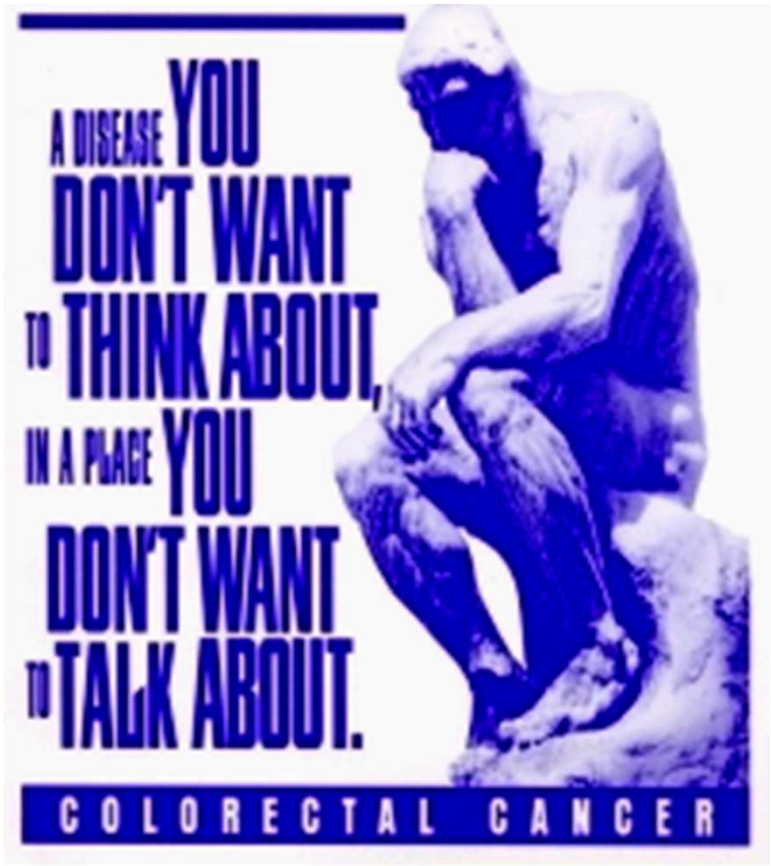
COLORECTAL CANCER
AWARENESS MONTH

Preventable. Treatable. Beatable!
Founded by the Cancer Research Foundation of America



Cancro colorettale (CR)

La maggior parte di CR avviene spontaneamente, in assenza di sindromi famigliari (1-3%), e come la maggior parte dei tumori ci sono condizioni associate al suo sviluppo.
Non considerando la causa scatenante, sono conosciute alterazioni genetiche che portano allo sviluppo di tale neoplasia.

98% dei carcinomi del colon sono adenocarcinomi

USA: 148.300 nuovi casi all'anno
56.600 decessi l'anno (10% dei decessi correlati al cancro)
I Paesi più colpiti sono USA; Australia, Nuova Zelanda, Europa dell'Est
Fattori ambientali, in particolare le abitudini alimentari, sono implicate nelle differenze di distribuzione geografica

Età: 60-79 anni (<20% prima dei 50 anni). Se si ritrova in una persona giovane, frequentemente presentava una colite ulcerativa o una sindrome poliposa precedenti.

Epidemiology

Each year in the United States there are more than 130,000 new cases and 55,000 deaths from colorectal adenocarcinoma. This represents nearly **15% of all cancer-related deaths**, and is second only to lung cancer. Colorectal cancer incidence peaks at 60 to 70 years of age, and fewer than 20% of cases occur before age 50. Males are affected slightly more often than females. **Colorectal carcinoma is most prevalent in the United States, Canada, Australia, New Zealand, Denmark, Sweden, and other developed countries**. The incidence of this cancer is as much as 30-fold lower in India, South America, and Africa. In Japan, where incidence was previously very low, rates have now risen to intermediate levels (similar to those in the United Kingdom), presumably as a result of changes in lifestyle and diet.

The dietary factors most closely associated with increased colorectal cancer rates are **low intake of unabsorbable vegetable fiber** and **high intake of refined carbohydrates and fat**. Although these associations are clear, the mechanistic relationship between diet and risk remains poorly understood. However, it is theorized that **reduced fiber content leads to decreased stool bulk** and **altered composition of the intestinal microbiota**. This change may increase **synthesis of potentially toxic oxidative by-products of bacterial metabolism**, which would be expected to remain in contact with the colonic mucosa for **longer periods of time** as a result of reduced stool bulk. Deficiencies of vitamins A, C, and E, which act as free-radical scavengers, may compound damage caused by oxidants. **High fat intake enhances the hepatic synthesis of cholesterol and bile acids**, which can be converted into carcinogens by intestinal bacteria.

Robbins 8th Edition

Base patologica: *sequenza adenoma-carcinoma*

-popolazioni con elevata prevalenza di adenoma hanno un'elevata prevalenza di carcinoma e viceversa

-la distribuzione degli adenomi nel colon-retto è simile a quella dei carcinomi

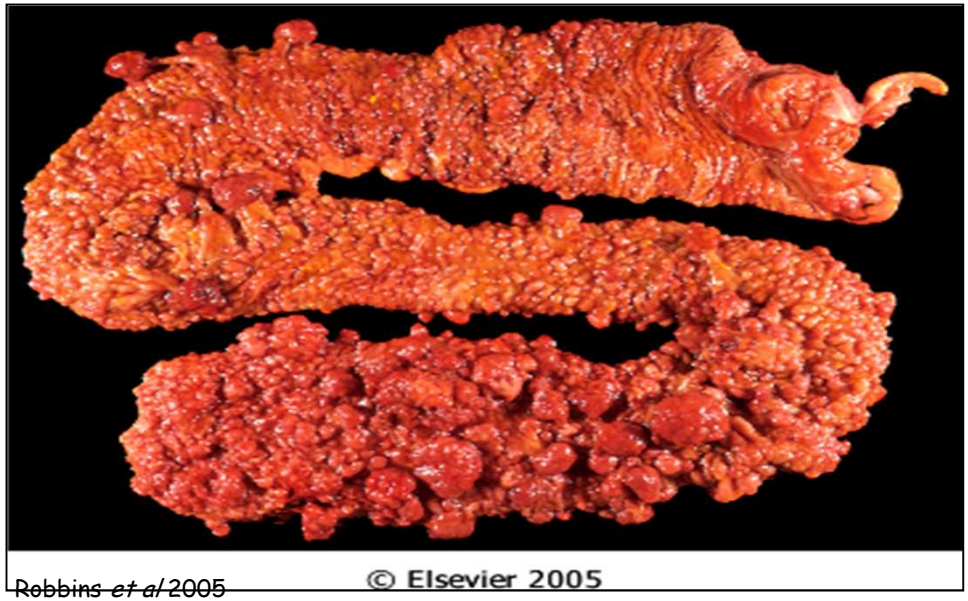
-il picco di incidenza dei polipi adenomatosi è sempre antecedente di alcuni anni rispetto al carcinoma

-quando si identifica precocemente un carcinoma invasivo intorno c'è tessuto adenomatoso

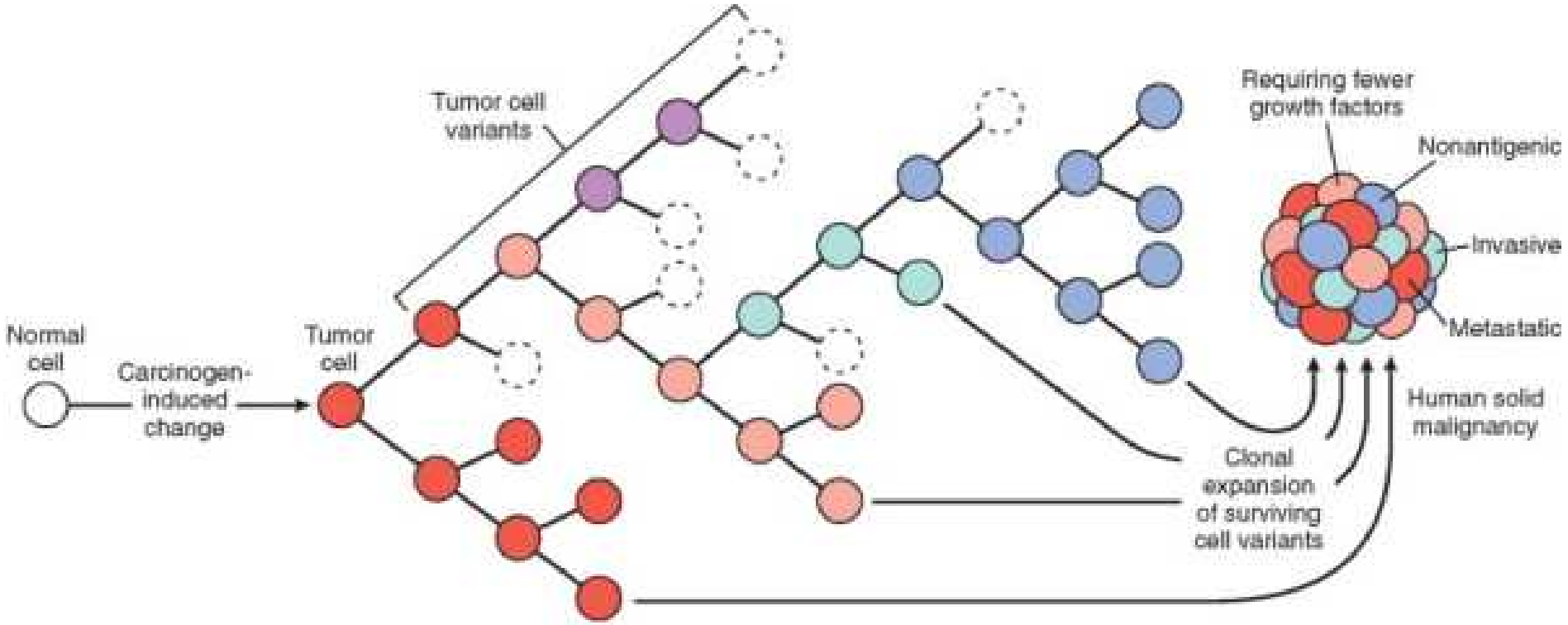
-il rischio di sviluppare il cancro è proporzionale al numero di adenomi presenti e quindi è molto elevato nei pazienti con sindromi polipose familiari.

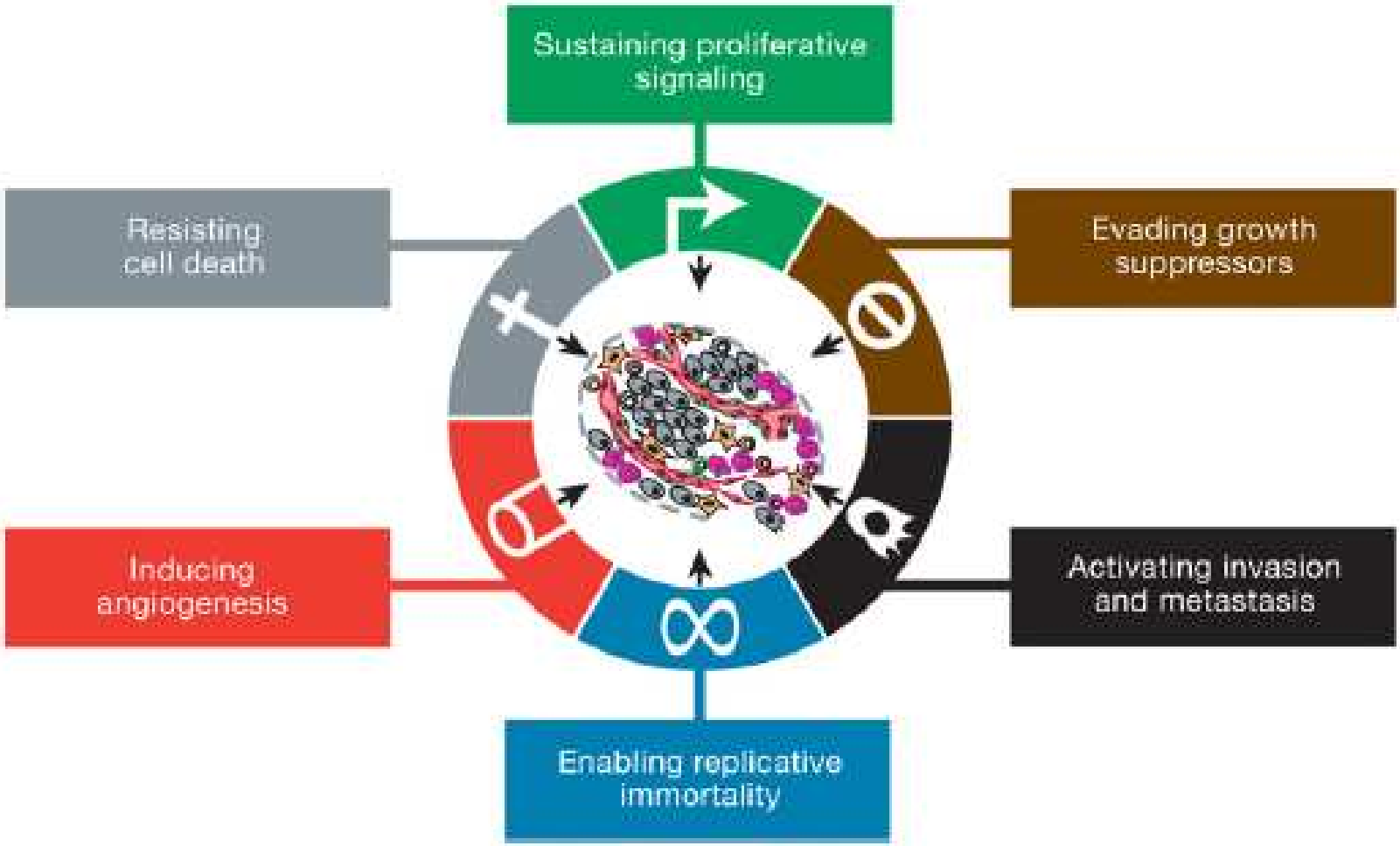
-i pazienti che sviluppano adenomi ed entrano in programmi preventivi che li fanno sottoporre a rimozione chirurgica anche se solo con sospetto, presentano una diminuita incidenza di carcinoma

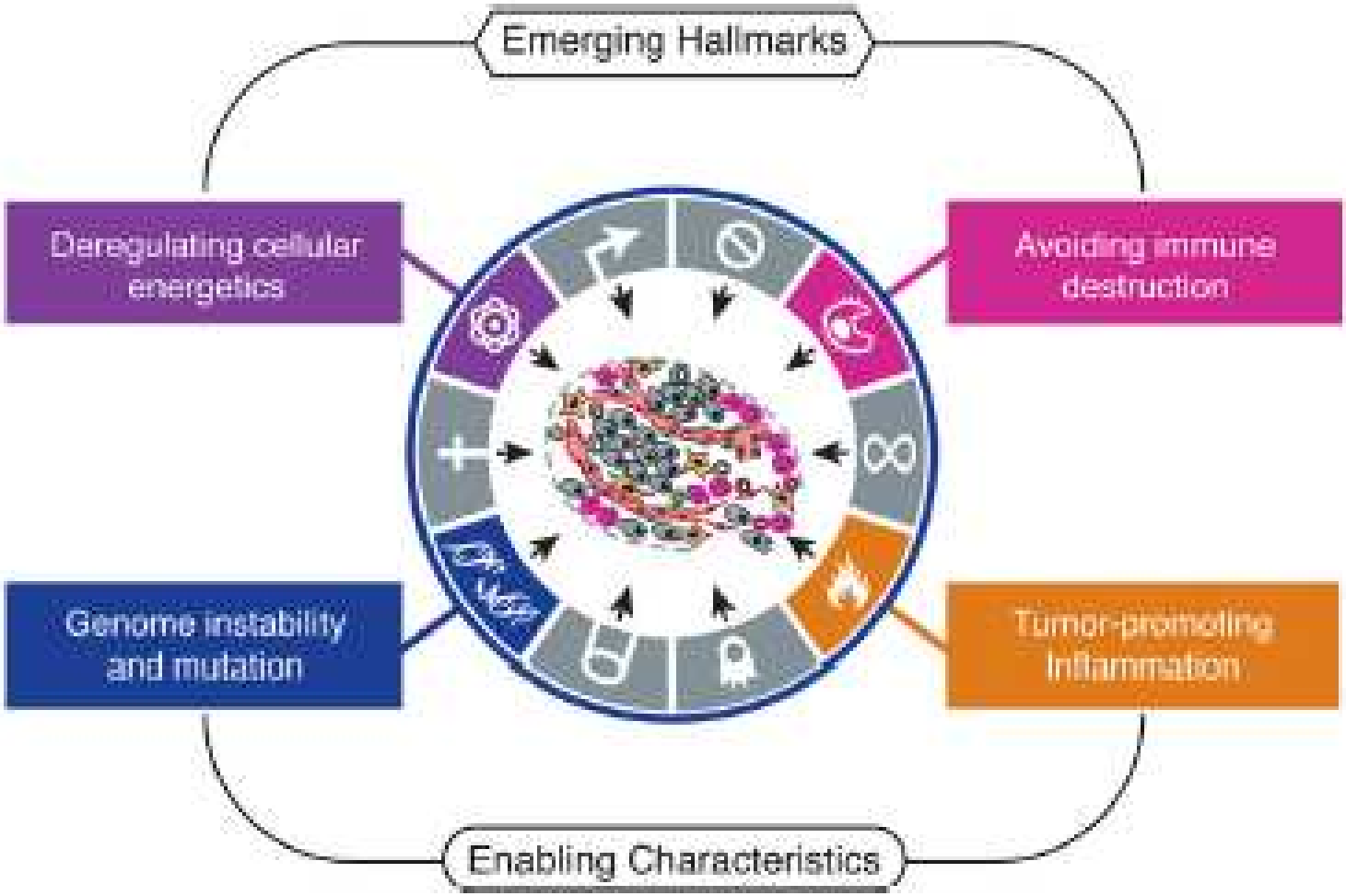
La presenza di carcinoma CR senza precedente adenoma suggerisce che alcune lesioni displastiche possono degenerare in neoplasie maligne senza passare attraverso uno stadio polipoide

