mostly non-sense or frameshift ⇒ truncated proteins
- WRN protein contributes to repair of DNA damage resulting from chronic **oxidative stress**, WRN is important in dealing with oxidative DNA damage that underlies normal aging



WS patient age 15 yrs



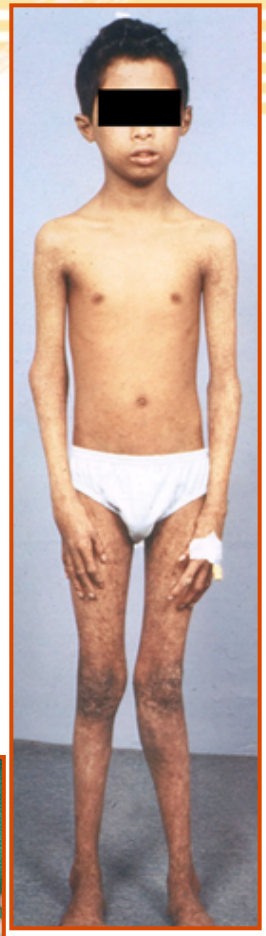
WS patient age 48 yrs

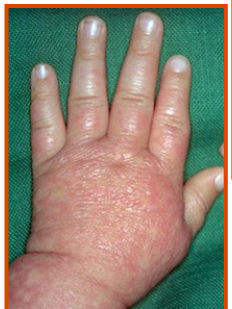
Penisi E, *Science* 272 (1996) 193-194

Rothmund-Thompson syndrome - RTS

- autosomal recessive inheritance
- Some RTS patients have mutation in ***RECQL4***, located **8q24.3**
- RECQL4: DNA helicase – problems during initiation of replication

- Characterized by defects in skin pigmentation, hair loss, skeletal defects
- Associated with increased cancer risk, common **osteosarcomas**, but also other tumors with early onset (before age 25 y)





Fanconi anemia - FA

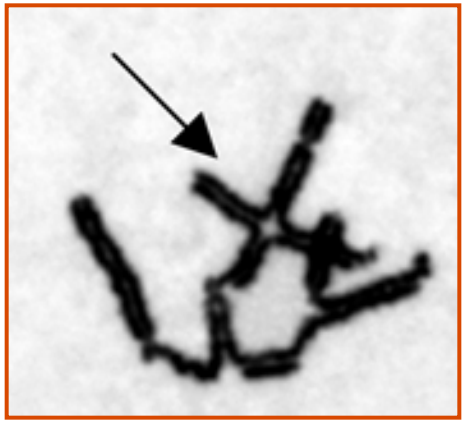
- autosomal recessive syndrome, very rare: common growth retardation, pigmentation defects, microcephaly, etc (heterogenous disease)
- Bone marrow failure syndrome leading to the congenital hematopoietic abnormalities
- Cancer predisposition, mostly: leukemia (AML), hepatocellular carcinoma,
- FA is rare, somatic mutations of „FA cluster“ also in some sporadic cancers – impaired response to DNA damage

Fanconi anemia

- **11** genes (*FANC**A*** to *FANC**L***) causing: no sequence homology, linked by functional interplay
- Encoding components of single tumor suppressor pathway and form **nuclear complex FA** that is required for activation of key protein **FANCD2**

➢ FANCD2 is monoubiquitinated in response to DNA damage, together with other proteins (BRCA1 and BRCA2) is localized in sites of DBSs, participates in homologous recombination

- At cellular level is for FA characteristic sensitivity to DNA crosslink agents



Skin cancer

- **Melanomas**: originate from transformation of melanocytes, cells producing

- **Other skin tumors**:

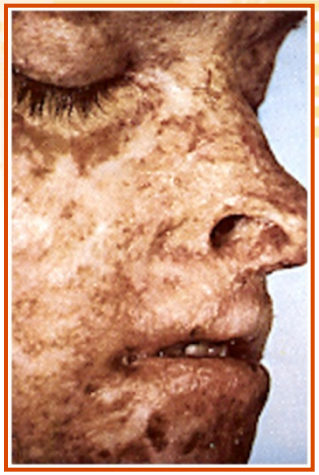
  - about 80 % represent basal cell carcinoma, - remaining 20 % squamous cell carcinomas

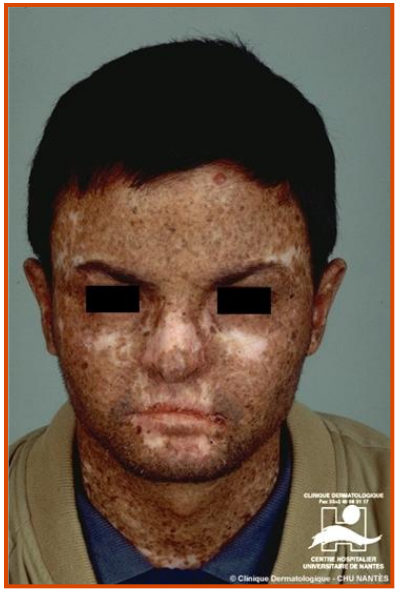
Xeroderma pigmentosum - XP

- Rare autosomal recessive syndrome
- Incidence rate 1:1.000.000 in USA and Europe and around 1:100.000 in Japan and north Africa
- High sensitivity to UV: Xeroderma (dry skin), irregular dark spots on the skin,severe sunburn, about **1000x** increased risk of „sunlight-induced“ skin cancer*, also predisposition to leukemias and gastric, brain, lung cancer
- In some patients (18%) associated with neurologic problems (hearing loss, poor coordination, etc)

* XP patients have higher risk for both melanomas (5%) and other skin cancers (squamous and basal cell derived) (95%)