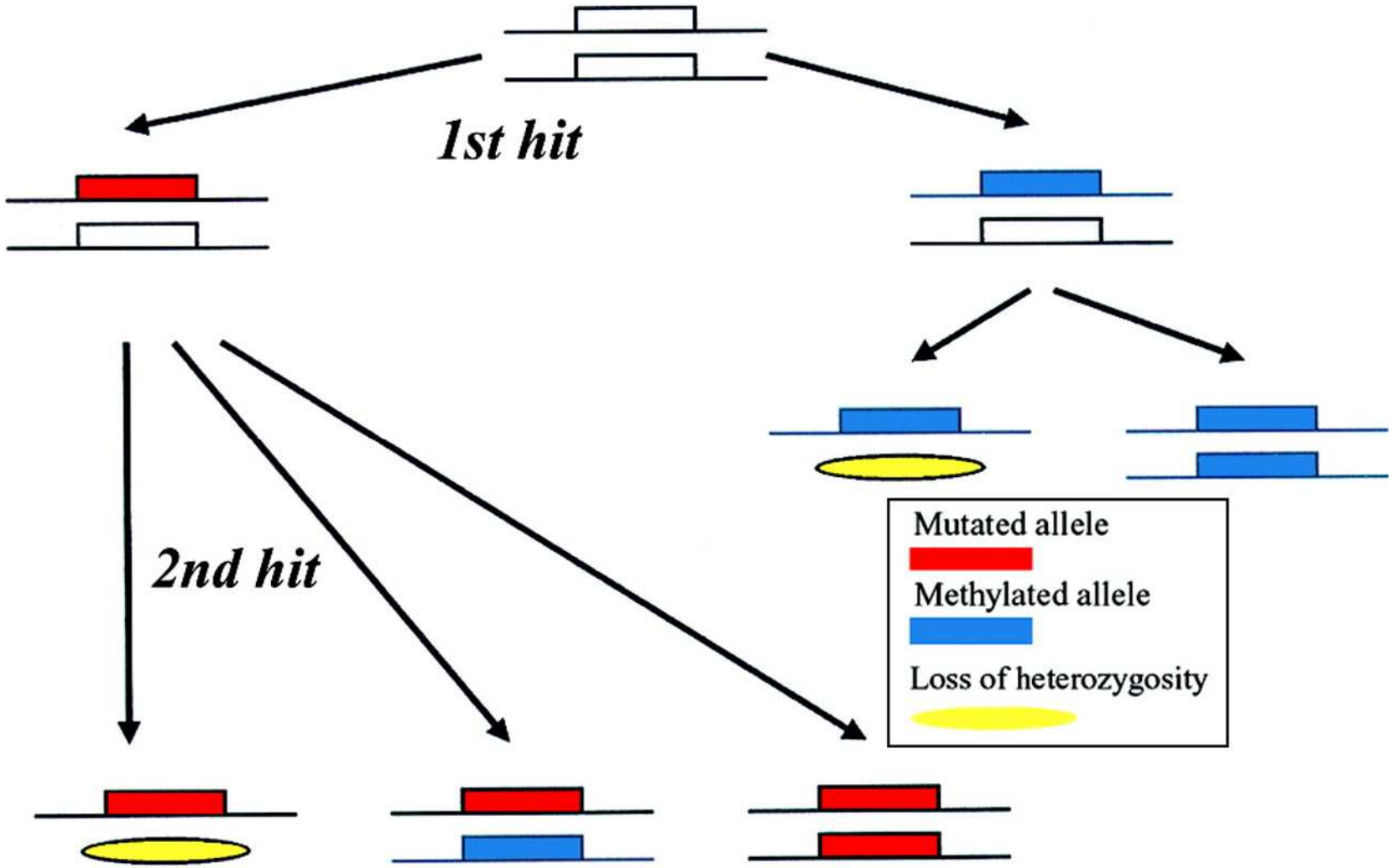


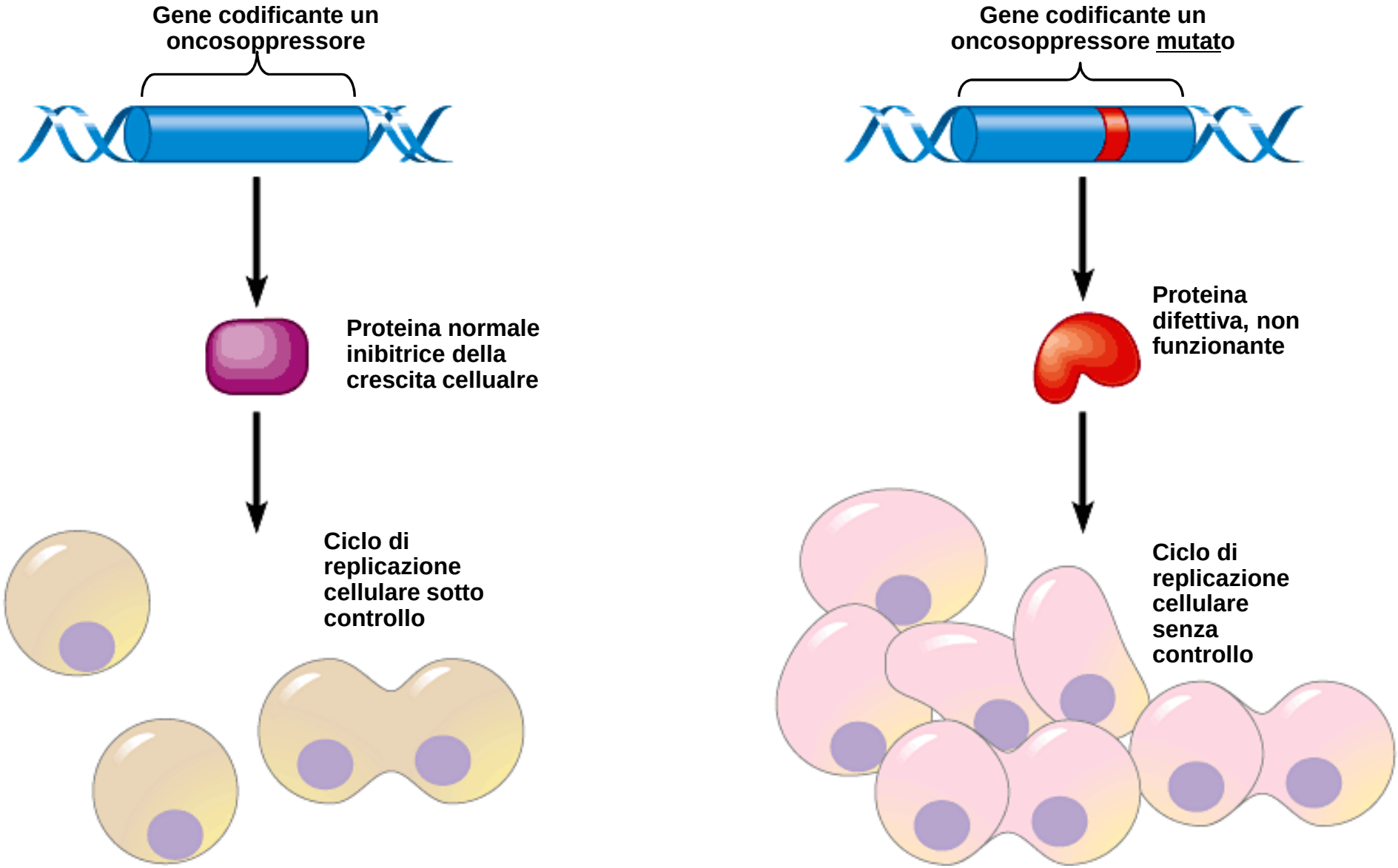
Adenomatosi poliposa familiare

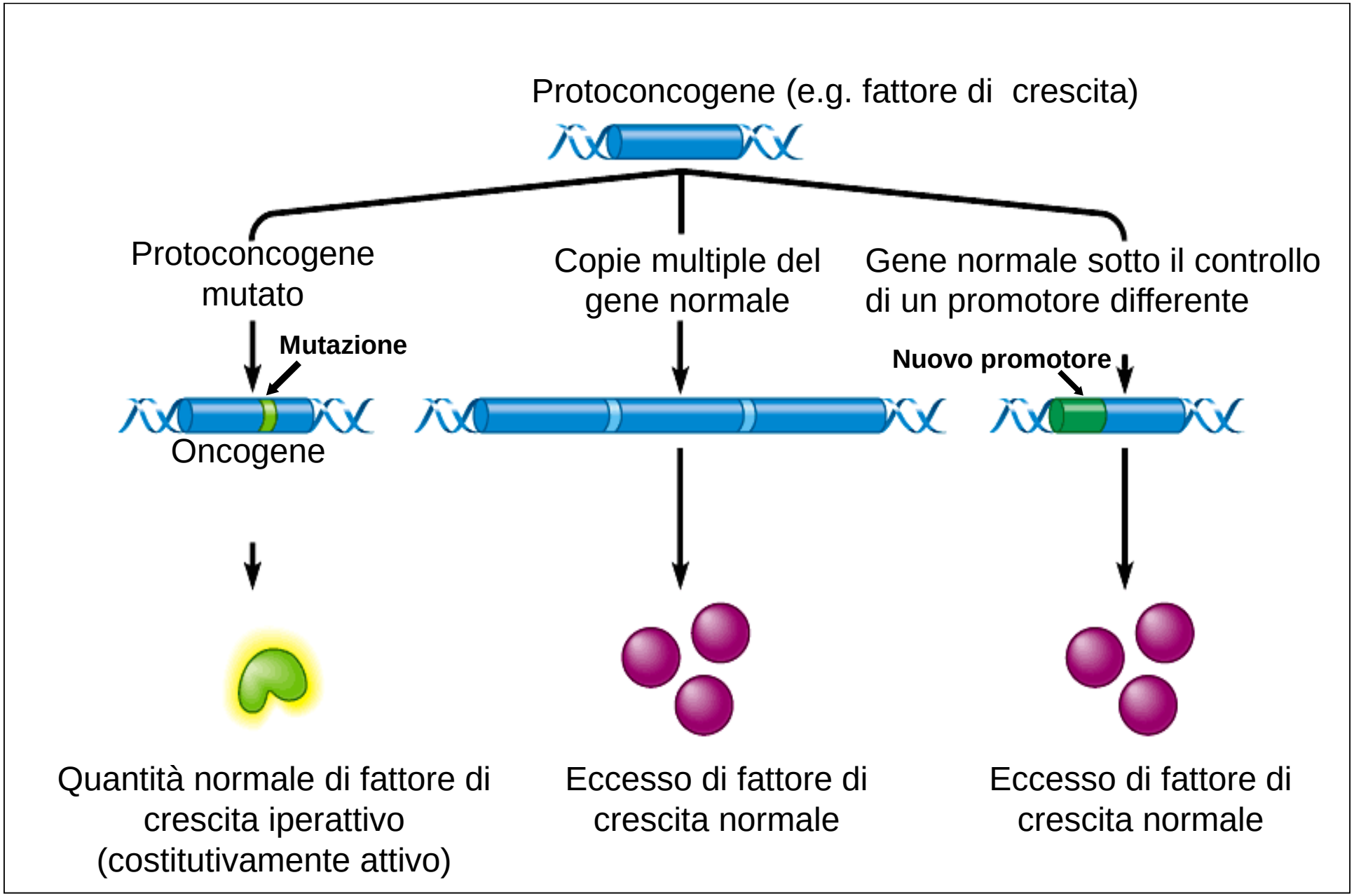

Tumor cell variants
Requiring fewer growth factors
Nonantigenic
Invasive
Metastatic
Normal cell
Carcinogen-induced change
Tumor cell
Human solid malignancy
Clonal expansion of surviving cell variants
TRANSFORMATION
PROGRESSION
PROLIFERATION OF GENETICALLY UNSTABLE CELLS
TUMOR CELL VARIANTS HETEROGENEITY


Sustaining proliferative signaling
Resisting cell death
Evading growth suppressors
Inducing angiogenesis
Activating invasion and metastasis
Enabling replicative immortality

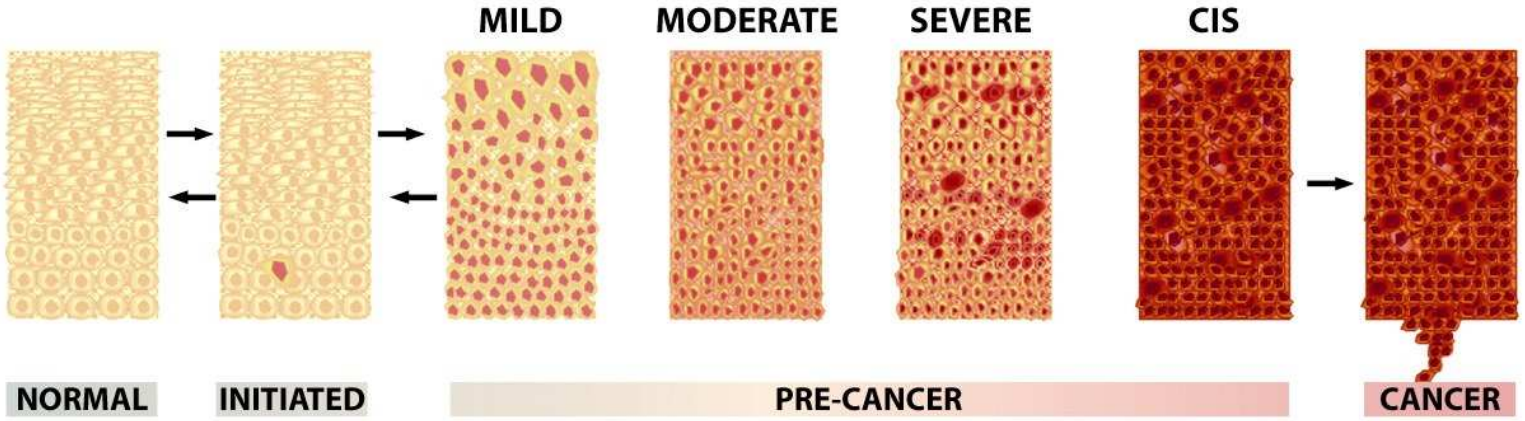

Emerging Hallmarks
Deregulating cellular energetics
Avoiding immune destruction
Genome instability and mutation
Tumor-promoting Inflammation
Enabling Characteristics


1st hit
2nd hit
Mutated allele
Methylated allele
Loss of heterozygosity

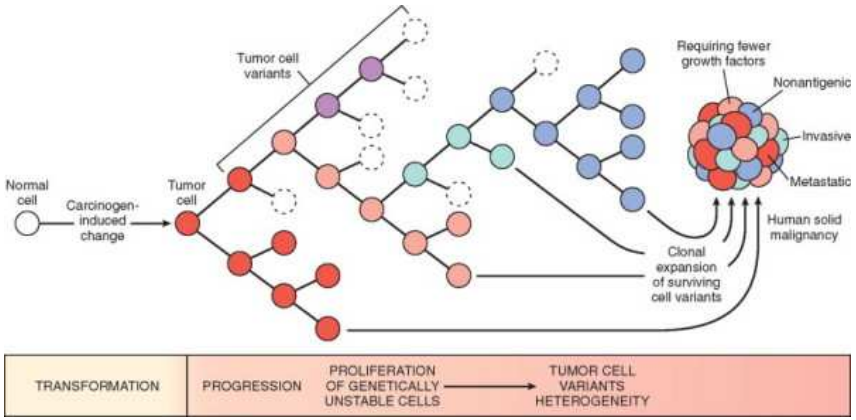

Gene codificante un oncosoppressore
Proteina normale inibitrice della crescita cellualre
Ciclo di replicazione cellulare sotto controllo
Gene codificante un oncosoppressore mutato
Proteina difettiva, non funzionante
Ciclo di replicazione cellulare senza controllo

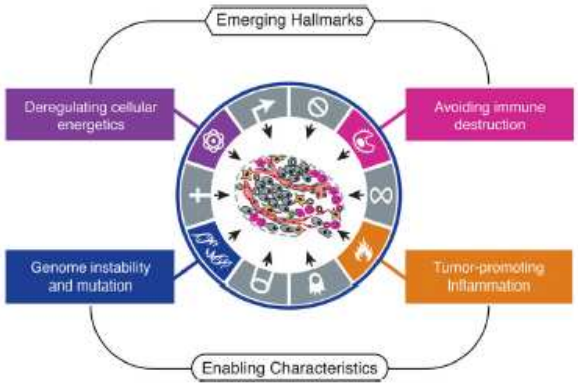


Mutazioni “dominanti”, “gain of function”

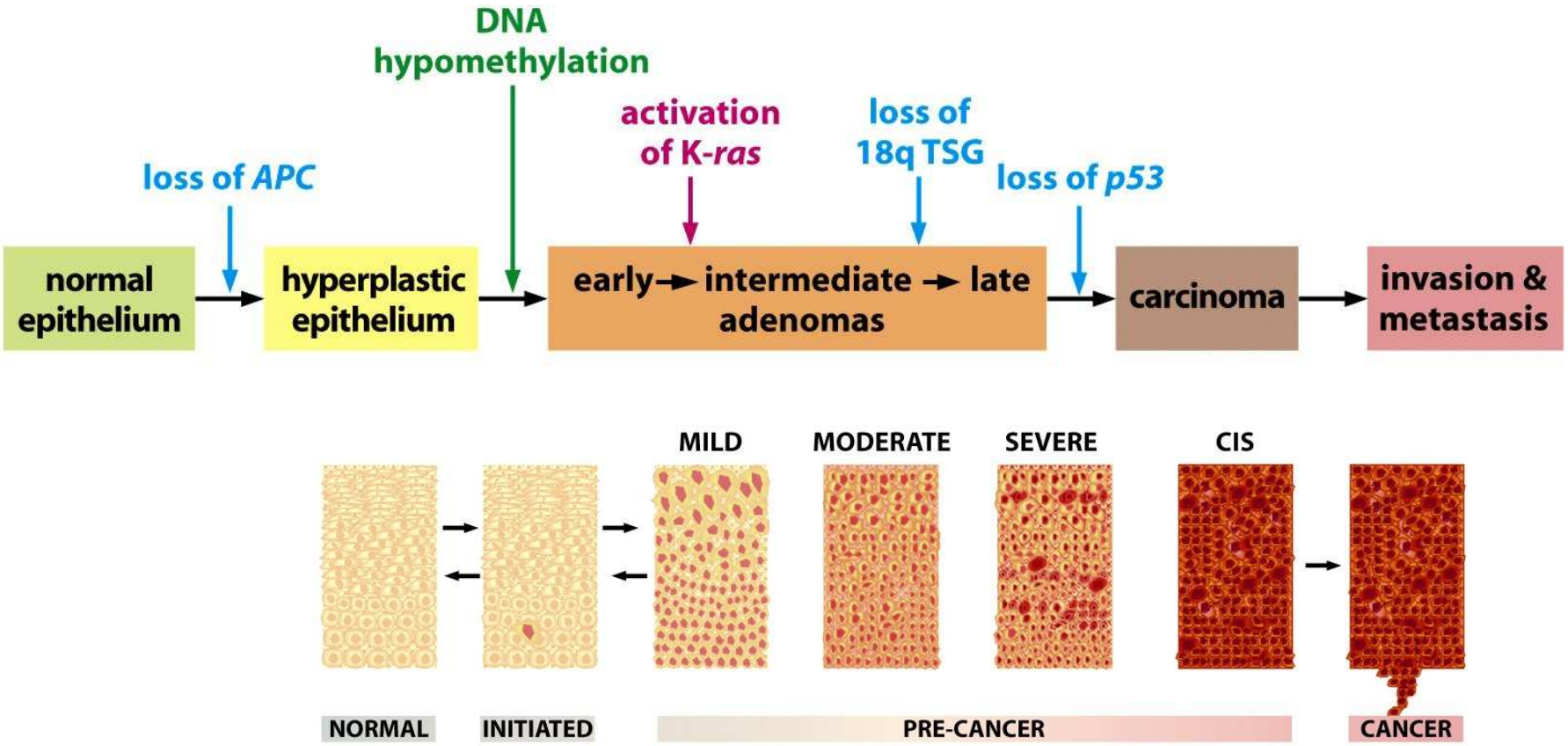


colon	5–20 years	adenoma	5–15 years			
head and neck	tobacco use 4–10 years	dysplastic oral leukoplakia	6–8 years			
cervix	CIN 1	9–13 years	CIN 3/CIS	10–20 years		
lung (smokers)	20–40 pack-years					
breast	atypical hyperplasia			DCIS	6–10 years	
prostate	20 years	PIN	≥10 years	latent cancer	3–15 years	





Vogelstein model

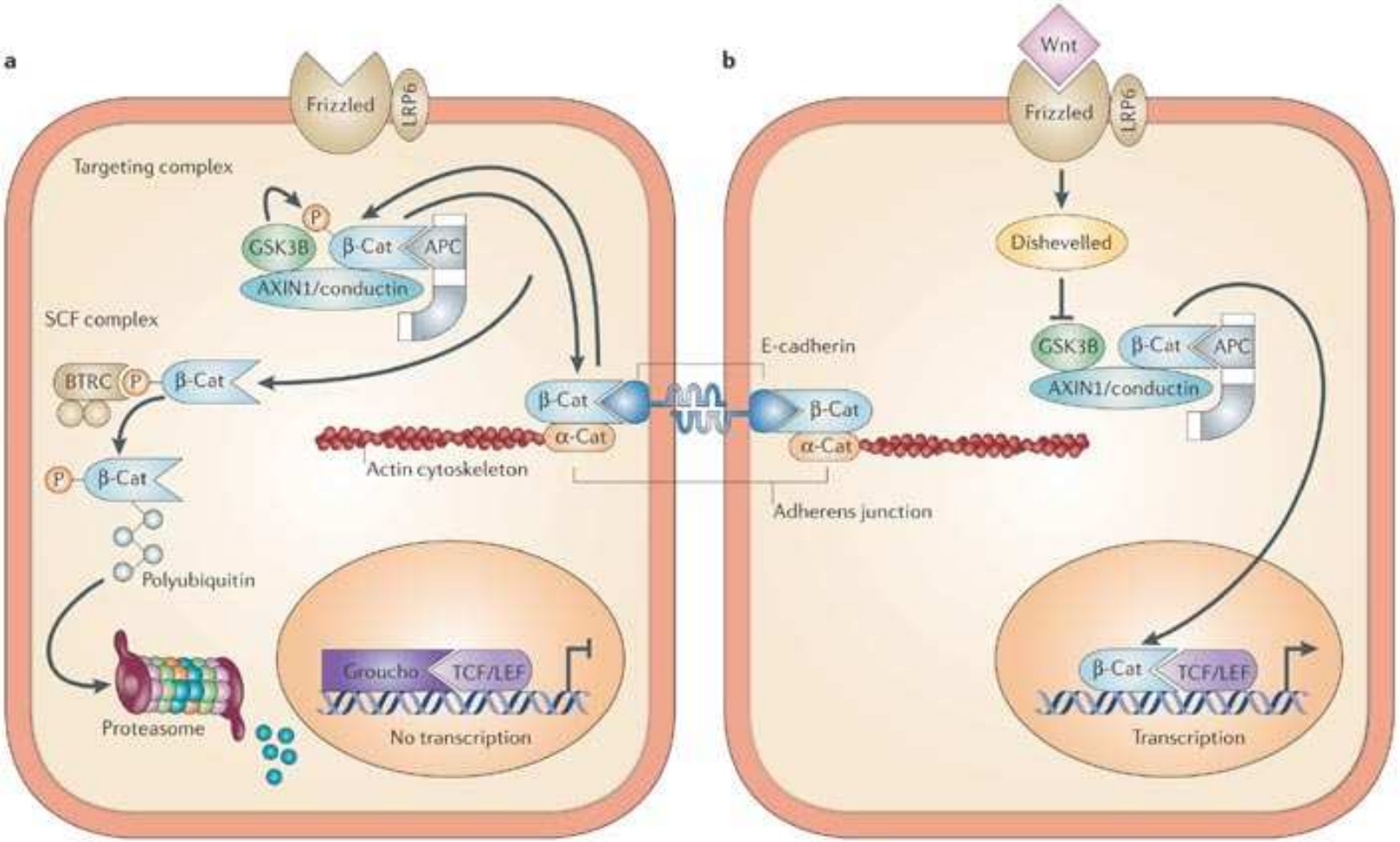


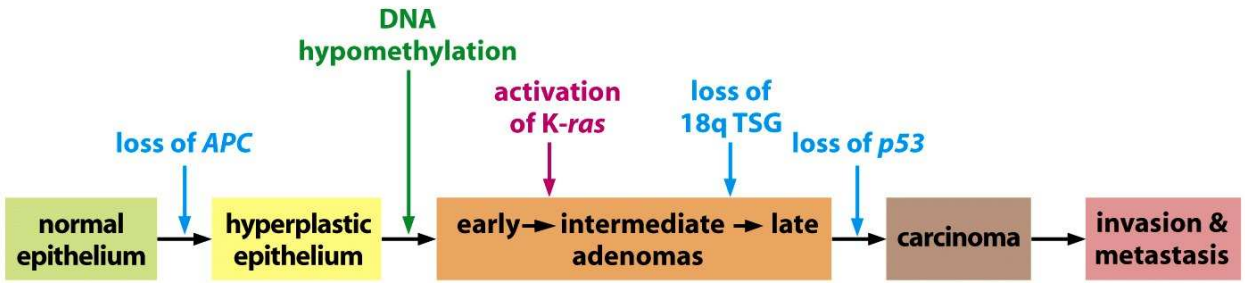
Patogenesi del cancro CR

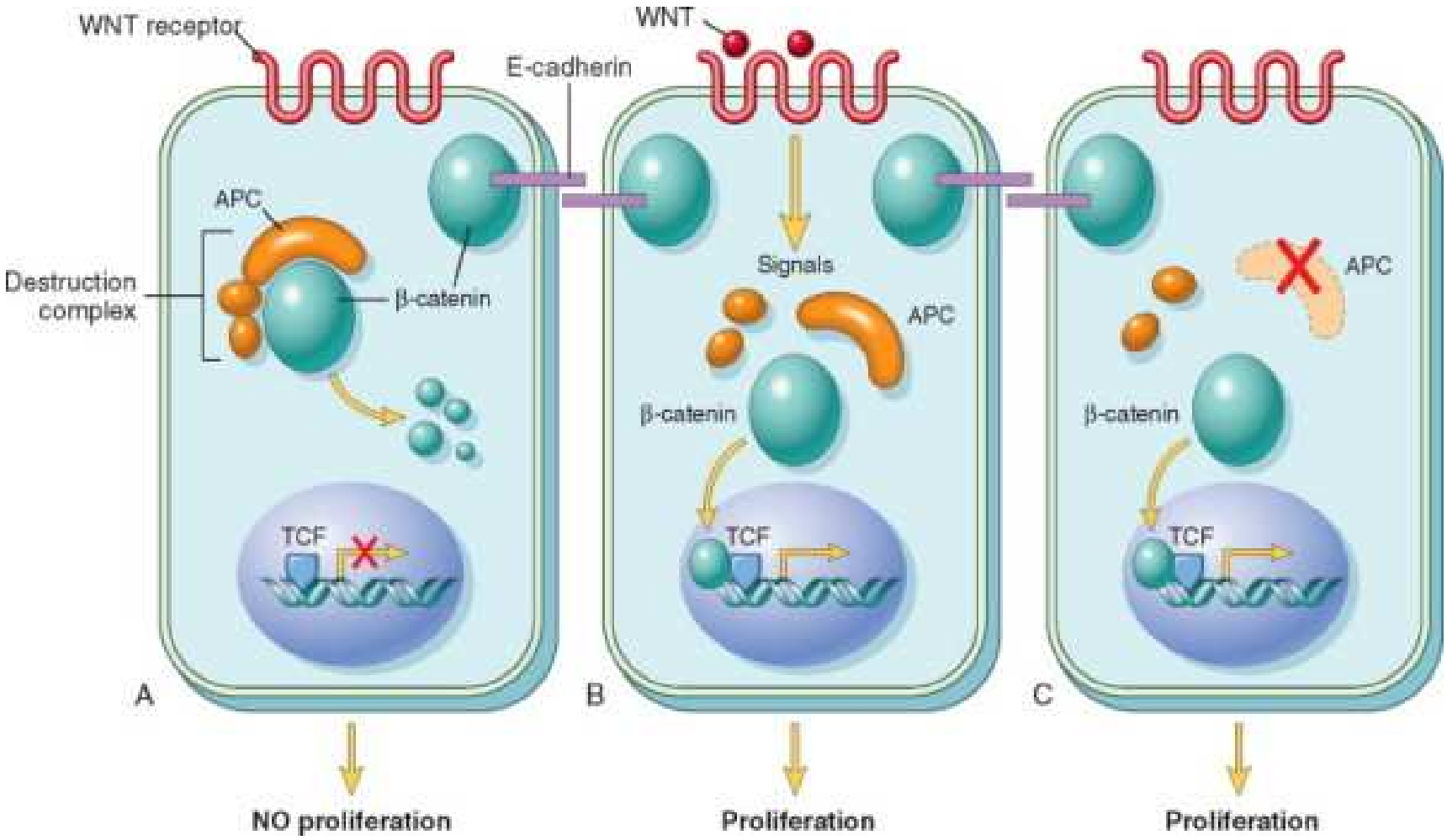
1)
APC/β-catenin: l'evoluzione molecolare del cancro CR avviene attraverso una serie di tappe morfologiche identificate: inizialmente è presente una proliferazione epiteliale localizzata, seguita dalla formazione di piccoli adenomi, che si ingrandiscono, diventano più displastici e determinano la formazione di un carcinoma invasivo.

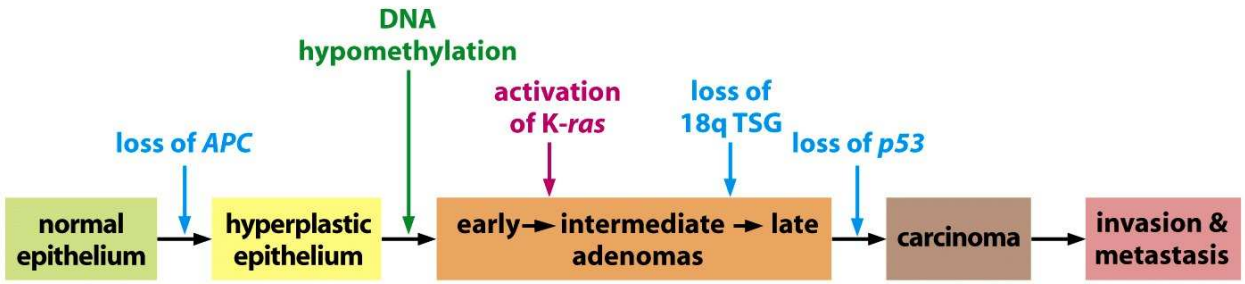
Le mutazioni genetiche correlate a questa via sono:
-perdita del gene APC (Adenomatous polyposis coli): cromosoma 5q21, è il primo evento che porta alla formazione di adenomi. Questo gene codifica per una proteina che è responsabile del legame tra i fasci dei microtubuli e promuove la migrazione cellulare e l'adesione. Inoltre regola la produzione di β-catenina, un importante mediatore del *Wnt/β-catenin signaling pathway* implicato nello sviluppo dell'epitelio intestinale. Più dell'80% dei casi di cancro CR ha APC inattivato e 50% dei casi senza APC presenta comunque mutazioni nella β-catenina.

La β-catenina fa parte del *cadherin-based cell adhesive complex*, che agisce come fattore di trascrizione se la proteina è traslocata nel nucleo: quando non è legata alla caderina e non partecipa all'adesione intercellulare, un complesso di degradazione citoplasmatica porta alla sua fosforilazione e degradazione. Se APC è mutatto, la β-catenina si accumula nel citoplasma, viene traslocata nel nucleo e si lega ad alcuni fattori di trascrizione chiamati T-cell factor (TCF) o lymphoid enhanced factor (LEF). I geni attivati dal complesso β-catenin-TCF sono quelli che regolano la proliferazione cellulare e l'apoptosi (c-MYC e CYCLIN D1). Quindi una normale funzione APC promuove l'adesione e la proliferazione cellulare, mentre l'assenza di APC porta ad una diminuita adesione cellulare ed ad un'aumentata proliferazione.

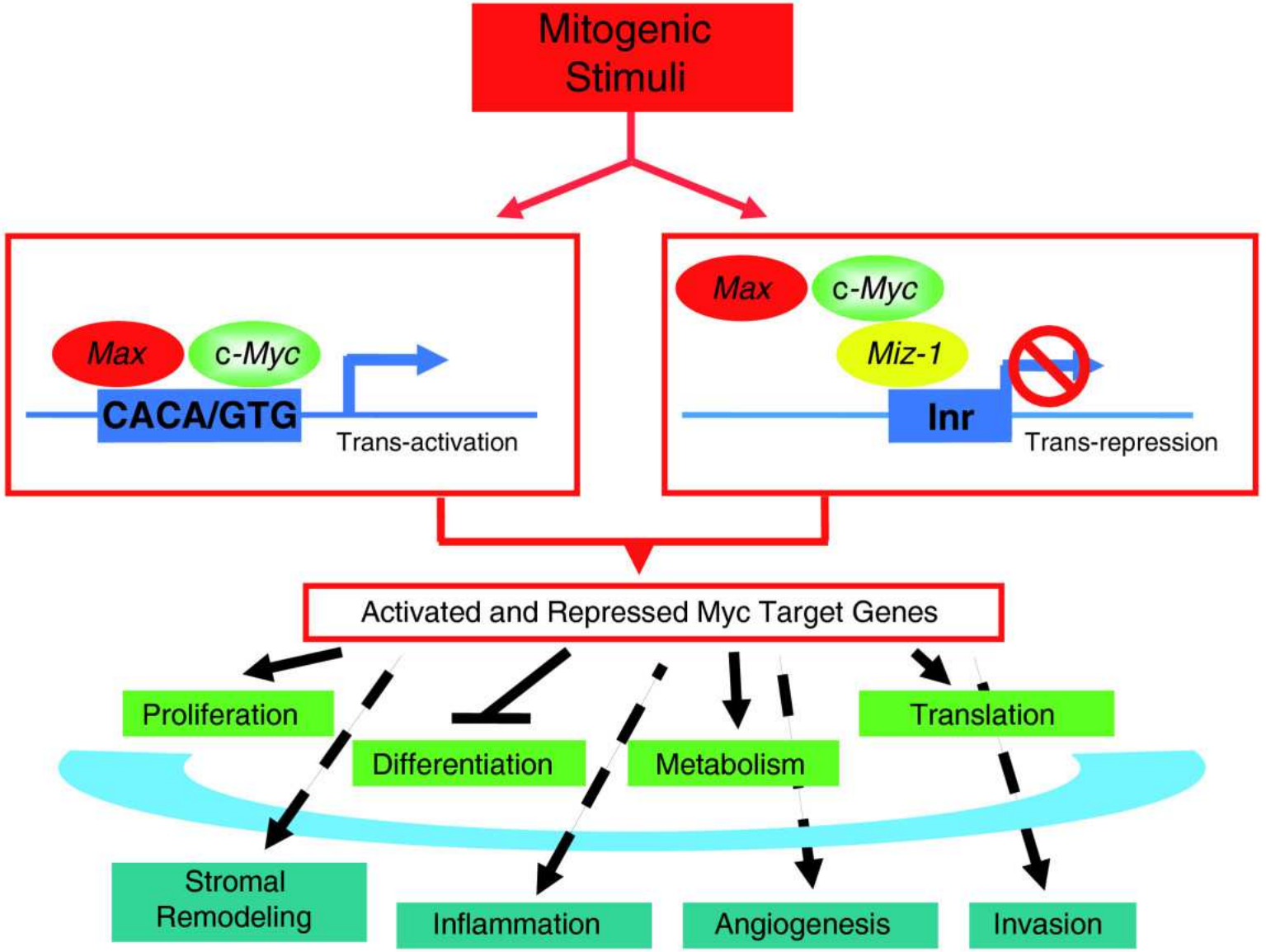


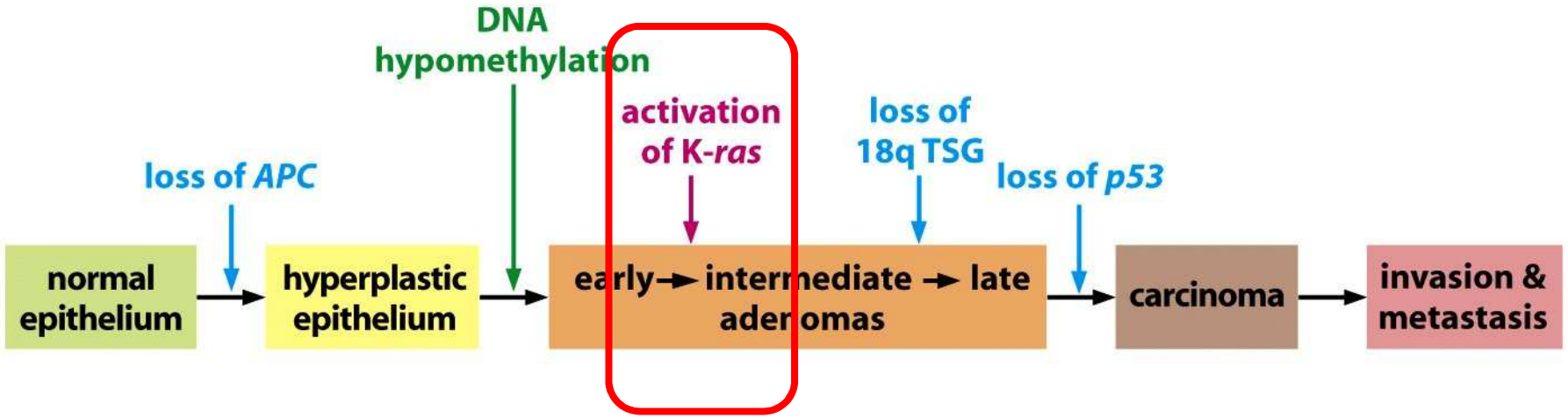



WNT receptor
WNT
E-cadherin
APC
Destruction complex
β-catenin
Signals
APC
APC
β-catenin
β-catenin
TCF
TCF
TCF
A
B
C
NO proliferation
Proliferation
Proliferation


DNA hypomethylation
activation of K-ras
loss of 18q TSG
loss of APC
loss of p53
normal epithelium
hyperplastic epithelium
early → intermediate → late adenomas
carcinoma
invasion & metastasis

Gene	Organism/system	up/down	Gene	Organism/system	up/down
c-myc	**human colon cancer**	**up**	claudin-1	human colon cancer	up
Tcf-1	human colon cancer	up	Survivin	human colon cancer	up
LEF1	human colon cancer	up	VEGF	human colon cancer	up
PPARdelta	human colon cancer	up	FGF18	human colon cancer	up
c-jun	human colon cancer	up	Hath1	human colon cancer	down
fra-1	human colon cancer	up	Met	human colon cancer	up
uPAR	human colon cancer	up	endothelin-1	human colon cancer	up
matrix metalloproteinase MMP-7	human colon cancer	up	c-myc binding protein	human colon cancer	up
Axin-2	human colon cancer	up	L1 neural adhesion	human colon cancer	up
Nr-CAM	human colon cancer	up	Id2	human colon cancer	up
ITF-2	human colon cancer	up	Jagged	human colon cancer	up
Gastrin	human colon cancer	up	EphB/ephrin-B	human colon cancer	up/down
CD44	human colon cancer	up	BMP4	human colon cancer	up


Mitogenic Stimuli
Max
c-Myc
CACA/GTG
Trans-activation
Max
c-Myc
Miz-1
Inr
Trans-repression
Activated and Repressed Myc Target Genes
Proliferation
Differentiation
Metabolism
Translation
Stromal Remodeling
Inflammation
Angiogenesis
Invasion


DNA
hypomethylation
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loss of
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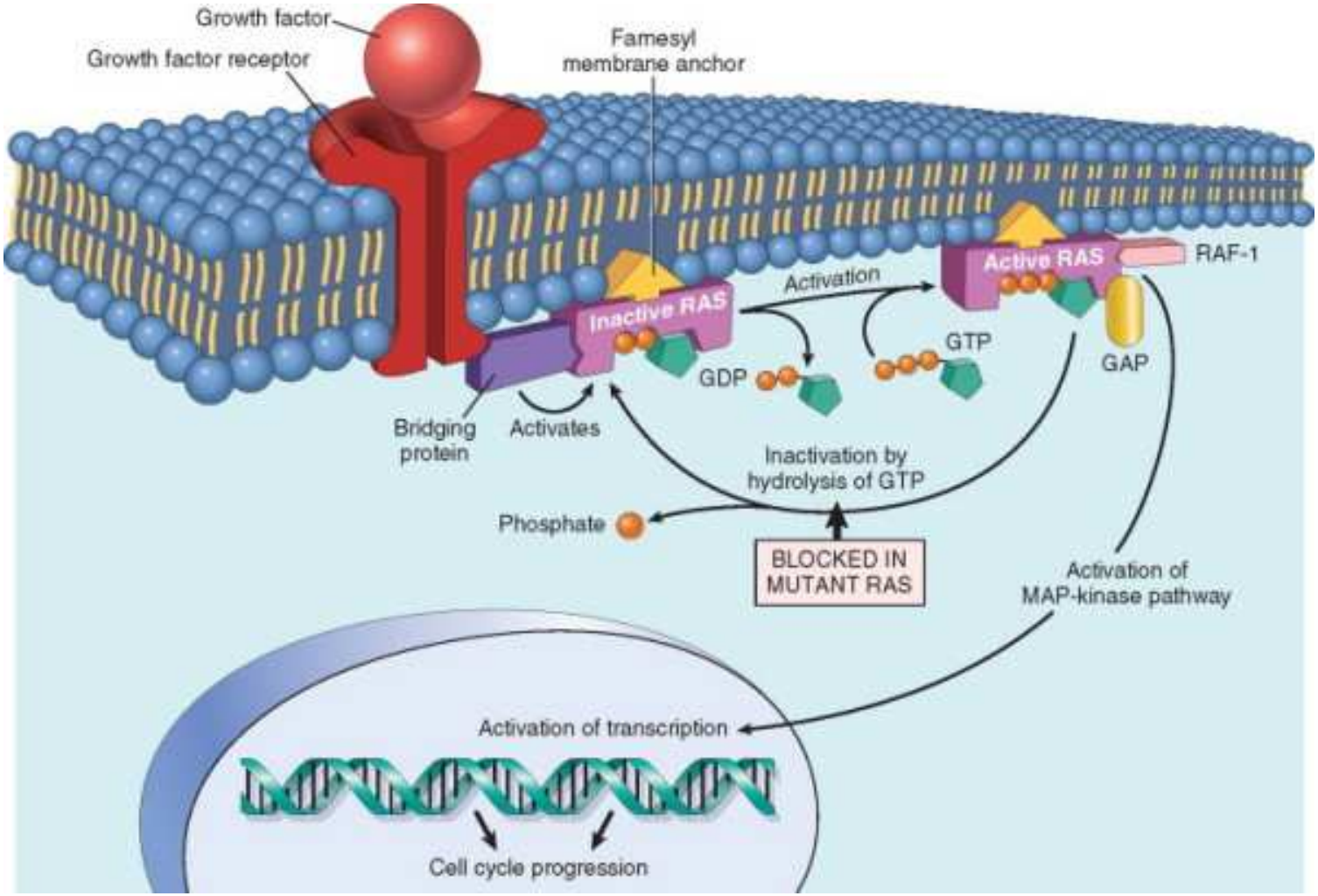
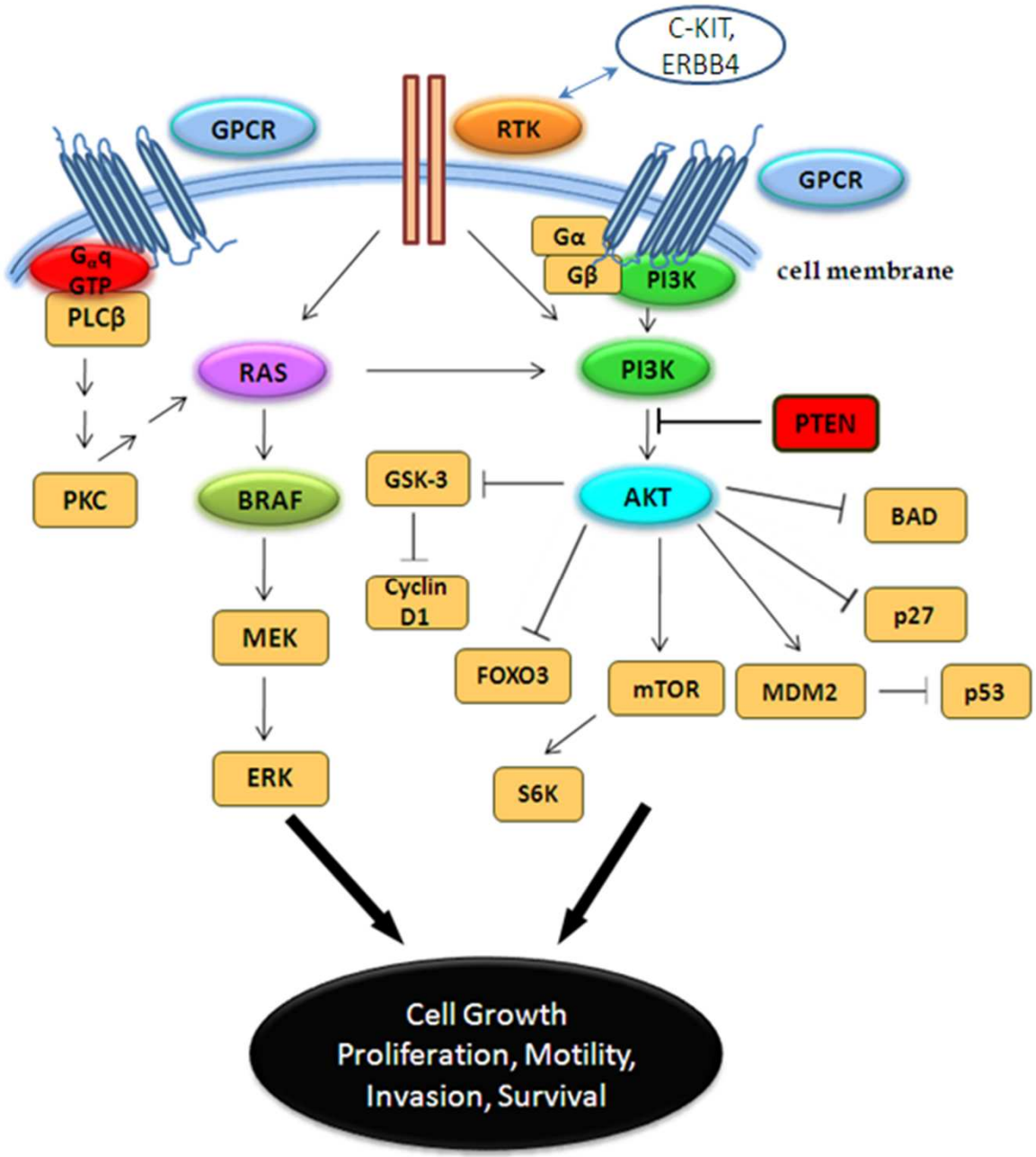
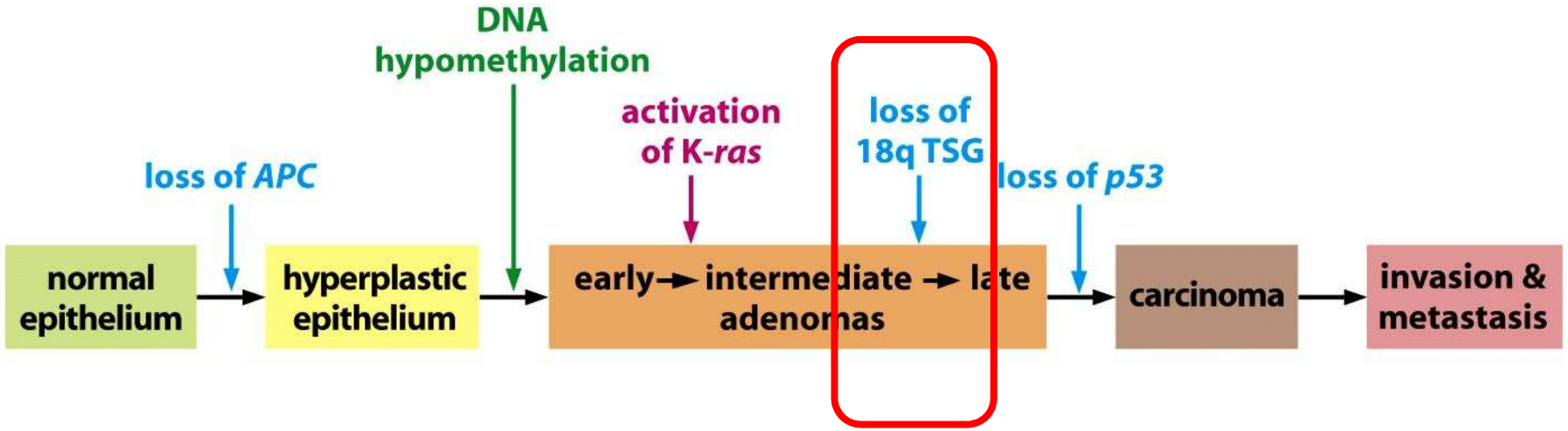
loss of APC
loss of p53
normal
epithelium
hyperplastic
epithelium
early → intermediate → late
adenomas
carcinoma
invasion &
metastasis

-<u>mutazione di K-RAS</u>: oncogene attivato più frequentemente riscontrato negli adenomi e carcinomi CR. Si ritrova in meno del 10% degli adenomi <1 cm, in 50% degli adenomi >1 cm e nel 50% dei carcinomi.




C-KIT,
ERBB4
RTK
GPCR
GPCR
cell membrane
Gα
Gβ
PI3K
Gαq
GTP
PLCβ
RAS
PI3K
PTEN
PKC
BRAF
GSK-3
AKT
BAD
Cyclin
D1
p27
MEK
FOXO3
mTOR
MDM2
p53
ERK
S6K
Cell Growth
Proliferation, Motility,
Invasion, Survival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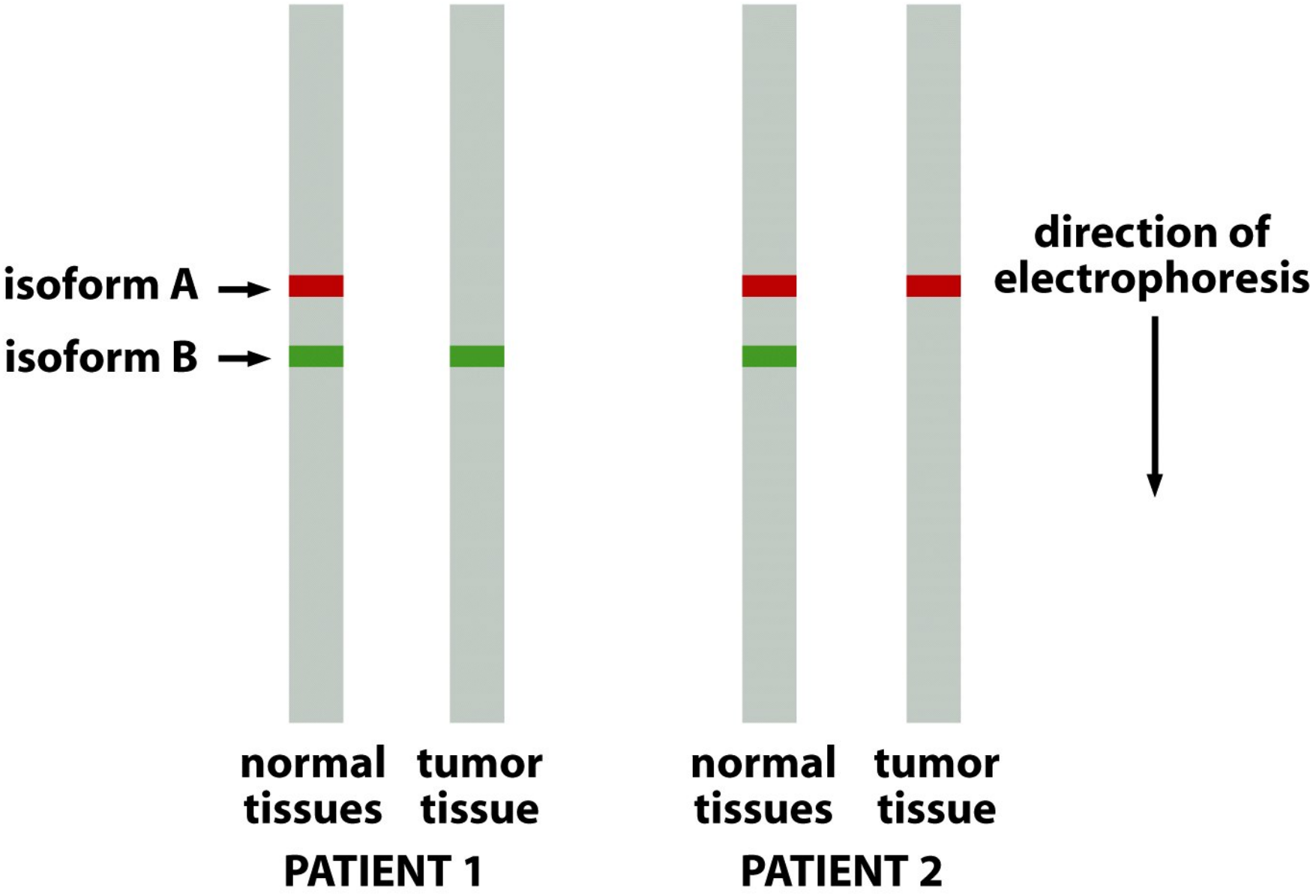
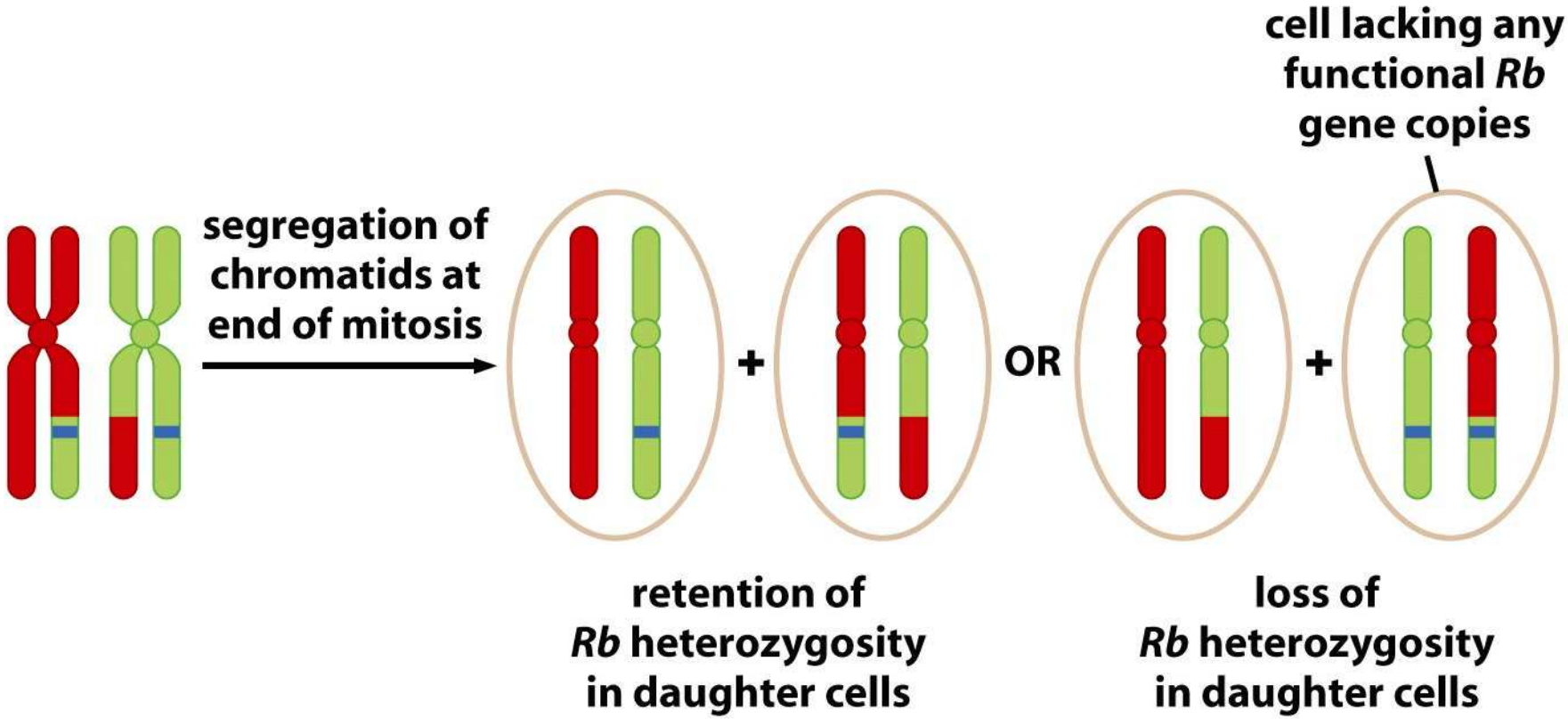