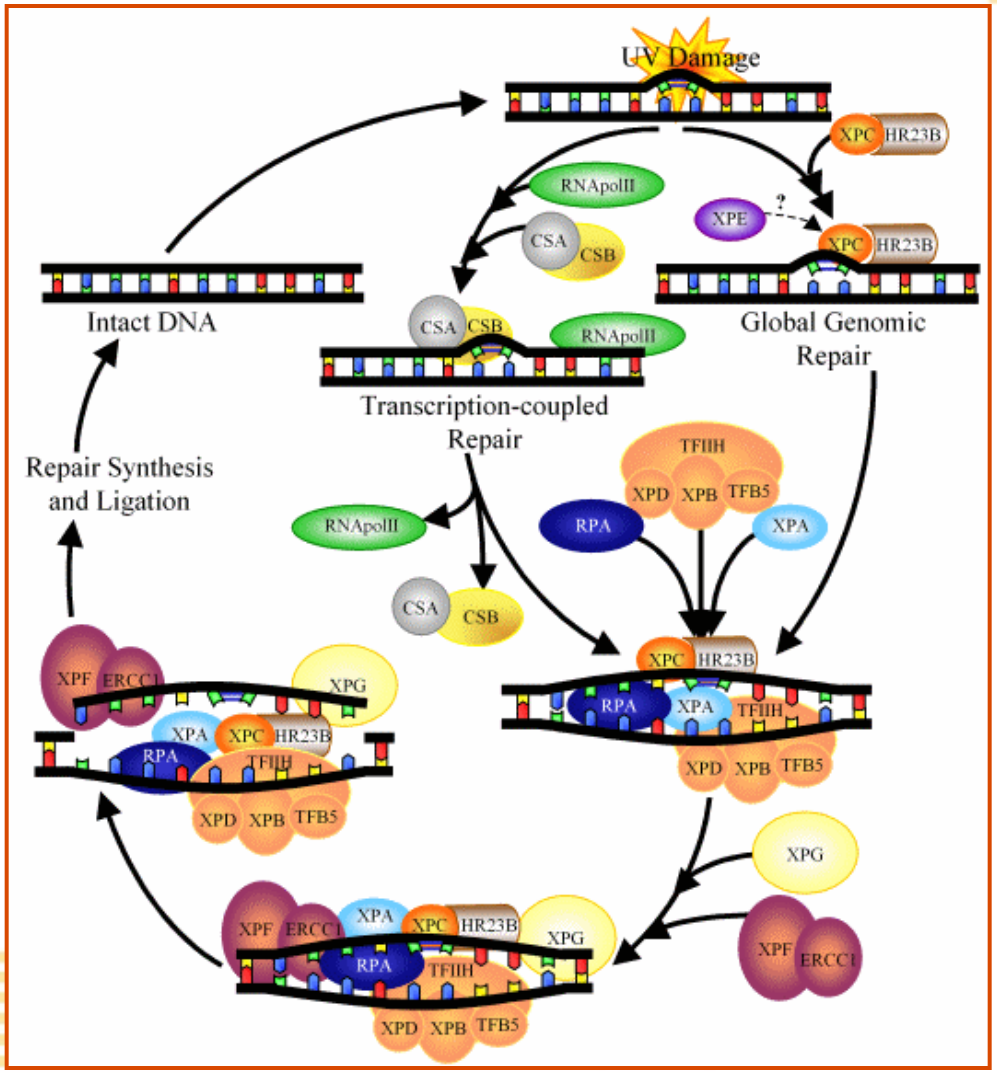
Xeroderma pigmentosum





- 90% of tumors appear at head, face and neck – skin highly exposed to sunlight
- Average age of tumor diagnosis is **8 years** (for sporadic tumors – not XP- average age is 60)
- Associated with germ-line mutations in one of 7 XP genes: ***XP-A*** to ***XP-G***
- Proteins endoded by these genes contribute to nucleotide excision repair (**NER**) as a part of multiprotein complex
- protein ***XP-V*** (POLH) encodes (error-prone) DNA polymerase pol-η – can replicate DNA over the lesion (→ 8 genes causally involved with XP syndrome)
- No therapy, prevention of UV exposure

Model of NER



NER is required for enzymatic removal of damaged nucleotides (common for bulky lesions such as thymine dimers induced by UV) and their replacement.

NER is mediated by multiprotein complex (of 24 subunits)

7 subunits encoded by XP genes (A, B, C, D, E, F, G), mutations cause different types of Xeroderma pigmentosum (type A..).

8th type (Type V) caused by XPV mutation – defects „by-pass (error-prone) polymerase“ pol-η.

von Hippel-Lindau (VHL) syndrome

- ***VHL*** (pVHL)

Hereditary diffuse gastric cancer

- ***CDH1*** (E-cadherin)

Secondary malignant neoplasias (SMN) induced by therapy

- Increasing numbers of patients (especially pediatric) that survive many years after treatment with cytotoxic therapy.
- Most common SMNs are sarcomas of bone and soft tissues
- Radiation therapy is frequently the cause of SMNs.
- Young age and low dose of radiation therapy increases risk of thyroid cancer
- Women that underwent radiation therapy of Hodgkin lymphoma often develop breast cancer with increasing age
- Patients that received alkylating agents and inhibitors of topoisomerase II have higher risk of secondary leukemias

Secondary malignant neoplasias (SMN) induced by therapy

Interation of therapy with genetic predispositions is critical:

RB (retinoblastom): sarkomy, melanomy

- NF1 (neurofibromas): neurofibrosarkomas, AML, JMML, gliomy
- RB (retinoblastoma): sarkomas, melanomas
- Sensitivity to ionizing radiation: AT, NBS, BS, WS
- Sensitivity to UV: XP, BS
- Sensitivity to alkylating agents: FA

⇒ **Therapy must be carefully considered especially in patients with genetic predispositions!!!**

Thank you for attention!