LOH Loss of heterozygosity


segregation of chromatids at end of mitosis
+
OR
+
cell lacking any functional *Rb* gene copies
retention of *Rb* heterozygosity in daughter cells
loss of *Rb* heterozygosity in daughter cells

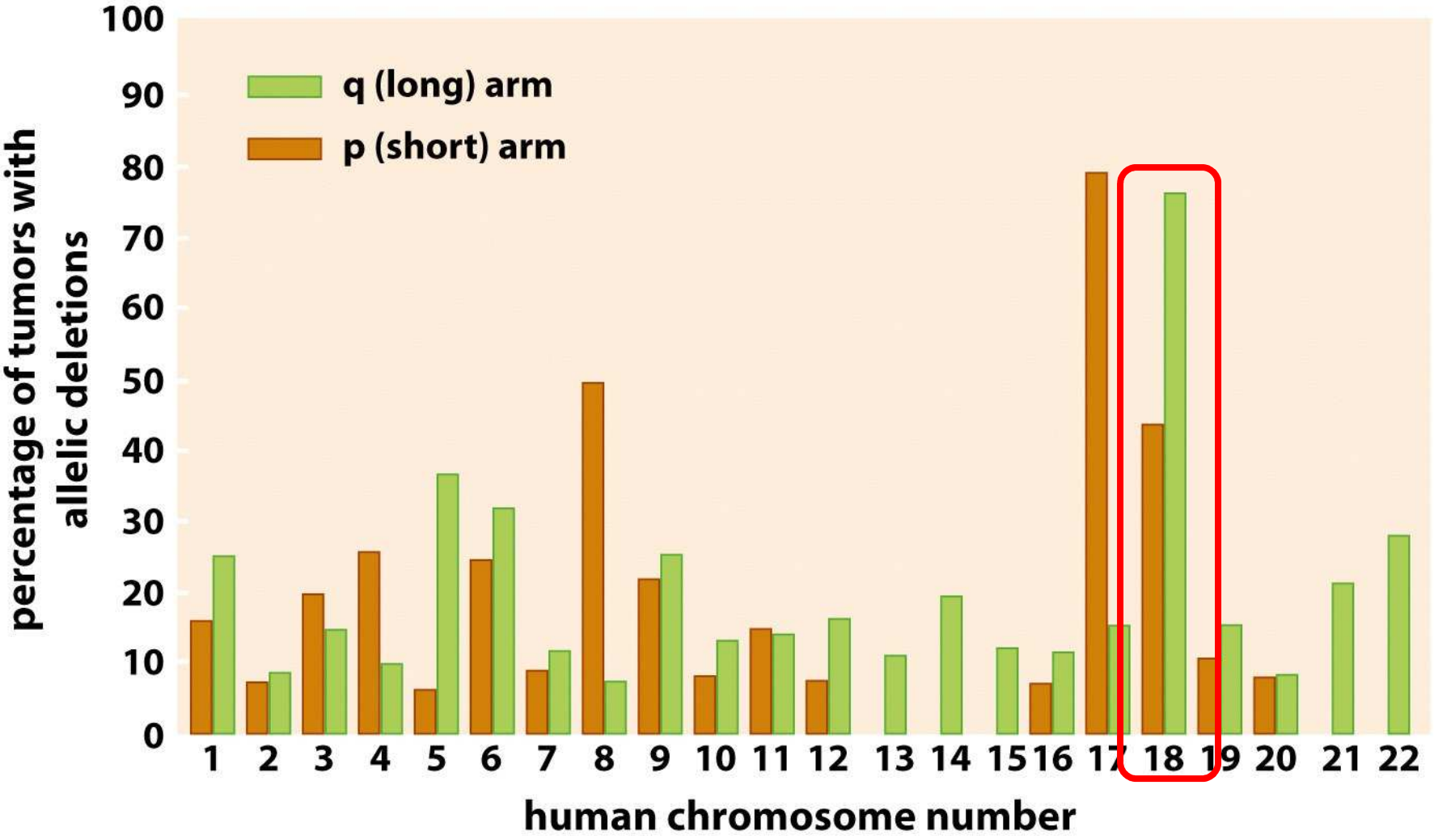

q (long) arm
p (short) arm
percentage of tumors with allelic deletions
100
90
80
70
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
human chromosome number

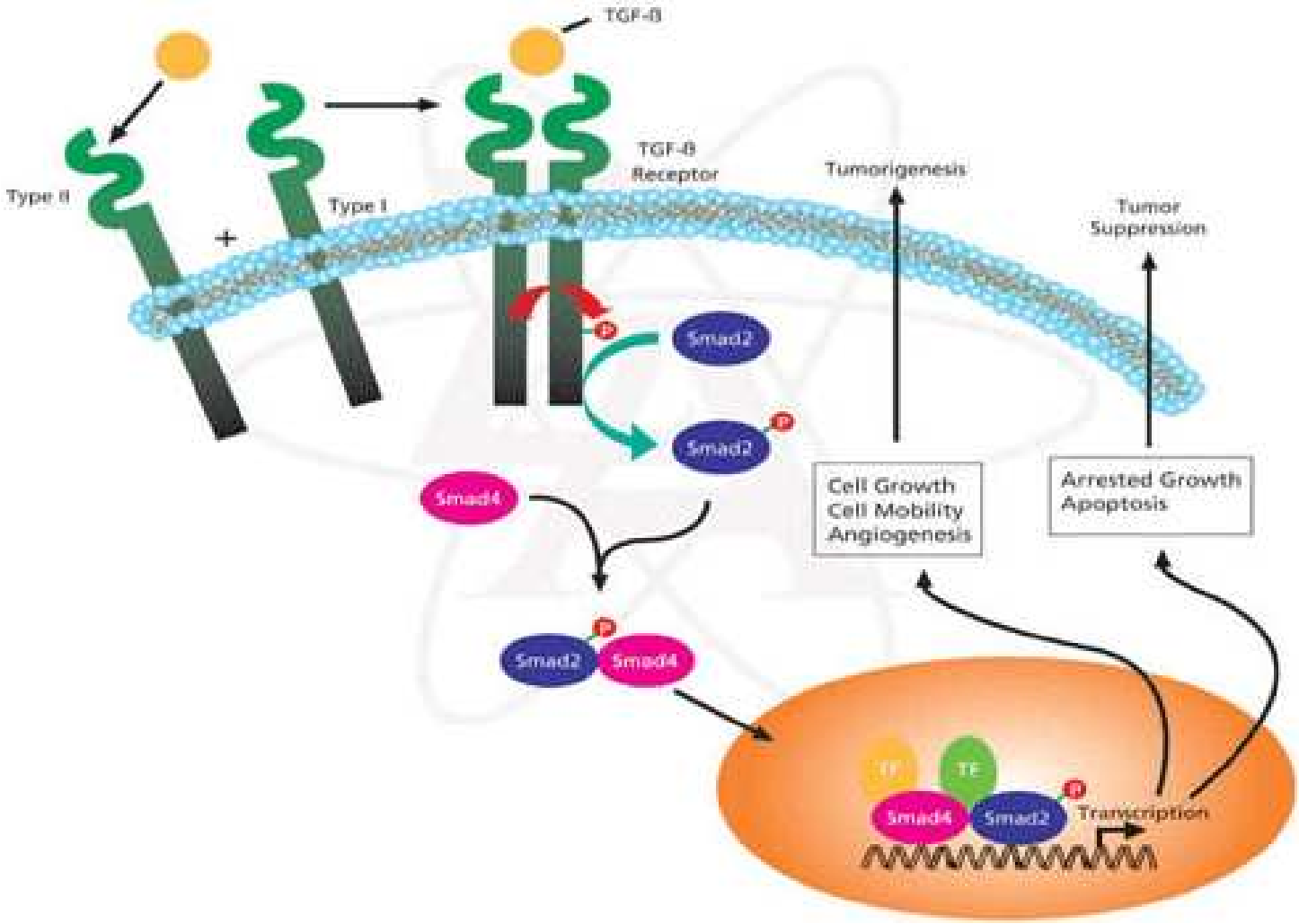
Table 7.1 Human tumor suppressor genes that have been cloned

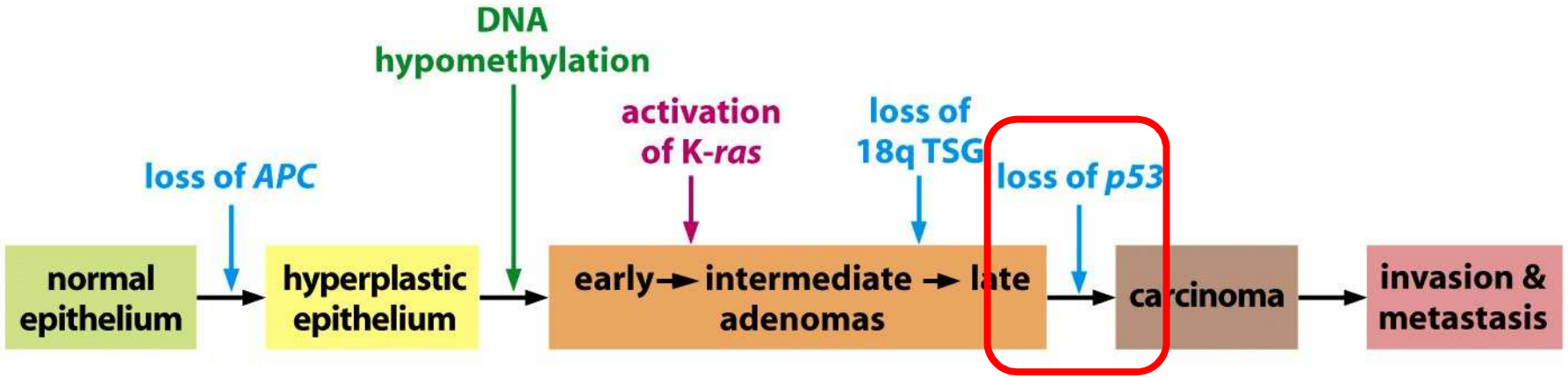
Name of gene	Chromosomal location	Familial cancer syndrome	Sporadic cancer	Function of protein
BWS/CDKN1C	11p15.5	Beckwith–Wiedemann syndrome	—	$p57^{Kip2}$ CDK inhibitor
SDHD	11q23	familial paraganglioma	pheochromocytoma	mitochondrial protein[e]
RB	13q14	retinoblastoma, osteosarcoma	retinoblastoma; sarcomas; bladder, breast, esophageal, and lung carcinomas	transcriptional repression; control of E2Fs
TSC2	16p13	tuberous sclerosis	—	inhibitor of mTOR[f]
CBP	16p13.3	Rubinstein–Taybi	AML[g]	TF co-activator
CYLD	16q12–13	cylindromatosis	—	deubiquitinating enzyme
CDH1	16q22.1	familial gastric carcinoma	invasive cancers	cell–cell adhesion
BHD	17p11.2	Birt–Hogg–Dube syndrome	kidney carcinomas, hamartomas	unknown
TP53	17p13.1	Li–Fraumeni syndrome	many types	TF
NF1	17q11.2	neurofibromatosis type 1	colon carcinoma, astrocytoma	Ras-GAP
BECN1	17q21.3	—	breast, ovarian, prostate	autophagy
PRKAR1A	17q22–24	multiple endocrine neoplasia[h]	multiple endocrine tumors	subunit of PKA
DPC4[i]	18q21.1	juvenile polyposis	pancreatic and colon carcinomas	TGF-β TF
LKB1/STK11	19p13.3	Peutz–Jeghers syndrome	hamartomatous colonic polyps	serine/threonine kinase
RUNX1	21q22.12	familial platelet disorder	AML	TF
SNF5[j]	22q11.2	rhabdoid predisposition syndrome	malignant rhabdoid tumors	chromosome remodeling
NF2	22q12.2	neurofibroma-position syndrome	schwannoma, meningioma; ependymoma	cytoskeleton–membrane linkage

[a]Familial leiomyomatosis includes multiple fibroids, cutaneous leiomyomas, and renal cell carcinoma. The gene product is a component of the tricarboxylic cycle.
[b]Also known as *MTS1*, *CDKN2*, and *p16*.
[c]The human homolog of the murine $p19^{ARF}$ gene.
[d]Also called *MMAC* or *TEP1*.
[e]*SDHS* encodes the succinate–ubiquinone oxidoreductase subunit D, a component of the mitochondrial respiratory chain complex II.
[f]mTOR is a serine/threonine kinase that controls, among other processes, the rate of translation and activation of Akt/PKB. TSC1 (hamartin) and TSC2 (tuberin) control both cell size and cell proliferation.
[g]The *CBP* gene is involved in chromosomal translocations associated with AML. These translocations may reveal a role of a segment of CBP as an oncogene rather than a tumor suppressor gene.
[h]Also termed Carney complex.
[i]Encodes the Smad4 TF associated with TGF-β signaling; also known as *MADH4* and *SMAD4*.
[j]The human SNF5 protein is a component of the large Swi/Snf complex that is responsible for remodeling chromatin in a way that leads to transcriptional repression through the actions of histone deactylases. The rhabdoid predisposition syndrome involves susceptibility to atypical teratoid/rhabdoid tumors, choroid plexus carcinomas, medulloblastomas, and extra-renal rhabdoid tumors.

Adapted in part from E.R. Fearon, *Science* 278:1043–1050, 1997; and in part from D.J. Marsh and R.T. Zori, *Cancer Lett.* 181:125–164, 2002.

-<u>perdita di SMADs</u>: una perdita allelica comune nel cancro CR è sul cromosoma 18q21. SMAD2 e SMAD4 sono coinvolti nel segnale del TGF-β, la loro perdita aumenta la tumorigenesi gastrointestinale.

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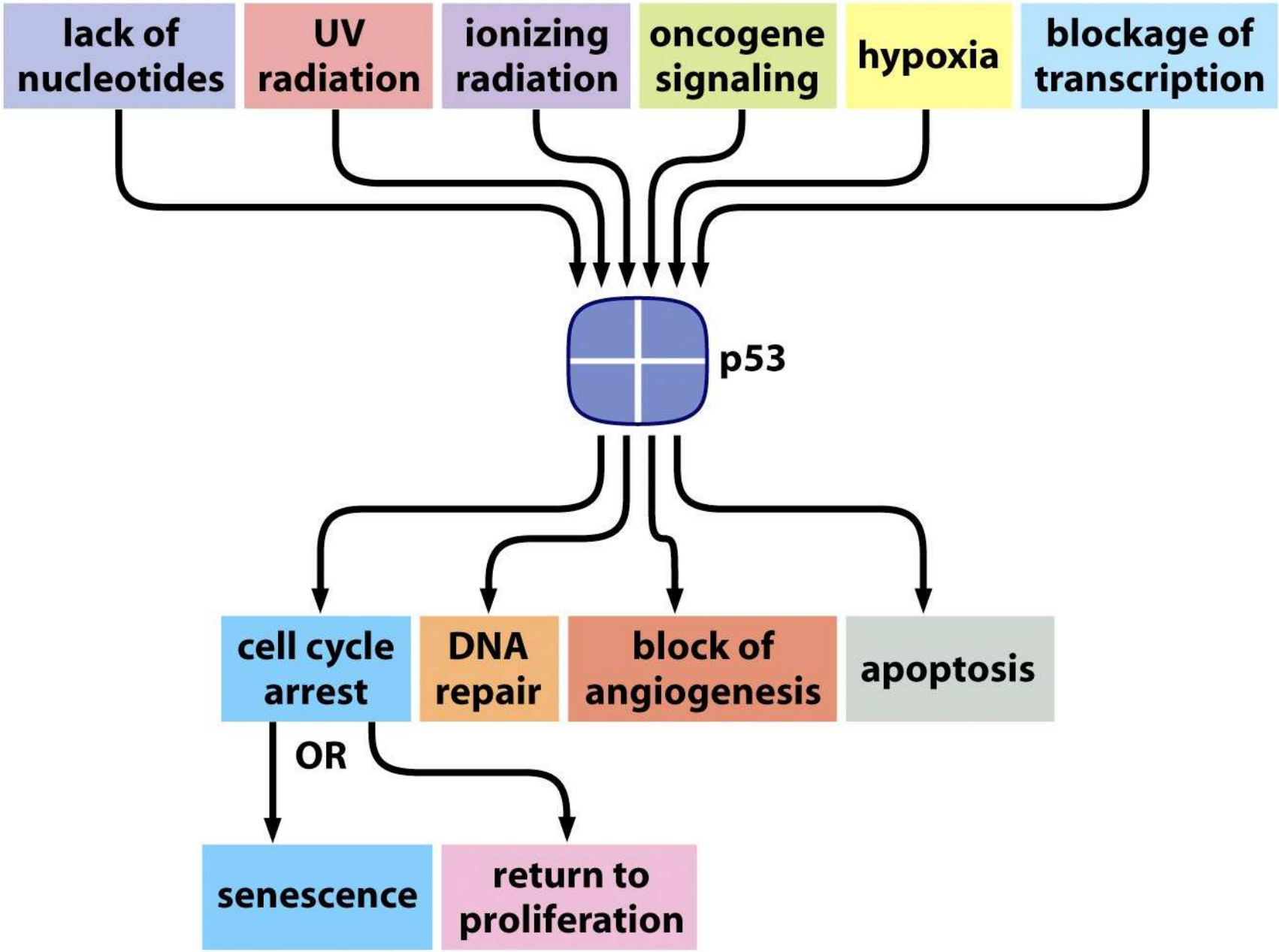
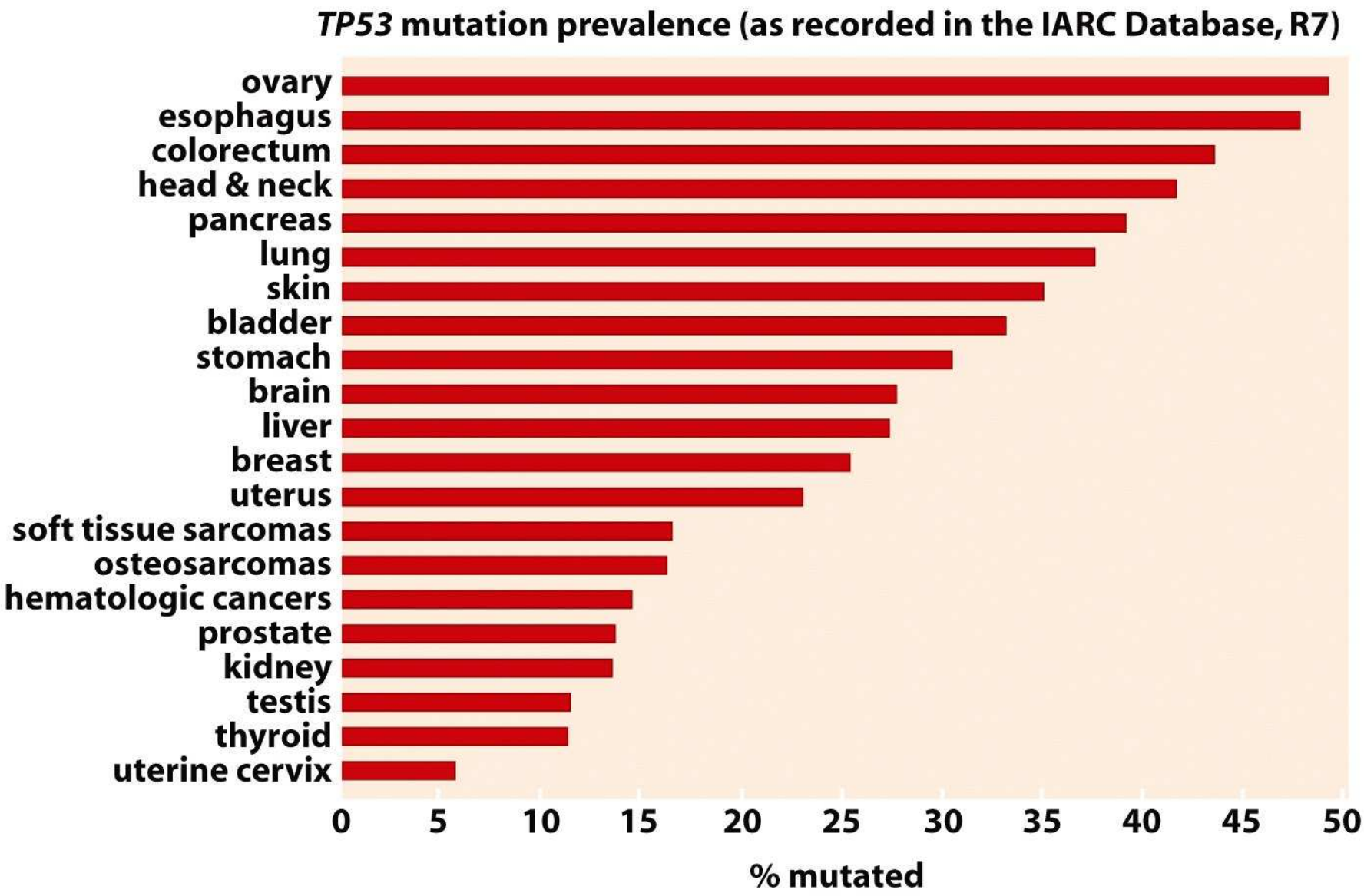
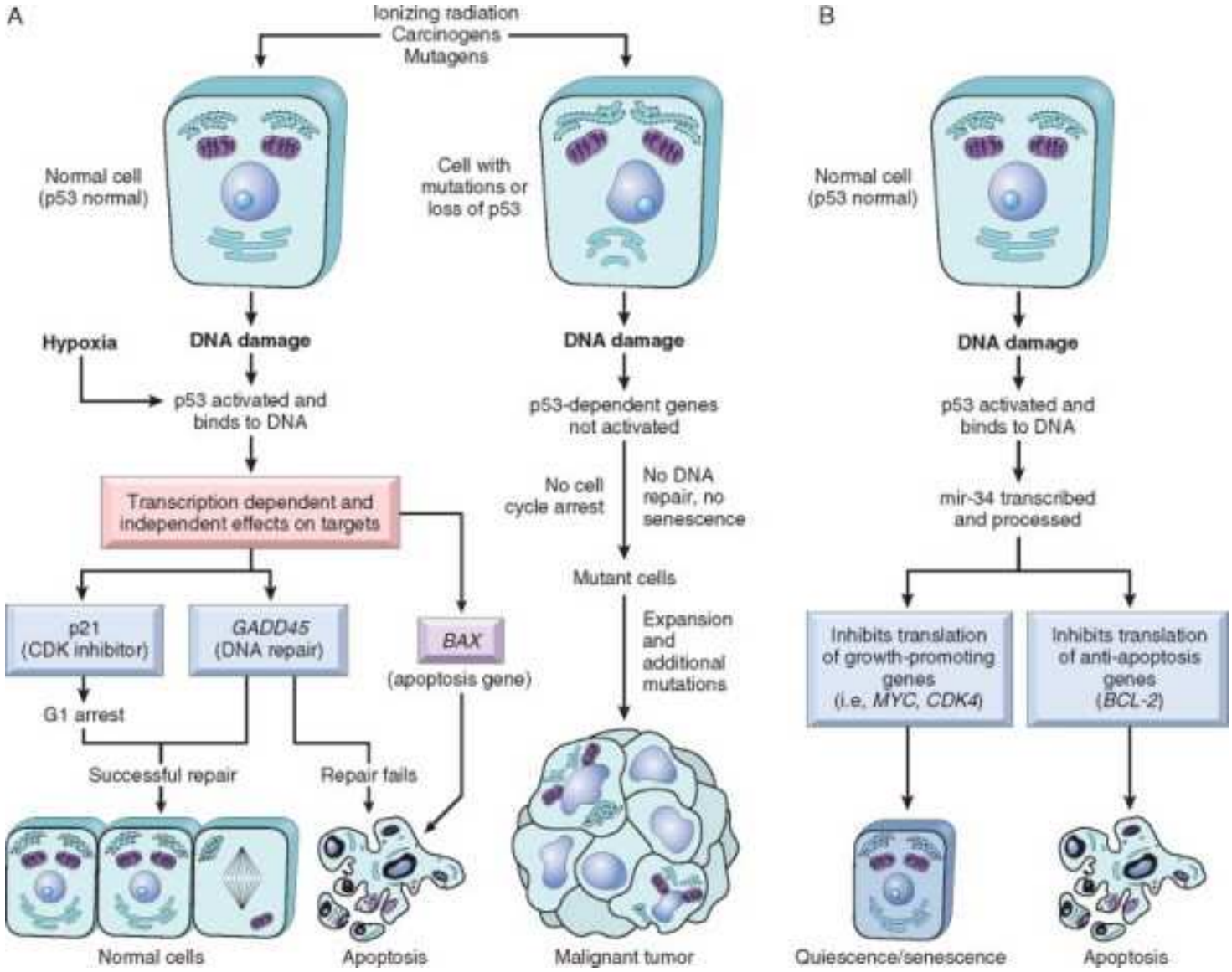
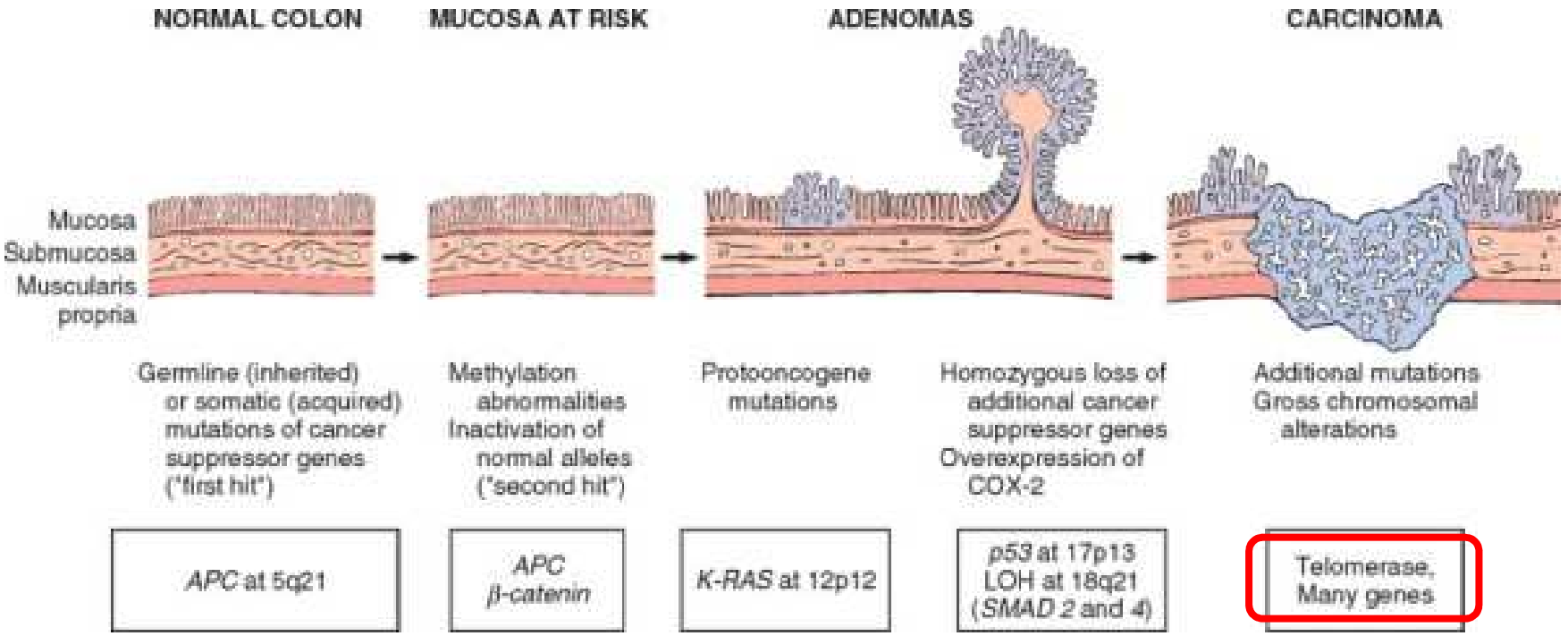
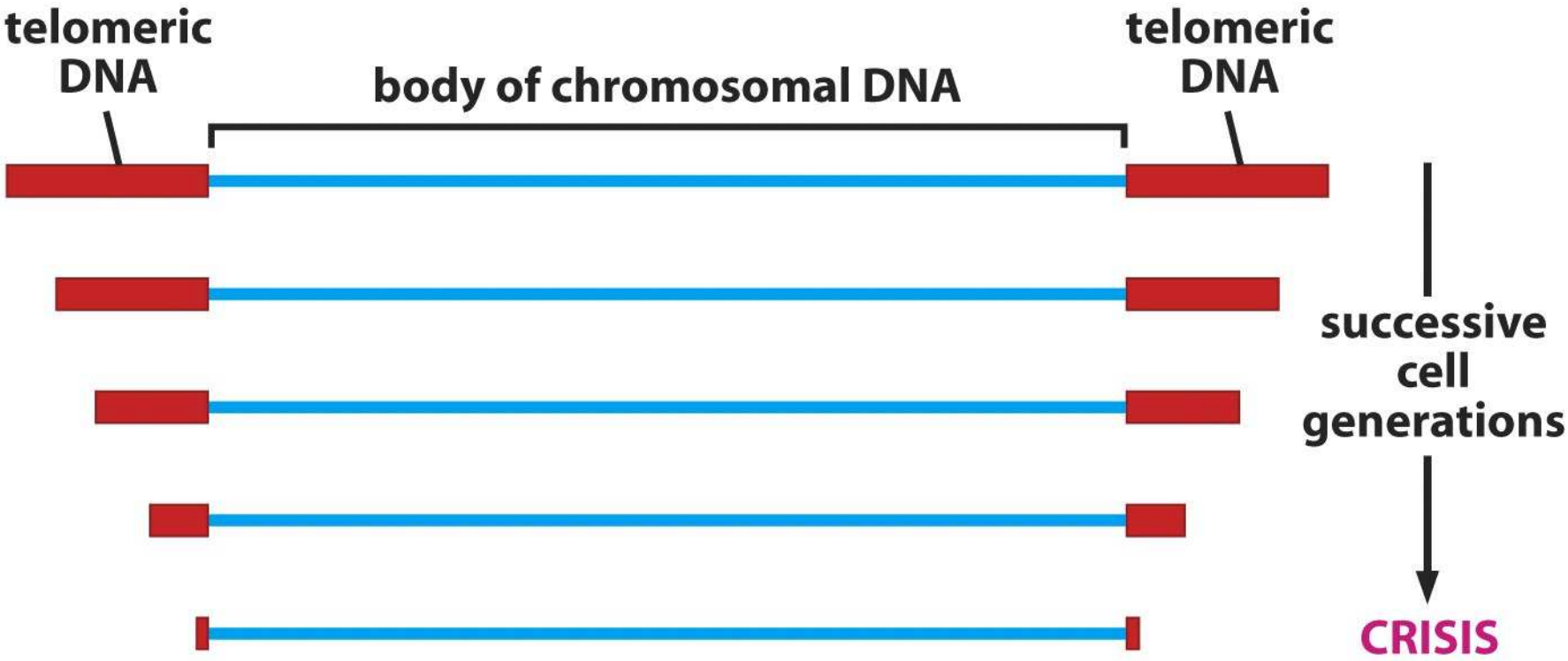

lack of nucleotides
UV radiation
ionizing radiation
oncogene signaling
hypoxia
blockage of transcription
p53
cell cycle arrest
DNA repair
block of angiogenesis
apoptosis
OR
senescence
return to proliferation

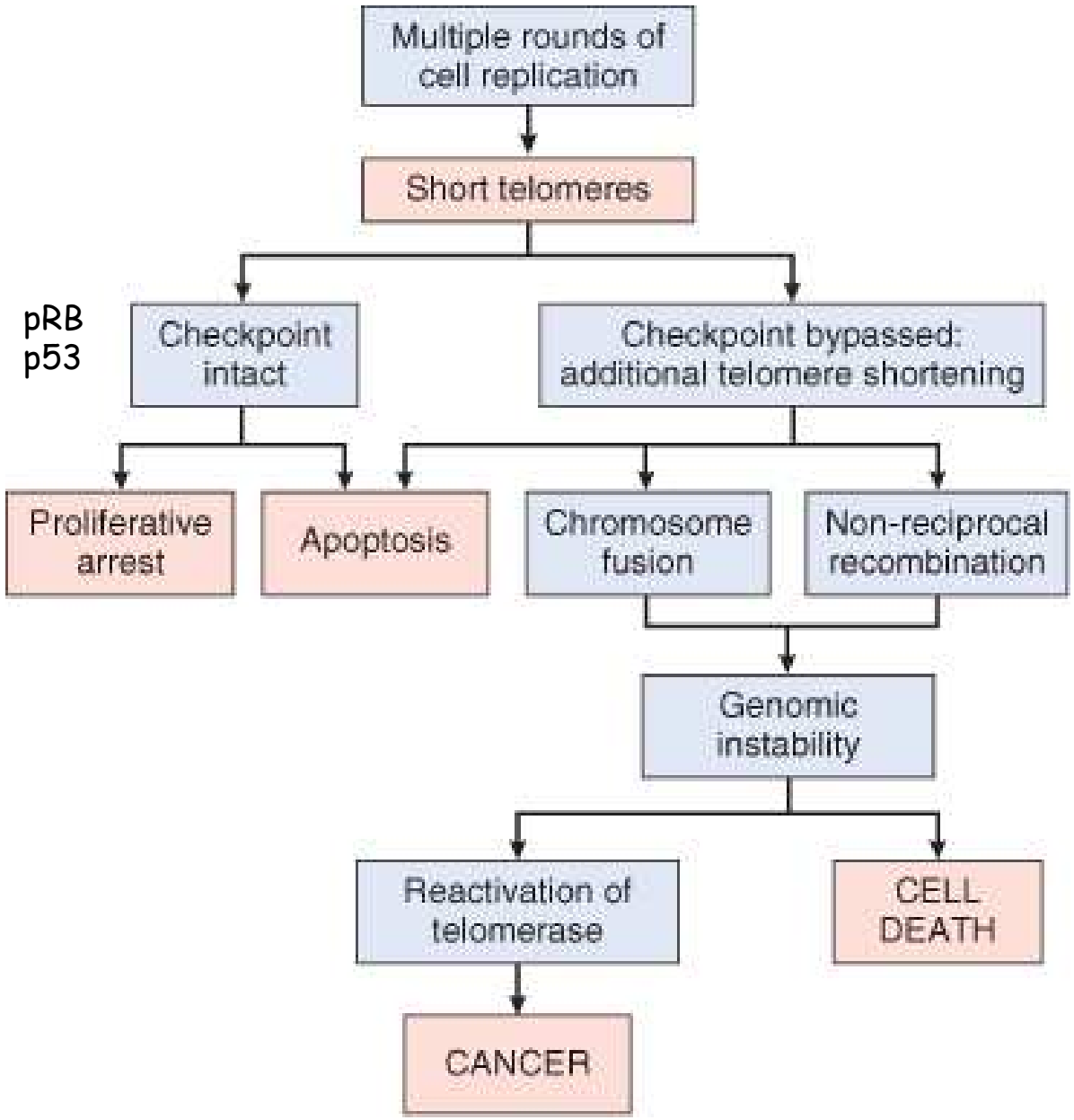

TP53 mutation prevalence (as recorded in the IARC Database, R7)
ovary
esophagus
colorectum
head & neck
pancreas
lung
skin
bladder
stomach
brain
liver
breast
uterus
soft tissue sarcomas
osteosarcomas
hematologic cancers
prostate
kidney
testis
thyroid
uterine cervix
0
5
10
15
20
25
30
35
40
45
50
% mutated

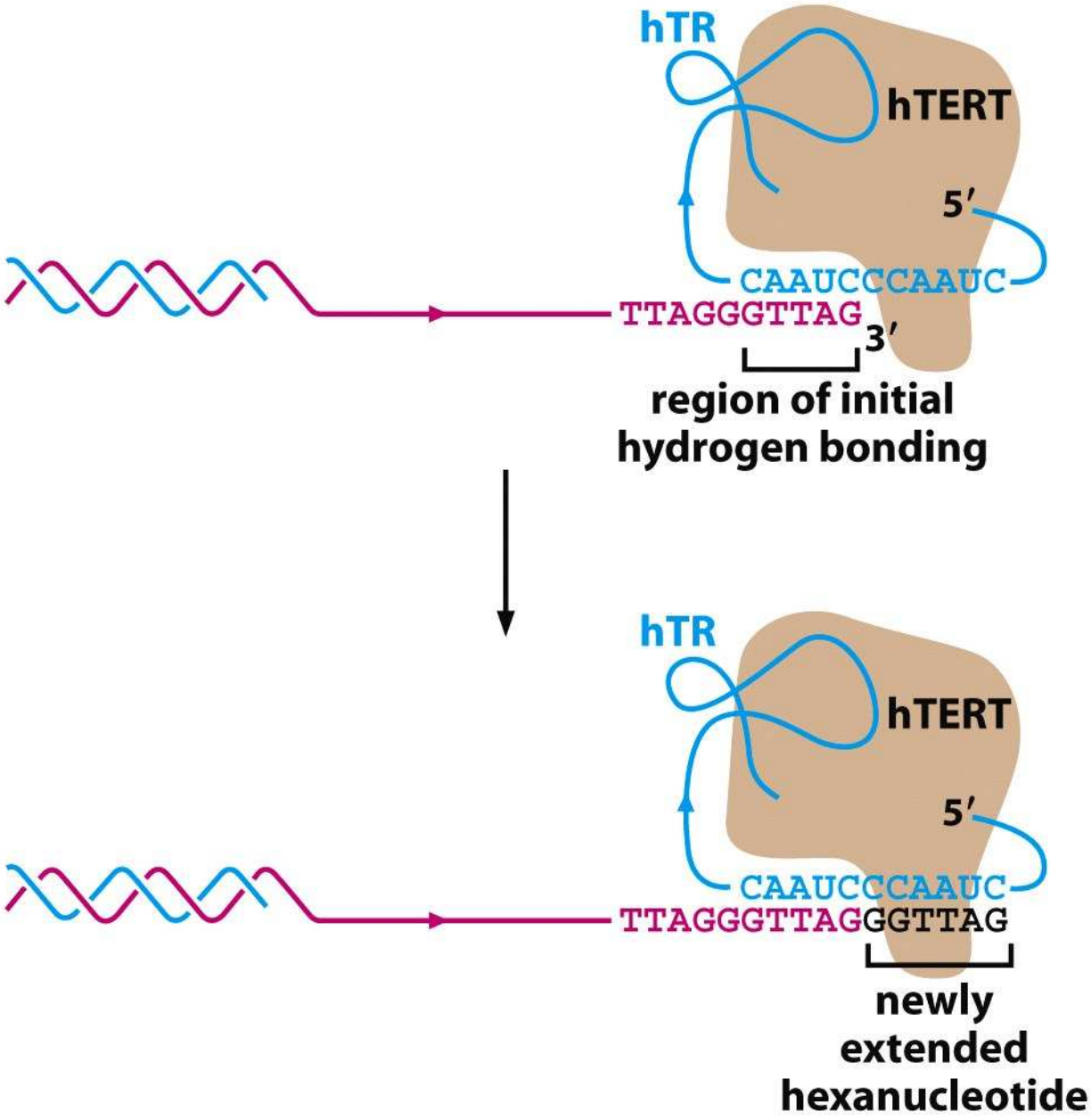
-<u>perdita di p53:</u> sul cromosoma 17p è stata riscontrata nel 70-80% dei casi di cancro CR (non paragonabile con la frequenza riscontrata negli adenomi, suggerendo che tale perdita avvenga tardivamente nella carcinogenesi)



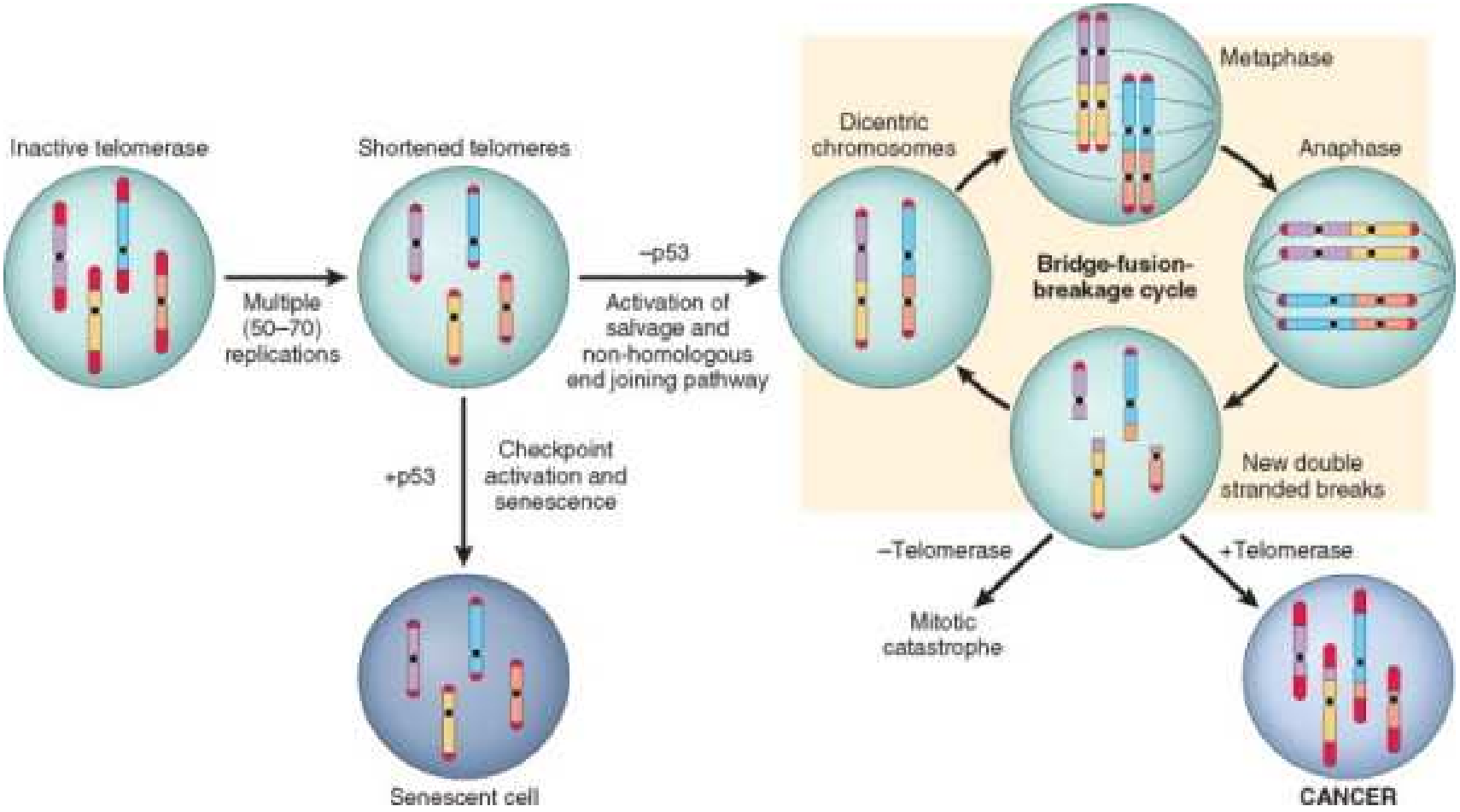

NORMAL COLON
MUCOSA AT RISK
ADENOMAS
CARCINOMA
Mucosa
Submucosa
Muscularis propria
Germline (inherited) or somatic (acquired) mutations of cancer suppressor genes ("first hit")
Methylation abnormalities
Inactivation of normal alleles ("second hit")
Protooncogene mutations
Homozygous loss of additional cancer suppressor genes
Overexpression of COX-2
Additional mutations
Gross chromosomal alterations
APC at 5q21
APC
β-catenin
K-RAS at 12p12
p53 at 17p13
LOH at 18q21
(SMAD 2 and 4)
Telomerase,
Many genes

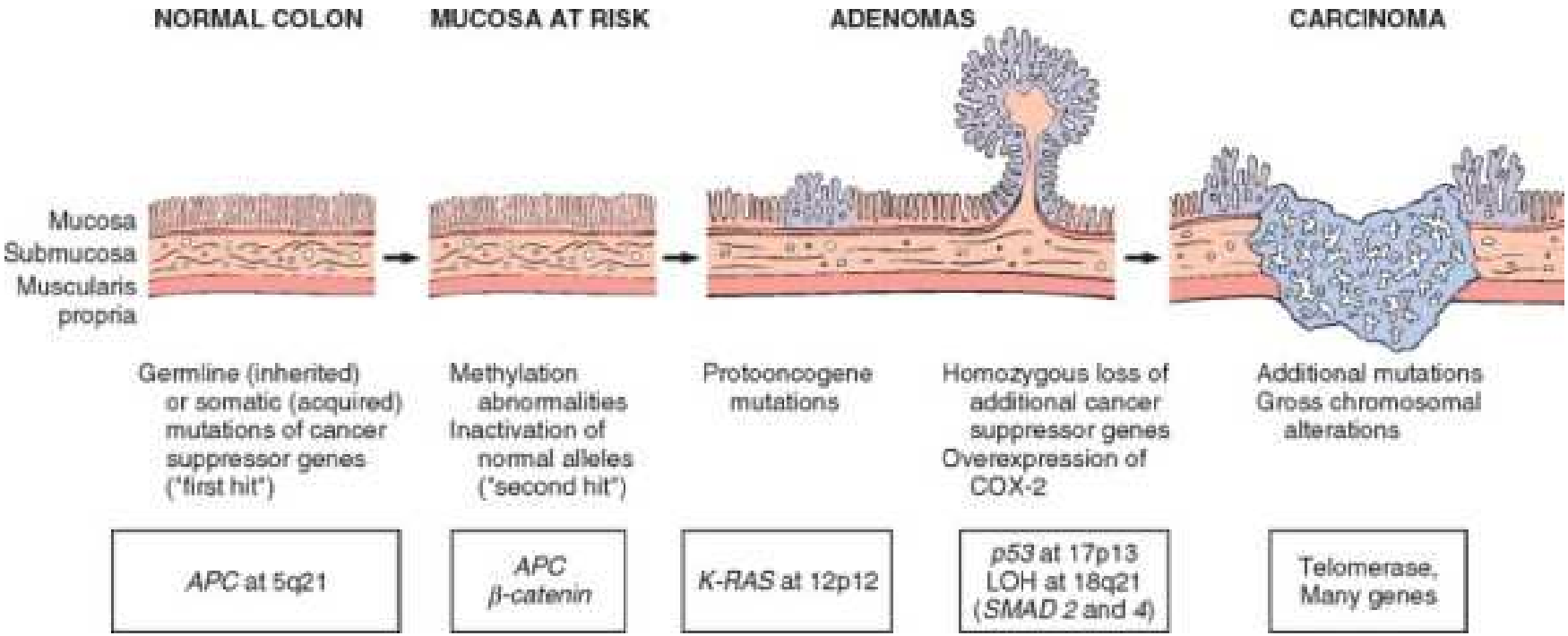

telomeric DNA
body of chromosomal DNA
telomeric DNA
successive cell generations
CRISIS




hTR
hTERT
5′
CAAUCCCAAUC
TTAGGGTTAG
3′
region of initial
hydrogen bonding
hTR
hTERT
5′
CAAUCCCAAUC
TTAGGGTTAGGGTTAG
newly
extended
hexanucleotide

-<u>attivazione della telomerasi:</u> richiesta per mantenere la stabilità dei telomeri e quindi l'immortalità cellulare, prerequisito per le cellule tumorali



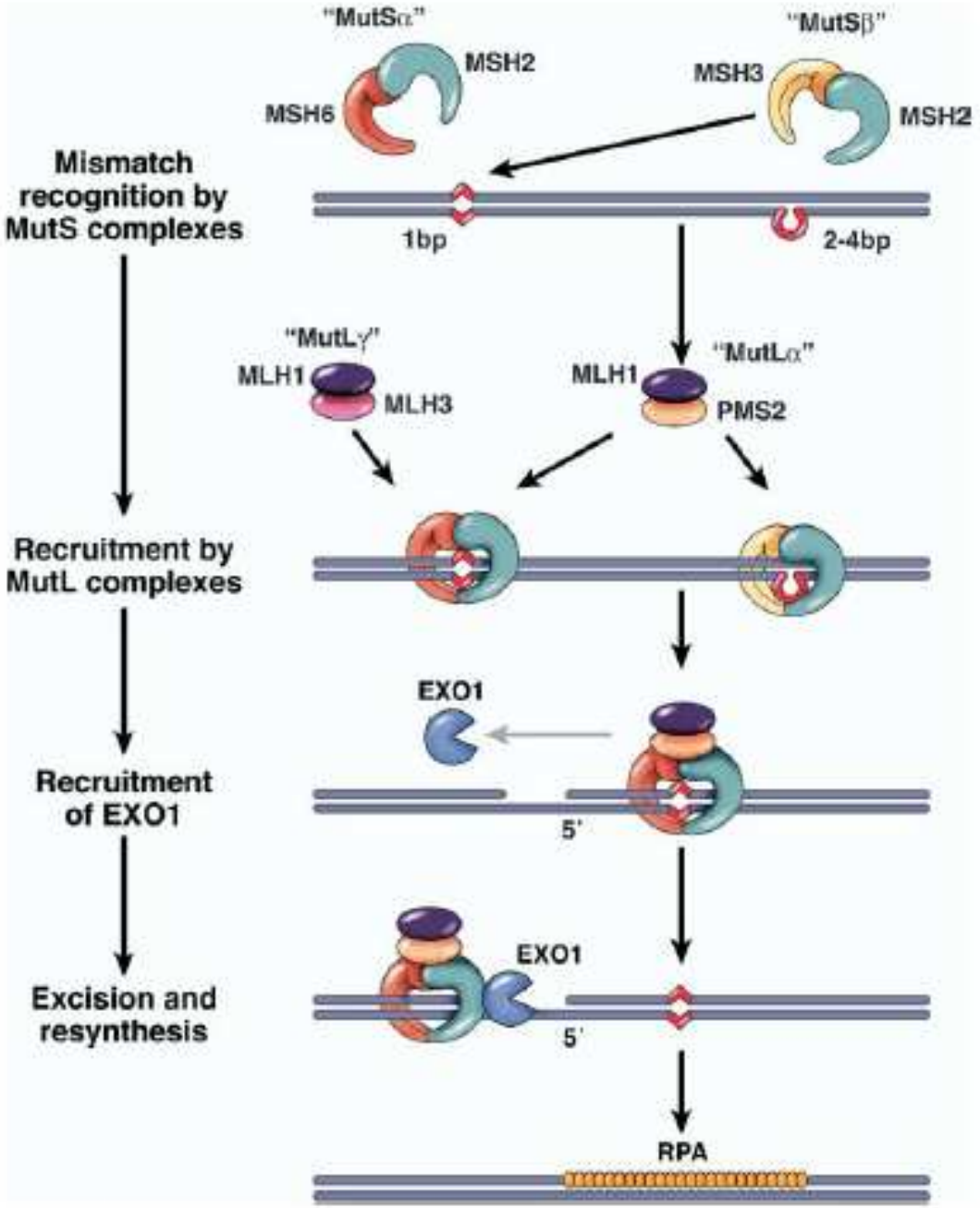

NORMAL COLON
MUCOSA AT RISK
ADENOMAS
CARCINOMA
Mucosa
Submucosa
Muscularis propria
Germline (inherited) or somatic (acquired) mutations of cancer suppressor genes ("first hit")
Methylation abnormalities
Inactivation of normal alleles ("second hit")
Protooncogene mutations
Homozygous loss of additional cancer suppressor genes
Overexpression of COX-2
Additional mutations
Gross chromosomal alterations
APC at 5q21
APC
β-catenin
K-RAS at 12p12
p53 at 17p13
LOH at 18q21
(SMAD 2 and 4)
Telomerase,
Many genes

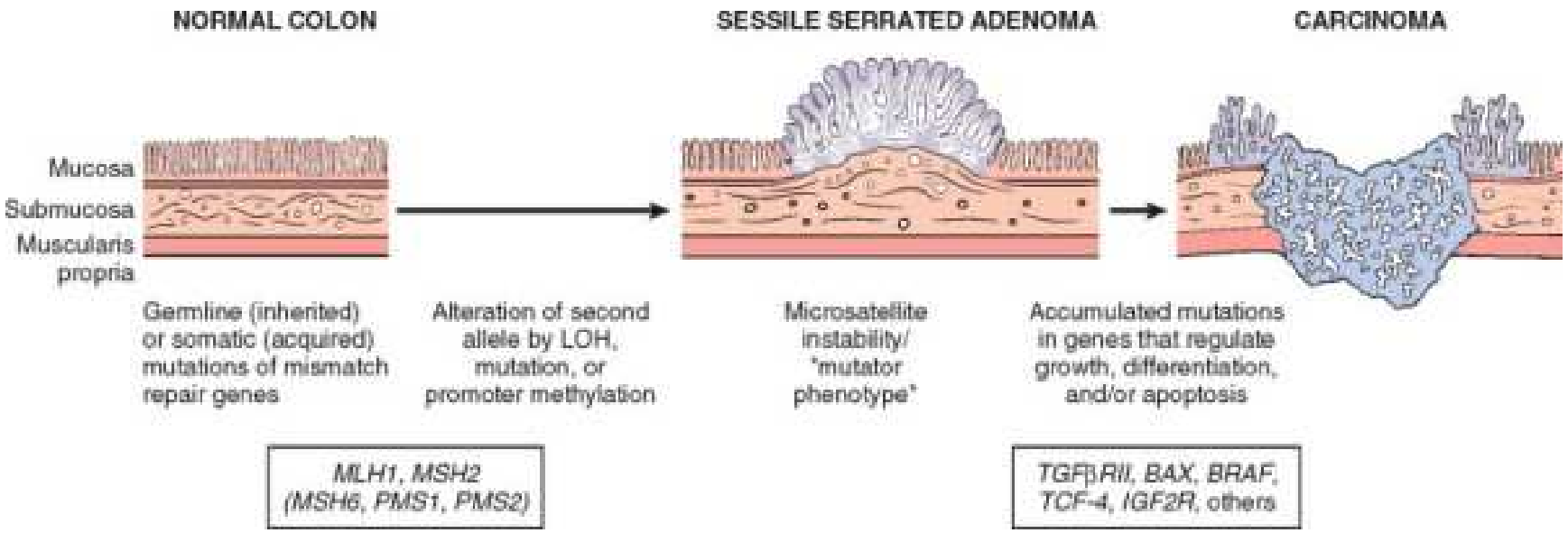
2)

Instabilità dei microsatelliti: frammenti di sequenze ripetute del genoma umano di 50.000-100000 microsatelliti. Queste sequenze possono non allinearsi correttamente durante la replicazione del DNA. Nelle cellule normali l'allineamento errato viene corretto dai geni *caretaker*. La mutazione ereditaria (*germ-line mutation*) di uno dei geni che sono coinvolti nella riparazione del DNA determina la sindrome familiare HNPCC (hMSH2 sul cromosoma 2p22, hMLH1 su 3p21, MSH6 su 2p21, hPMS1 su 2q31-33 e hPMS2 su 7p22. Il 90% delle mutazioni coinvolgono MSH2 e MLH1). I pazienti con HNPCC ereditano un allele di riparazione del DNA mutante e un allele normale. Le cellule di alcuni organi (colon, stomaco, endometrio)
sono suscettibili ad una seconda mutazione che inattiva anche l'allele normale.
Sebbene non ci sia una chiara correlazione morfologica come nella sequenza adenoma-carcinoma si è notato che, alcuni dei cosiddetti polipi iperplastici, che si localizzano nel colon ascendente, presentano un'instabilità dei microsatelliti e possono essere considerati precancerosi.
Inoltre i tumori derivanti dalla via del mismatch repair, spesso localizzati nel colon ascendente, sono carcinomi mucinosi con infiltrazione linfocitaria e in genere per questi tumori la prognosi è più favorevole.

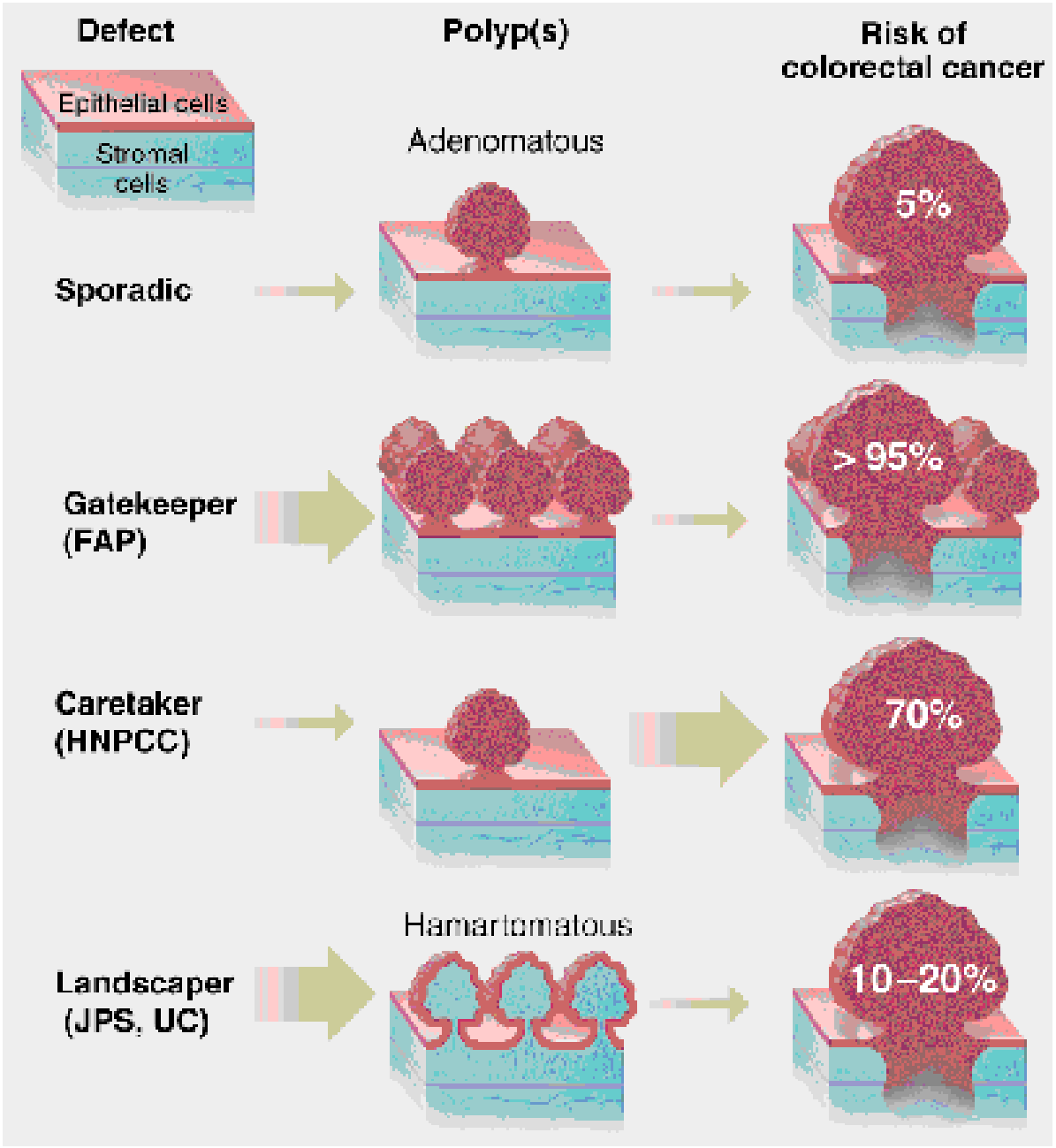
Hereditary Nonpolyposis Colorectal Cancer (HNPCC) Syndrome: sindrome autosomica dominante familiare (descritta da Henry Lynch e per questo chiamata sindrome di Lynch), caratterizzata da un aumento del rischio di cancro CR ed endometriale. Il numero di neoplasie maligne per individuo è elevato e spesso non sono associate a pre-esistenti adenomi, la maggior parte dei carcinomi sono nel cieco e nel colon prossimale

HNPCC determina 2-4% di tutti i carcinomi CR, ma l'instabilità dei microsatelliti si ritrova nel 15% dei CRC sporadici. I geni regolatori della crescita, mutati nei pazienti con HNPCC non sono stati ancora completamente caratterizzati, ma includono il gene che codifica per il recettore II TGF-β, il componente TCF della via della β-catenin pathway, *BAX,* e altri oncogeni e oncosoppressori.


"MutSα"
MSH2
MSH6
"MutSβ"
MSH3
MSH2
Mismatch recognition by MutS complexes
1bp
2-4bp
"MutLγ"
MLH1
MLH3
"MutLα"
MLH1
PMS2
Recruitment by MutL complexes
EXO1
Recruitment of EXO1
5'
EXO1
Excision and resynthesis
5'
RPA


NORMAL COLON
SESSILE SERRATED ADENOMA
CARCINOMA
Mucosa
Submucosa
Muscularis propria
Germline (inherited) or somatic (acquired) mutations of mismatch repair genes
Alteration of second allele by LOH, mutation, or promoter methylation
Microsatellite instability/ "mutator phenotype"
Accumulated mutations in genes that regulate growth, differentiation, and/or apoptosis
MLH1, MSH2 (MSH6, PMS1, PMS2)
TGFβRII, BAX, BRAF, TCF-4, IGF2R, others

Ascending colon		Descending colon/rectum
Microsatellite instability	**Predominant initiation mechanism**	Chromosomal instability
Often arise *de novo* or out of hyperplastic serrated adenomas	**Precursor lesion**	Predominantly arise out of benign adenomatous polyps
Exophytic or sessile growths with poor differentiation and excess mucin production	**Typical morphology**	Well differentiated, pedunculated tubular adenomas
Lynch syndrome	**Classic human familial disease**	Familial adenomatous polyposis
β-Catenin stabilizing mutations	**WNT signaling**	*APC* inactivating mutations
Inactivating mutations common	**DNA mismatch repair genes**	Mutations uncommon
BRAF mutations	**Growth factor receptor pathways**	*KRAS* mutations
TGFβIIR mutations common	**TGFβ signaling**	*SMAD* mutation or deletion
Activating mutations of *BAX*	**Apoptosis/cell survival**	*TP53* inactivating mutations


Defect
Polyp(s)
Risk of colorectal cancer
Epithelial cells
Stromal cells
Adenomatous
Sporadic
5%
Gatekeeper (FAP)
> 95%
Caretaker (HNPCC)
70%
Hamartomatous
Landscaper (JPS, UC)
10–20%

Animal models of human colorectal cancer

While it seems obvious, the goal of modeling human colorectal cancer in animals is to recapitulate the molecular etiology, pathology, and clinical progression of the disease.

As a result, the known diversity of human colorectal cancer makes it impossible for a single animal model to adequately represent all forms of the disease. Nonetheless, we believe that **three characteristics** are important to maintain the translational potential of the studies conducted in the animal models. **First**, the cancer that develops in the animal model should be **limited to the large intestine** so that researchers can study the development of the disease without the confounding effects of disease in other tissues. **Second**, the **histologic** and **molecular features** of colorectal lesions should be **similar** to those observed in human tissue. **Third**, the models should **capture the complex cellular interactions** that are relevant to human colon cancer. For example, while xenografts of human tumor into nude mice are often cited as highly relevant to the study of human cancer, these mice are immune-compromised, and this eliminates the impact of this critical system on the tumors.

Johnson e Fleet Cancer Metastasis Rev (2013) 32:39–61

Animal models of human colorectal cancer

Potential animal models for colorectal cancer fit into three broad categories:

1) spontaneous intestinal cancers in various animal species,
2) chemically or environmentally induced cancers in rodents,
3) cancers induced by genetic manipulation of mice.

Johnson e Fleet Cancer Metastasis Rev (2013) 32:39–61

Animal models of human colorectal cancer

Canine intestinal cancer occurs more commonly in the large intestine than in the small intestine [46]. Like humans, pedunculated adenomas are more prevalent in the distal colon/rectum, whereas tumors in the middle or proximal colon are more likely to exhibit a sessile, annular phenotype that cause luminal stricture. Immunohistochemical evaluation of canine colorectal adenomas revealed cytoplasmic and nuclear accumulation of β-catenin, suggesting that dysregulation of the WNT signaling pathway is also an important driver of colorectal carcinogenesis in the dog. Canine colorectal adenomas demonstrate a tendency to progress to malignancy, but unlike human colorectal tumors, this malignant behavior is **not accompanied by acquisition of Tp53 mutations**. Despite all of the similarities between colon tumors in dogs and humans, the utility of dogs for colorectal cancer research is severely limited by the low prevalence of the disease in the pet dog population (**less than 1 %**).

The incidence of feline gastrointestinal adenocarcinoma is **also less than 1 %,** and over 70 % of these tumors occur in the **small intestine**. The low incidence and disparity of tumor location from the human condition make feline gastrointestinal adenocarcinoma a poor model for colorectal cancer research.

Vet Pathol 36:228–236 (1999)

Dysregulation of β-Catenin is Common in Canine Sporadic Colorectal Tumors

M. F. McEntee and K. A. Brenneman

Department of Pathology, College of Veterinary Medicine, University of Tennessee, Knoxville, TN

Abstract. Human colorectal tumorigenesis is often initiated by *APC* (adenomatous polyposis coli) or β-catenin (*CTNNB1*) mutations, which result in dysregulation of β-catenin expression, followed by alterations in E-cadherin and/or p53. We examined 32 canine intestinal tumors for expression and intracellular distribution of β-catenin, E-cadherin, and p53 using immunohistochemistry. β-Catenin in normal mucosal epithelial cells was restricted to lateral cell membranes, but 13/13 (100%) colorectal adenomas had intense cytoplasmic and/or nuclear reactivity. Three of six (50%) colorectal carcinomas, 2/13 (15%) small intestinal carcinomas, and dysplastic cells in 1/2 focal hyperplastic lesions in the small intestine had a similar pattern of staining; remaining tumors had normal membranous β-catenin reactivity. There was a correlation ($P = 0.007$) between abnormal β-catenin and E-cadherin staining with 11/13 (85%) colorectal adenomas, 3/6 (50%) colorectal carcinomas, and 3/13 (23%) small intestinal carcinomas showing decreased membranous reactivity compared with normal mucosal epithelium. E-cadherin staining was reduced more often in adenomas than in carcinomas ($P = 0.04$). There were two patterns of nuclear p53 staining: >60% of nuclei in 2/26 (8%) carcinomas (one colorectal, one small intestinal) were strongly labeled, whereas three colorectal adenomas and one small intestinal carcinoma had fainter staining in 10–20% of cells. Dysregulation of β-catenin appears to be as important in canine colorectal tumorigenesis as it is in the human disease and could be due to analogous mutations. Malignant progression in canine intestinal tumors does not appear to be dependent on loss of E-cadherin or β-catenin expression or strongly associated with overexpression of nuclear CM1 antibody-reactive p53.

Table 1. Summary of canine lesions.

Diagnosis	Small Intestine	Colon–Rectum	Total
Hyperplastic focus	2	0	2
Tubular adenoma	0	7	7
Tubulovillous adenoma	0	6	6
Acinar adenocarcinoma	8	4	12
Solid/signet ring carcinoma	4	2	6
Mucinous carcinoma	1	0	1
Total	15	19	34

Table 2. Summary of abnormal immunohistochemical staining (+) by location and diagnosis.

Location	Diagnosis	β-Catenin	E-Cadherin	p53	No. Tumors
Colon–rectum	Polyp	+	+	+*	2
	Polyp	+	−	+*	1
	Polyp	+	−	−	3
	Polyp	+	×	−	6
	Polyp, carcinoma	+†	+†	−	1
	Carcinoma	+	+	+‡	1
	Carcinoma	+	+	−	1
	Carcinoma	−	−	−	3
Total polyps		100% (13)	85% (11)	23% (3)	13
Total carcinomas		50% (3)	50% (3)	17% (1)	6
Small intestine	Polyp§, carcinoma	+‖	+‖	−	1
	Polyp§, carcinoma	−	−	−	1
	Carcinoma	−	−	+‡	1
	Carcinoma	+	−	+*	1
	Carcinoma	−	+	−	2
	Carcinoma	−	−	−	7
Total polyps		50% (1)	50% (1)	0% (0)	2
Total carcinomas		15% (2)	23% (3)	15% (2)	13

* Moderate, patchy nuclear staining.
† Abnormal for polyp and carcinoma.
‡ Strong, widespread nuclear staining.
§ Focal hyperplastic lesion interpreted as nonneoplastic because of lack of significant dysplasia.
‖ Abnormal only in small population of dysplastic cells of hyperplastic lesion; the carcinoma had normal membranous staining.

Vet Pathol 34:394–404 (1997)

Immunohistochemical Detection of p53 Tumor Suppressor Gene Protein in Canine Epithelial Colorectal Tumors

J. C. Wolf, P. E. Ginn, B. Homer, L. E. Fox, and I. D. Kurzman

Departments of Pathobiology (JCW, PEG, BH) and Small Animal Clinical Sciences (LEF), College of Veterinary Medicine, University of Florida, Gainesville, FL; and Department of Medical Sciences, School of Veterinary Medicine, University of Wisconsin, Madision, WI (IDK)

Abstract. Eighty canine epithelial colorectal tumors obtained by excisional biopsy were evaluated immunohistochemically for p53 tumor suppressor gene protein. Dogs in the study averaged 6.9 years of age (range, 1–12.5 years). A standard avidin–biotin immunohistochemical protocol incorporated a polyclonal antibody of rabbit origin (CM-1) as the primary antibody. Positive staining was observed within all subcategories of lesions, including hyperplastic polyps 1/2 (50%), adenomas 14/29 (48%), carcinomas in situ 9/22 (41%), adenocarcinomas 3/4 (75%), and invasive carcinomas 8/23 (35%). A total of 35/80 (44%) positive tumors were identified. Fifteen of 31 (48%) benign tumors labeled for p53 protein compared to 20/49 (41%) malignant tumors. Survival data was available for 57/80 (71%) dogs. The average age of dogs within the group with survival data was 4.4 years. Males predominated 34/57 (60%). Mean survival time was 20.6 months. There was no significant difference in survival time between dogs grouped according to p53 immunoreactivity, cellular stain location, or tumor site. A statistically significant increase in survival time was observed between dogs with clean surgical margins and those without ($P < 0.018$) and for dogs with adenomas or carcinomas in situ over dogs with invasive carcinomas ($P < 0.02$). In this study, the overall greater positive staining frequency of benign lesions compared to malignant lesions is contrary to data derived from similar immunohistochemical analyses of human tumors and is incongruous with the theorized late-stage participation of the p53 protein in the development of human colorectal cancers. The results of this study suggest that if the p53 tumor suppressor gene protein is involved in the progression of canine colorectal tumors, it may play a relatively early role, possibly analogous to the early appearance of p53 overexpression in precancerous lesions of human ulcerative colitis. Immunohistochemical detection of p53 was not useful prognostically.

Table 2. Distribution of positive immunohistochemical staining among canine colorectal tumors.

Total positive	35/80 (44%)
Benign	15/31 (48%)
Malignant	20/49 (41%)
Hyperplastic polyp	1/2 (50%)
Adenoma	14/29 (48%)
Adenoma with carcinoma in situ	9/22 (41%)
Adenocarcinoma	3/4 (75%)
Invasive carcinoma	8/23 (35%)

The only other domestic veterinary species with a significant incidence of intestinal cancer is sheep. In New Zealand, intestinal adenocarcinomas were found in **1.6 % of normal adult sheep**. Sheep are an attractive model for human colorectal cancer because their adenocarcinomas share **many histologic features** with the human lesion and mimic many aspects of the metastatic behavior of the human disease. However, in contrast to the human disease, **100 % of the intestinal adenocarcinomas of sheep** are found in the **small intestine**. An obvious weakness of sheep as a model of human intestinal disease is the unique physiology of the ruminant forestomachs. The potential influence of this anatomic variant on intestinal carcinogenesis is unclear.

Comparative Medicine
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Pages 136-141

Ovine Intestinal Adenocarcinomas: Histologic and Phenotypic Comparison with Human Colon Cancer

John S Munday,[1,*] Moira M Brennan,[1] Azhar M Jaber,[2] Matti Kiupel[3]

Approximately 7% of old, unthrifty sheep (*Ovis aries*) in New Zealand have intestinal adenocarcinomas. To investigate whether these sheep might be used as a model of human colonic neoplasia, the biologic behavior and histologic appearance of ovine intestinal adenocarcinomas were compared with those reported for human colonic adenocarcinomas. We collected 50 intestinal tracts with grossly visible intestinal neoplasia from slaughtered sheep. Neoplasms were assessed using World Health Organization guidelines for assessment of human colonic adenocarcinomas. All ovine adenocarcinomas developed in the small intestine. In contrast, only 4% of human intestinal tumors develop at this location, whereas the majority develop in the colon. A visible polyp is present within 89% of human colonic adenocarcinomas, whereas polyps were present in only 46% of the ovine neoplasms. Intestinal wall infiltration by the neoplastic cells and rates of lymph node (84% in sheep; 61% in humans) and distant (52% in sheep; 17% in humans) metastases were comparable between ovine and human adenocarcinomas. However, ovine adenocarcinomas developed more peritoneal and fewer hepatic metastases than human adenocarcinomas. Histologic grading of ovine tumors revealed cell differentiation similar to that reported within human colonic adenocarcinomas. In conclusion, ovine intestinal adenocarcinomas, like human colonic adenocarcinomas, typically arise spontaneously and consistently develop widespread metastases. In addition, tumors appear histologically similar between these species. Therefore, sheep may provide a model of advanced human colonic cancer, possibly allowing evaluation of novel therapeutics and surgical procedures.

Abbreviations: TNM system, tumor–node–metastasis system of classifying neoplasms

Fattore prognostico staging della lesione al momento della diagnosi (TNM: tumor-nodes-metastasis)

TNM Classification of Carcinoma of the Colon and Rectum

Tumor Stage	Histologic Features of the Neoplasm
Tis	Carcinoma in situ (high-grade dysplasia) or intramucosal carcinoma (lamina propria invasion)
T1	Tumor invades submucosa
T2	Extending into the muscularis propria but not penetrating through it
T3	Penetrating through the muscularis propria into subserosa
T4	Tumor directly invades other organs or structures
Nx	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in 1 to 3 lymph nodes
N2	Metastasis in 4 or more lymph nodes
Mx	Distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis

Robbins et al 2005

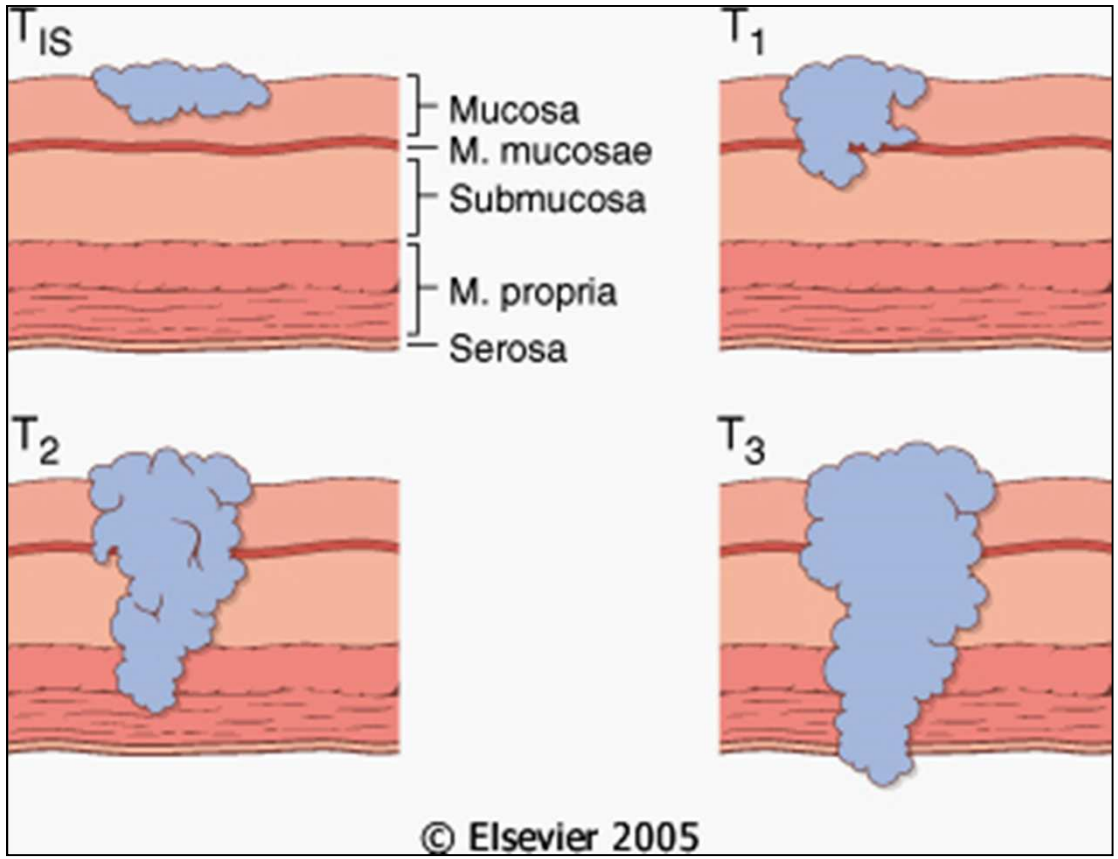

T_{IS}
Mucosa
M. mucosae
Submucosa
M. propria
Serosa
T_1
T_2
T_3
© Elsevier 2005

Table 1. Summary of gross and histologic evaluation of sheep intestinal adenocarcinomas

Feature of neoplasm	Ovine	Reported human[a] (reference no.)
Proportion of intestinal neoplasms in the small intestine (%)	100	4 (9)
Visible polyp present (%)	46	89 (17)
TNM classification (%)		
Primary tumor (T)		
T1	0	1 (12)–2 (24)
T2	0	4 (24)–7 (12)
T3	70	35 (24)–49 (12)
T4	30	43 (12)–59 (24)
Nodal status (N)		
N0	16	39 (11)
N1 and N2	84	61 (11)
Distant metastasis		
M0	48	83 (12)
M1	52	17 (12)
Hepatic metastases[b] (%)	9	10–25 (19)
Stage (%)		
I	0	7 (12)–20(4)
II	16	30 (4)–59(12)
III	32	16 (4)–26(12)
IV	52	18 (4)–23(12)
Histological grade (%)		
Well differentiated	10	4 (12)
Moderately differentiated	48	56 (12)
Poorly differentiated	42	40 (12)
Mucinous adenocarcinomas (%)	0	10 (12)

[a]At initial diagnosis
[b]Livers from only 22 affected sheep were examined.

Vet Pathol 43:613–621 (2006)

Altered Expression of β-catenin, E-cadherin, Cycloxygenase-2, and p53 Protein by Ovine Intestinal Adenocarcinoma Cells

J. S. Munday, M. M. Brennan, and M. Kiupel

Abstract. Around 1.6% of sheep in New Zealand develop small-intestinal adenocarcinomas. These neoplasms typically develop widespread metastases. The common development of these neoplasms and their subsequent behavior suggests that sheep could be a useful animal model of human colonic cancer. However, for an animal model of human disease to be relevant, similar genetic mutations should be present. Genetic mutations within human colonic cancers frequently result in expression of cycloxygenase-2 (COX-2), loss of membranous expression of β-catenin and E-cadherin, and accumulation of p53 protein within the neoplastic cells. Immunohistochemistry was used to investigate the presence of these 4 proteins within 26 ovine intestinal adenocarcinomas. Loss of membranous β-catenin reactivity was observed in 14 of 26 ovine intestinal adenocarcinomas (54%). The loss of membranous β-catenin reactivity was accompanied by cytoplasmic and nuclear reactivity in 2 neoplasms. Loss of E-cadherin was observed within 8 of 26 neoplasms (31%). Neoplastic cell expression of COX-2 was observed in 12 of 26 neoplasms (46%), whereas cells within 3 of 26 neoplasms (11%) contained visible p53 protein. In conclusion, all 4 proteins that commonly have altered expression in human colonic cancers were also altered in a proportion of the ovine intestinal adenocarcinomas. These results provide additional evidence that sheep could be useful for the study of human colonic cancer.

Intro: circa 1,6% delle pecore della Nuova Zelanda sviluppa adenocarcinoma intestinale (intestino tenue), invasivo con frequenti metastasi. Il comportamento biologico di tali neoplasie suggerisce che possano essere considerate un modello animale per CRC umano.
Le mutazioni genetiche in CRC umano risultano in espressione di ciclossigenasi-2 (COX-2), perdita dell'espressione della β-catenina e della E-caderina sulla membrana e accumulo della proteina p-53 nelle
cellule neoplastiche

Methods: IHC per investigare la presenza di queste 4 proteine in 26 adenocarcinomi intestinali di pecora

Results:
-perdita dell'attività della **β-catenina** nel 54% dei casi ovini (nell'uomo circa 84%, nel cane 26%).
La perdita della β-catenina è stata riscontrata tramite PCR in *APC-defective mice* e in circa 80% delle neoplasie indotte da azoxymetano nei roditori. Come E-caderina, β-catenina è rapidamente degradata dalla
proteina APC. Mutazioni nei geni APC e β-catenina inibiscono la sua degradazione e come risultato si ha un
accumulo nella cellula, l'ingresso nel nucleo e attivazione del *Wnt signaling pathway* i cui prodotti sono
c-myc (promotore di crescita), ciclina D1 (regolatore del ciclo cellulare), survivin (inibitore dell'apoptosi)
implicati nella carcinogenesi
In una cellula normale la β-catenina lega la proteina transmembrana E-caderina al citoscheletro e pertanto
rimane collegata alla membrana cellulare. Un accumulo cellulare di β-catenina interrompe il legame con E-caderina quindi si perde la connessione della β-catenina alla membrana cellulare. All'IHC un accumulo di β-catenina appare come perdita della positività membranaria e aumento della positività citoplasmatica e nucleare (uomo, cane, roditore, nella pecora solo 2 casi)

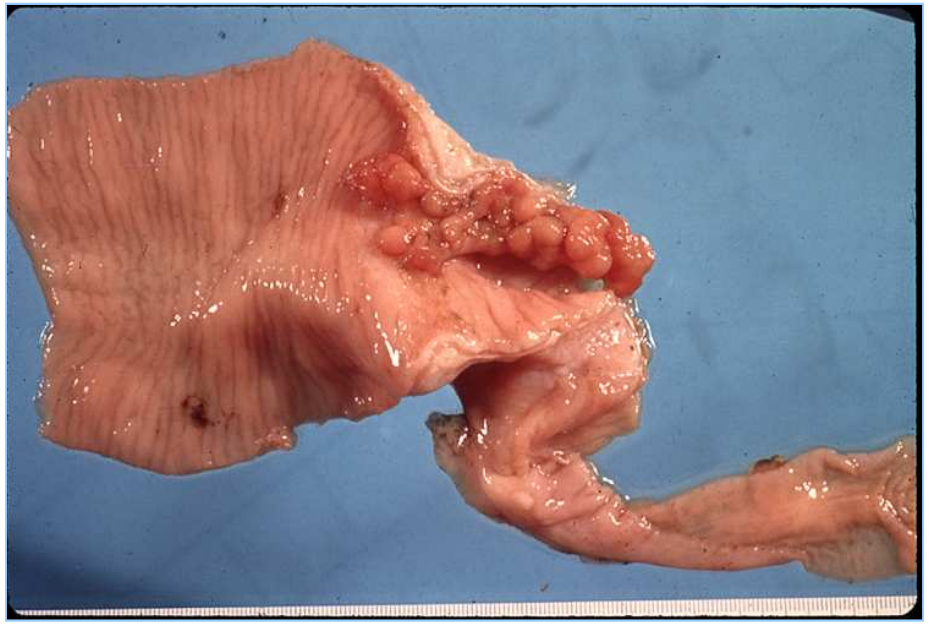
-perdita dell'attività di **E-caderina** nel 31% dei casi ovini (nell'uomo circa 44%, nel cane 31%).
La perdita di positività IHC di E-caderina è stata riportata in *APC-mutant mice*. E-caderina mantiene l'adesione tra le cellule epiteliali: la sua perdita riduce l'adesione cellulare, permette l'invasione e le metastasi da parte delle cellule neoplastiche. Inoltre inibisce il ciclo cellulare, quindi una sua riduzione determina proliferazione cellulare
Nel CRC umano la perdita di E-caderina si determina per inibizione del gene di trascrizione in seguito ad aumento della concentrazione intracellulare di β-catenina. Nella pecora la perdita dell'espressione di E-caderina nelle cellule tumorali è stata correlata con la perdita dell'espressione della β-catenina membranaria, tuttavia poiché l'aumento dell'espressione di β-catenina cellulare è raro (2 casi), evidentemente un'espressione alterata di β-catenina riduce quella di E-caderina con un meccanismo diverso

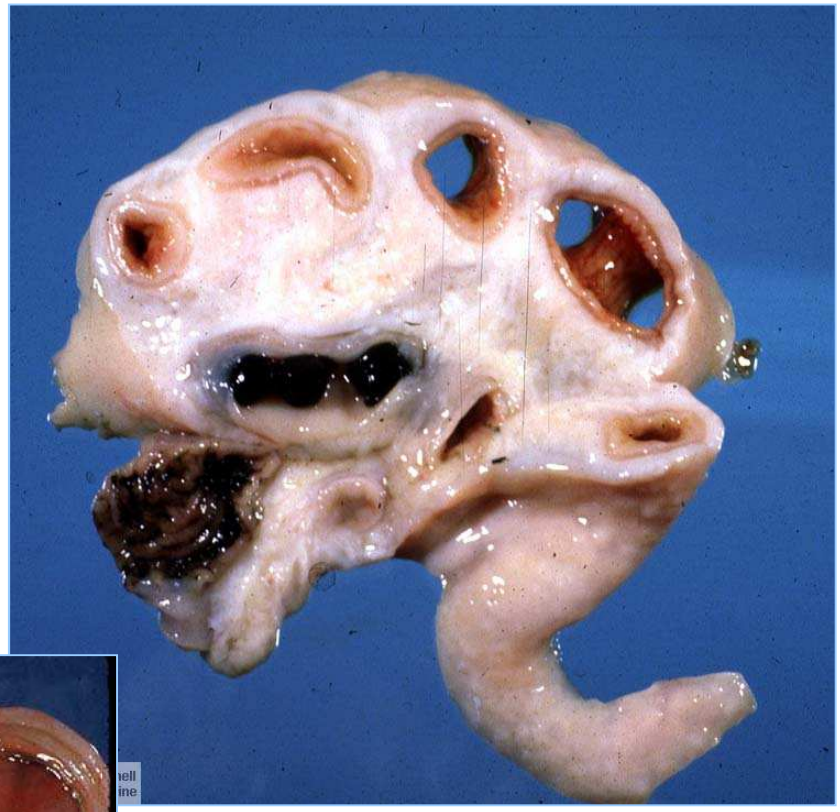
-**COX-2** era presente nel 46% dei casi ovini, inoltre molte sezioni contenevano cellule stromali che esprimevano COX-2 (correlata all'infiammazione per l'ulcerazione e la necrosi) (nell'uomo circa 83%, nel cane 47%).
L'espressione di COX-2 è stata frequentemente riportata nei modelli di roditori *APC-mutant* e *carcinogen-induced* (dubbi su quale popolazione realmente esprima COX-2).
Studi epidemiologici hanno evidenziato come un'inibizione della COX-2 determini una riduzione del 30-50% del rischio di CRC, inoltre determina regressione degli adenomi CR nelle persone con poliposi familiare.
Il meccanismo dell'induzione di COX-2 non è del tutto chiarito. A differenza di COX-1 che è un costituente tissutale, l'espressione di COX-2 è indotta da numerosi mediatori tra cui quelli dell'infiammazione, IGF, *ras*, β-catenina. Una volta espressa, COX-2 promuove la proliferazione cellulare, inibisce l'apoptosi, promuove l'angiogenesi e inibisce la risposta antitumorale dell'ospite. Tutti questi effetti forse sono determinati da un'aumentata produzione di prostaglandina E2.

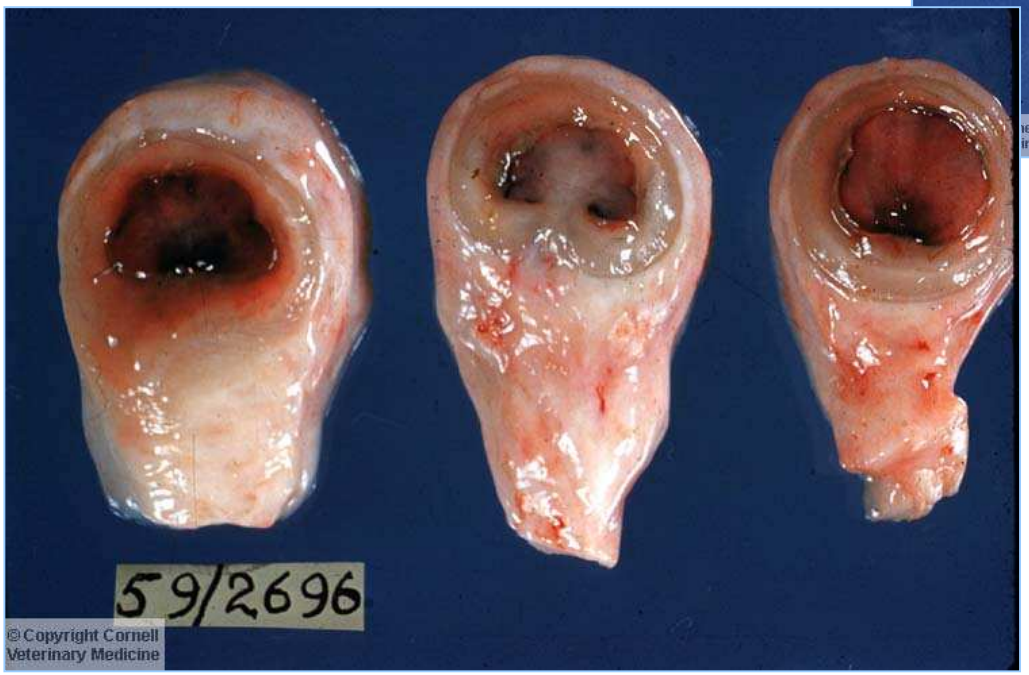
-**p53** era visibile nell'11% dei casi ovini. A differenza della proteina p-53 wild-type, la proteina mutata ha una lunga emivita nella cellula, quindi la positività IHC per p53 è suggestiva nella mutazione del gene di p53 (nell'uomo circa 50%, nel cane 41%). Mutazioni del gene p53 sono rare nei modelli murini CRC *APC-mutant* o nei modelli di CRC di ratto indotti chimicamente.
La proteina p53 mantiene la stabilità genetica nella cellula regolando l'arresto del ciclo cellulare, l'apoptosi, l'integrità e la riparazione del genoma. Nelle pecore la presenza di p53 non è correlata con l'espressione di COX-2 o con la perdita di β-catenina e di E-caderina

Table 3. Comparison of immunohistochemically-detectable alterations in cell protein expression within ovine intestinal and human colonic neoplasia.

	Ovine Small-intestinal Adenocarcinomas	Human Colonic Adenocarcinomas (from published reports)
Percent tumors with loss of membranous β-catenin expression	54	84[15]
Percent tumors with loss of E-cadherin expression	31	44[10]
Percent tumors with neoplastic cells expressing COX-2	46	83[14]
Percent tumors with neoplastic cells expressing p53	11	50[25]







w3.vet.cornell.edu/nst/

Conclusions: nonostante che la percentuale di espressione di β-catenina, E-caderina, COX-2 e p53 sia inferiore nell'adenocarcinoma intestinale ovino rispetto al CRC umano, viene ipotizzato che i meccanismi alla base delle alterazioni della loro espressione siano simili sia nell'uomo che nella pecora.
Pertanto la pecora potrebbe essere utilizzata come modello animale del CRC umano (large animal)

Vet Pathol 45:3–6 (2008)

BRIEF COMMUNICATIONS and CASE REPORTS

Mismatch Repair Protein Expression in Ovine Intestinal Adenocarcinomas

J. S. MUNDAY, F. A. FRIZELLE, AND M. R. WHITEHEAD

Abstract. Sheep in New Zealand develop small intestinal adenocarcinomas more frequently than sheep elsewhere in the world. This high rate of neoplasm development could be due to a genetic predisposition or due to an environmental carcinogen. Differentiation between a genetic and an environmental factor is important as, if an environmental carcinogen is present, people could be exposed directly or by consuming sheep meat. In humans, germline defects in the mismatch repair (MMR) genes cause hereditary nonpolyposis colorectal cancer (HNPCC). Affected people are predisposed to neoplasm development, most commonly colonic adenocarcinomas. It was hypothesized that MMR defects are common within the New Zealand sheep flock, and these defects predispose New Zealand sheep to intestinal neoplasia. To investigate this, immunohistochemistry was used to evaluate the expression of the MMR proteins MSH2, MSH6, MLH1, and PMS2 within 49 ovine intestinal adenocarcinomas. Neoplastic cells within all sheep tumors expressed MSH2, MSH6, and MLH1. Expression of PMS2 could not be assessed, most likely because of insufficient affinity of the anti-human PMS2 antibody to ovine PMS2. The consistent expression of MSH2, MSH6, and MLH1 within the ovine intestinal adenocarcinomas does not support the hypothesis that defects in the MMR genes are common in New Zealand sheep.

Among non-human primates, the cotton-top tamarin (*Saguinus oedipus*) is predisposed to idiopathic ulcerative colitis, and a high percentage of animals with colitis develop colorectal adenocarcinomas. **In some colonies, the incidence of colorectal adenocarcinomas at death can be as high as 39 %.** The adenocarcinomas arose within the cotton-top tamarin, exhibit **mucinous or signet-ring morphology, and rarely originate from adenomatous polyps**. In addition, they metastasize early and aggressively to regional lymph nodes. The average age of death due to colonic adenocarcinoma ranges from 5 to 7 years. Efforts to identify a heritable or familial cause for this syndrome have failed, and there are no reports detailing the molecular nature of these adenocarcinomas. The cause of the syndrome is unknown, but there is evidence that **environmental stress and luminal bacteria** may play a role in its pathogenesis. The similarities to the human disease and spontaneous nature of these adenocarcinomas are features beneficial in modeling therapeutic and preventative interventions. However, the long latency of carcinogenesis, costs, and ethical concerns of using non-human primates for biomedical research are barriers to widespread use of this model.

Spontaneous rodent models

Spontaneous gastrointestinal neoplasia is rare in rodents. In a 1969 survey of three early sublines of C57BL mice, 9.5 % of aged mice had neoplastic or hyperplastic lesions in the gastrointestinal tract. However, the majority of epithelial glandular adenomas or adenocarcinomas were found in the small intestine, and only two epithelial glandular adenomas were found in the colon.

A more contemporary study reported that the incidence of intestinal tumors in C57Bl/6J mice fed a common diet was 1 % in the large intestine and 4 % in the small intestine

Western-diet induced rodent models

Dietary patterns and colorectal cancer: systematic review and meta-analysis

Bruno Magalhães[a,b], Bárbara Peleteiro[b,c] and Nuno Lunet[b,c]

Studies on the association between single foods or nutrients and colorectal cancer have provided inconsistent results. Previous reviews did not conduct a quantitative synthesis of the relation with dietary patterns. We conducted a systematic review and meta-analysis of studies addressing the association between dietary patterns and colorectal cancer. Studies quantifying the association between dietary patterns (defined *a posteriori*) and colorectal cancer were identified in PubMed (until 01.08.2010) and through backward and forward citation tracking (ISI Web of Science and Scopus). Summary relative risk (RR) estimates and 95% confidence intervals (95% CI) were computed for highest versus lowest levels of exposure, for colon cancer (CC) and rectal cancer (RC), and for proximal and distal CC, by random effects meta-analysis. Heterogeneity was quantified using the I^2 statistic. Eight cohort and eight case–control studies defining patterns through principal components and factor analyses were included in the systematic review. Meta-analyses were conducted for three patterns: (i) 'drinker,' characterized by high alcohol consumption (CC: $RR_{combined}=0.96$, 95% CI: 0.82–1.12, $I^2=0.6\%$; RC: $RR_{combined}=0.83$, 95% CI: 0.47–1.45, $I^2=65.1\%$); (ii) 'healthy,' characterized by high fruit/vegetables consumption (CC: $RR_{combined}=0.80$, 95% CI: 0.70–0.90, $I^2=55.1\%$; RC: $RR_{combined}=1.02$, 95% CI: 0.89–1.17, $I^2=10.8\%$); (iii) 'western,' characterized by high red/processed meat consumption (CC: $RR_{combined}=1.29$, 95% CI: 1.13–1.48, $I^2=31.7\%$; RC: $RR_{combined}=1.13$, 95% CI: 0.92–1.39, $I^2=40.6\%$). Summary estimates for proximal and distal CC were similar. The risk of CC was increased with patterns characterized by high intake of red and processed meat and decreased with those labelled as 'healthy.' No significant associations were observed for RC. *European Journal of Cancer Prevention* 21:15–23

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Keywords: colon cancer, colorectal cancer, dietary patterns, eating patterns, foods, rectal cancer

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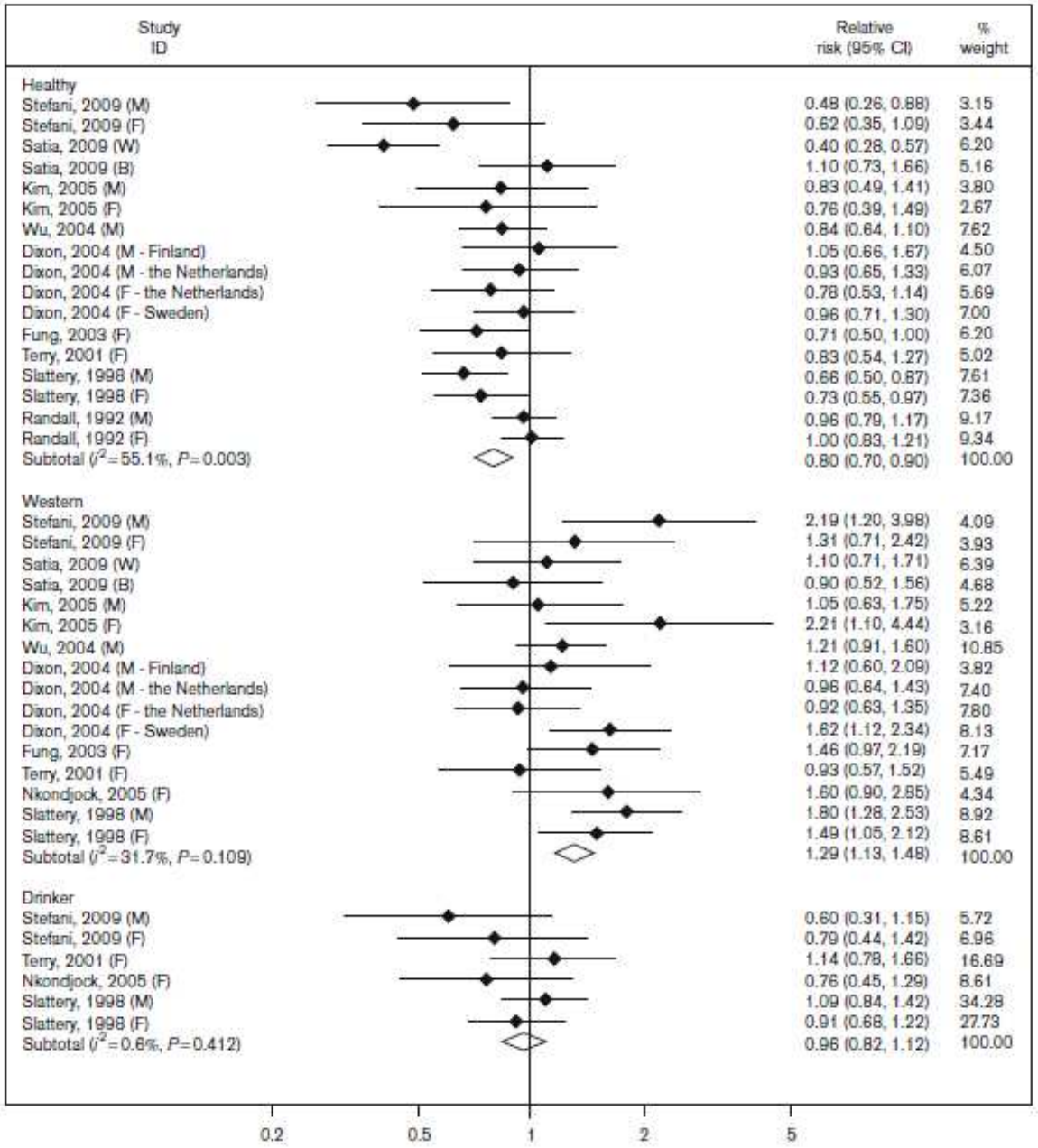

Meta-analyses of studies quantifying the association between dietary patterns and colon cancer. B, blacks; CI, confidence interval; F, females; M, males; W, whites.

Table 1 Dietary patterns identified in the original studies and considered for meta-analysis

Reference and country	Dietary patterns as labeled in the original articles	Dietary patterns considered for meta-analysis and respective labels
Kurotani *et al.*, 2010 Japan	'Prudent'	'Healthy'
	'High fat'	Not used in meta-analysis
	'Light-meal'	Not used in meta-analysis
Miller *et al.*, 2010b USA	'Fruits and vegetables' (M, F)	'Healthy'
	'Meat, potatoes, and refined grain' (M, F)	'Western'
	'Alcohol and sweetened beverages' (M)	'Drinker'
Williams *et al.*, 2009 USA	'High fat, meat, and potatoes' (B, W)	'Western'
	'Vegetable, fish, and poultry' (W)	'Healthy'
	'Fruit and vegetables' (B)	'Healthy'
	'Fruit, whole grain, and dairy' (W)	Not used in meta-analysis
	'Fruit and dairy' (B)	Not used in meta-analysis
De Stefani *et al.*, 2009 Uruguay	'Prudent'	'Healthy'
	'Traditional'	Not used in meta-analysis
	'Western'	'Western'
	'Drinker'	'Drinker'
Satia *et al.*, 2009 USA	'Western-Southern'	'Western'
	'Fruit-vegetable'	'Healthy'
	'Metropolitan'	Not used in meta-analysis
Butler *et al.*, 2008 China	'Vegetable-fruit-soy'	Not used in meta-analysis
	'Meat-dim sum'	Not used in meta-analysis
Flood *et al.*, 2008 USA	'Fruit and vegetables'	'Healthy'
	'Fat reduced and diet foods'	Not used in meta-analysis
	'Red meat and potatoes'	'Western'
Kesse *et al.*, 2006 France	'Healthy'	'Healthy'
	'Western'	'Western'
	'Drinker	'Drinker'
	'Meat eaters'	'Western'

Kim *et al.*, 2005 Japan	'Healthy'	'Healthy'
	'Traditional'	Not used in meta-analysis
	'Western'	'Western'
Nkondjock and Ghadirian 2005 Canada	'Chocolate–cereal'	Not used in meta-analysis
	'Pork and processed meat'	'Western'
	'Drinker'	'Drinker'
Wu *et al.*, 2004 USA	'Prudent'	'Healthy'
	'Western'	'Western'
Dixon *et al.*, 2004 Finland, Netherlands, Sweden	'Vegetables'	'Healthy'
	'Pork, processed meat, potatoes'	'Western'
Fung *et al.*, 2003 USA	'Prudent'	'Healthy'
	'Western'	'Western'
Terry *et al.*, 2001 Sweden	'Healthy'	'Healthy'
	'Western'	'Western'
	'Drinker'	'Drinker'
Slattery *et al.*, 1998 USA	'Western'	'Western'
	'Prudent'	'Healthy'
	'Drinker'	'Drinker'
	'Substituters'	Not used in meta-analysis
Randall et al., 1992 USA	'Salad'	Not used in meta-analysis
	'Fruit'	Not used in meta-analysis
	'Healthful'	'Healthy'
	'High fat'	Not used in meta-analysis
	'Whole grain'	Not used in meta-analysis
	'Traditional'	Not used in meta-analysis
	'Low cost'	Not used in meta-analysis
	'Snacks' (M)	Not used in meta-analysis
	'Light' (F)	Not used in meta-analysis

B, blacks; F, females; M, males; W, whites.

Dietary Induction of Colonic Tumors in a Mouse Model of Sporadic Colon Cancer

Kan Yang,[1] Naoto Kurihara,[1] Kunhua Fan,[1] Harold Newmark,[2] Basil Rigas,[3] Laura Bancroft,[4] Georgia Corner,[4] Elayne Livote,[6] Martin Lesser,[6] Winfried Edelmann,[5] Anna Velcich,[4] Martin Lipkin,[1] and Leonard Augenlicht[4,5]

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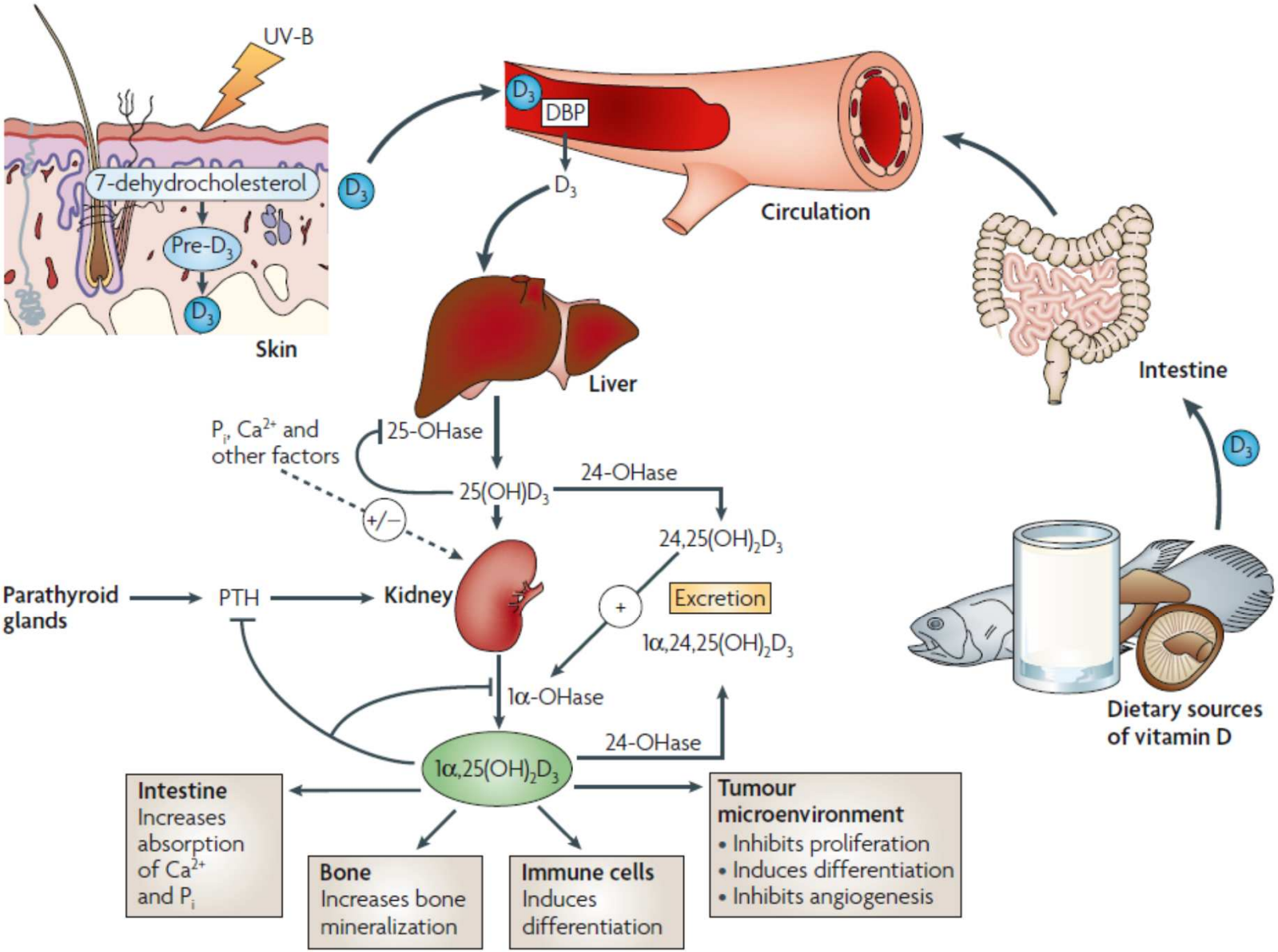
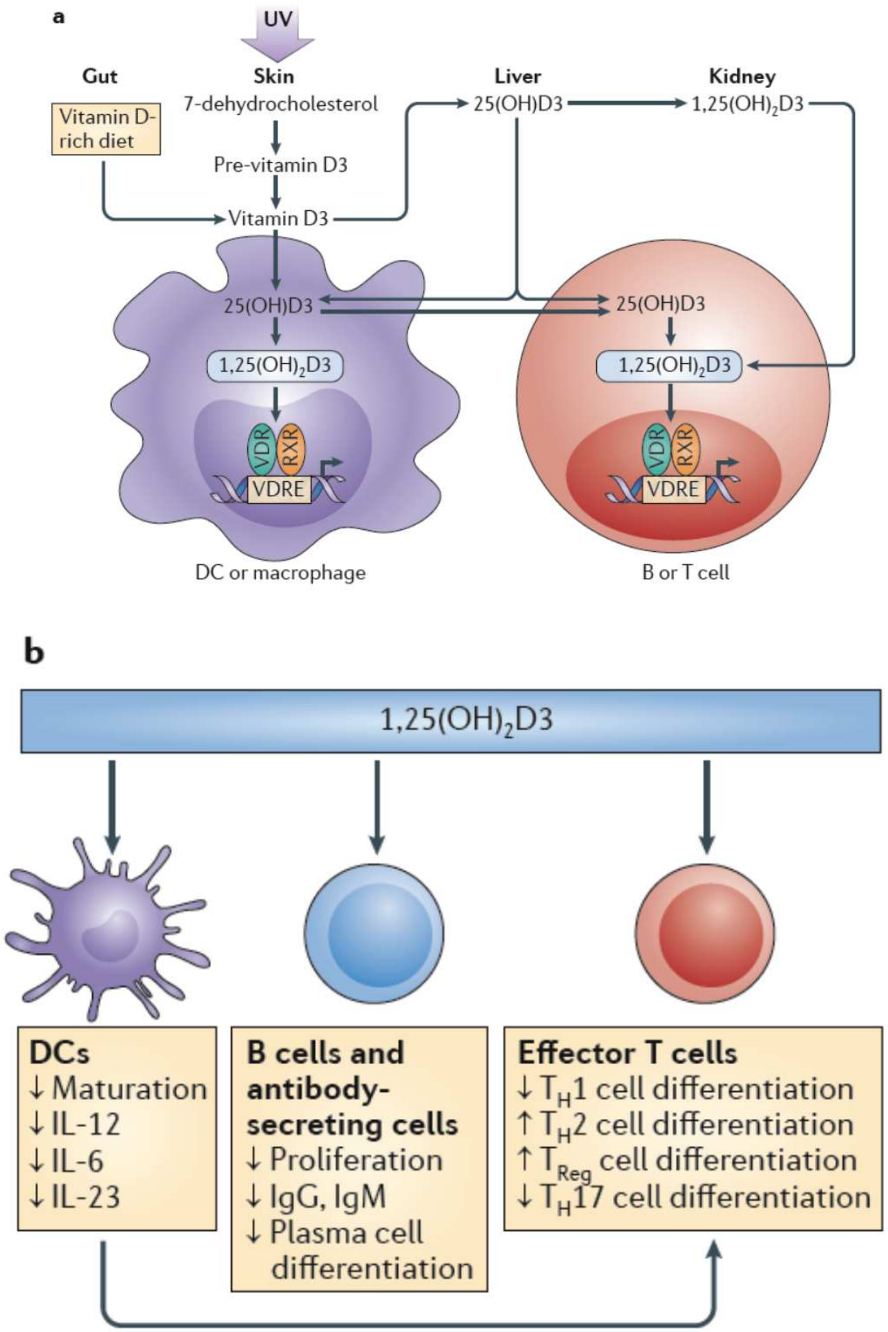
A defined rodent ‘‘new Western diet’’ (NWD), which recapitulates intake levels of nutrients that are major dietary risk factors for human colon cancer, induced colonic tumors when fed to wild-type C57Bl/6 mice for 1.5 to 2 years from age 6 weeks (two-thirds of their life span). Colonic tumors were prevented by elevating dietary calcium and vitamin D3 to levels comparable with upper levels consumed by humans, but tumorigenesis was not altered by similarly increasing folate, choline, methionine, or fiber, each of which was also at the lower levels in the NWD that are associated with risk for colon cancer. The NWD significantly altered profiles of gene expression in the flat colonic mucosa that exhibited heterogeneity among the mice, but unsupervised clustering of the data and novel statistical analyses showed reprogramming of colonic epithelial cells in the flat mucosa by the NWD was similar to that initiated by inheritance of a mutant Apc allele. The NWD also caused general down-regulation of genes encoding enzymes involved in lipid metabolism and the tricarboxylic acid cycle in colonic epithelial cells before tumor formation, which was prevented by the supplementation of the NWD with calcium and vitamin D3 that prevented colon tumor development, demonstrating profound interaction among nutrients. This mouse model of dietary induction of colon cancer recapitulates levels and length of exposure to nutrients linked to relative risk for human sporadic colon cancer, which represents the etiology of >90% of colon cancer in the United States and other Western countries. [Cancer Res 2008;68(19):7803–10]

Table 1. Intestinal tumor incidence (% of mice with tumors) and multiplicity (number of tumors/mouse) for animals fed the indicated diets from ages 6 wk to 2 y

Diet	*n*	Intestinal tumors	
		Incidence (%)	Multiplicity
AIN76A	15	27	0.27 ± 0.15
NWD	15	53	0.67 ± 0.19
NWD+Ca/vitD	18	6	0.06 ± 0.06
NWD+folic acid	18	44	0.56 ± 0.17
NWD+choline	18	33	0.44 ± 0.19
NWD+methionine	16	38	0.63 ± 0.27
NWD+fiber	17	35	0.59 ± 0.30

The NWD increases lipid content, and decreases calcium and vitamin D3, fiber, and methyl-donor nutrients (folic acid, choline, and methionine) to nutrient-density levels associated with risk for colon cancer that are consumed by large segments of human Western populations.


UV-B
7-dehydrocholesterol
Pre-D3
D3
Skin
D3
DBP
D3
Circulation
Liver
Intestine
D3
Pi, Ca2+ and other factors
25-OHase
25(OH)D3
24-OHase
+/−
24,25(OH)2D3
Excretion
Parathyroid glands
PTH
Kidney
+
1α,24,25(OH)2D3
1α-OHase
24-OHase
1α,25(OH)2D3
Dietary sources of vitamin D
Intestine
Increases absorption of Ca2+ and Pi
Bone
Increases bone mineralization
Immune cells
Induces differentiation
Tumour microenvironment
• Inhibits proliferation
• Induces differentiation
• Inhibits angiogenesis


a
UV
Gut
Skin
Liver
Kidney
Vitamin D-rich diet
7-dehydrocholesterol
25(OH)D3
1,25(OH)2D3
Pre-vitamin D3
Vitamin D3
25(OH)D3
1,25(OH)2D3
VDR
RXR
VDRE
25(OH)D3
1,25(OH)2D3
VDR
RXR
VDRE
DC or macrophage
B or T cell
b
1,25(OH)2D3
DCs
↓ Maturation
↓ IL-12
↓ IL-6
↓ IL-23
B cells and antibody-secreting cells
↓ Proliferation
↓ IgG, IgM
↓ Plasma cell differentiation
Effector T cells
↓ TH1 cell differentiation
↑ TH2 cell differentiation
↑ TReg cell differentiation
↓ TH17 cell differentiation

Chemical-induced models of colorectal cancer

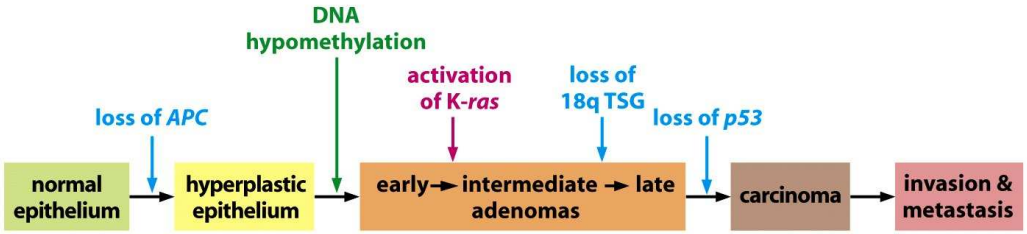
A large number of chemicals are known to have mutagenic potential, and many cancer studies have used this characteristic to controllably induce cancer.

DMH and AOM The compound 1,2-dimethylhydrazine (**DMH**) and its metabolite, azoxymethane (**AOM**), are the two most commonly used carcinogens to induce and promote colorectal cancer in rats and mice. **DMH** and **AOM** are **alkylating agents** that are typically injected intraperitoneally or subcutaneously over several weeks to induce development of tumors in the distal colon. The majority of these tumors harbor **mutations in the β-catenin gene** (Ctnnb1), which is **similar to HNPCC**. These mutations affect the N-terminal amino acids of the β-catenin gene product, making the protein resistant to regulatory degradation, stabilizing β-catenin, and increasing WNT signaling to drive tumorigenesis. In addition, tumor incidence and multiplicity can be altered by both genetic background and by diet. This makes the models useful for the study of gene–gene and gene–environment interactions that influence the pathogenesis of colorectal cancer. However, there is **little evidence that a large proportion of human sporadic colorectal cancer results from exposure to alkylating agents** so some have questioned the translational potential of data generated with the model.

Chemical-induced models of colorectal cancer

PhIP **PhIP** (2-amino-1-methyl-6-phenylimidozopyridine) is a **heterocyclic am**ine produced during the **cooking of meat and fish** that is a colon cancer causing **mutagen in rats**. In mice, **PhIP induces formation of colonic aberrant crypt foci** but **not colon tumors**. However, combining PhIP with either DSS treatment or treating ApcMin mice with PhIP can enhance tumorigenesis. Epidemiologic evidence links PhIP from cooked meat to increased colorectal cancer risk and so the data obtained from the study of PhIP in rodents is highly relevant to human cancer. At typical PhIP doses (100–400 ppm), approximately 50 % of male rats develop aberrant crypt foci or colonic adenomas within 1–2 years. In PhIP-induced tumors, **mutations** in the **Cnntb1and Apc genes** are **common while Kras and Tp53 mutations are rare**. Colon cancers develop typically in the middle to distal regions of the colon and exhibit a polypoid, tubular adenoma morphology. **Invasion and metastasis are rare**.

Similar to what others have shown in mice fed a Western diet, PhIP induces colonic adenomas in rats with a gene expression profile that includes markers of Paneth cell differentiation. PhIP-induced cancer is **modulated by genetic background**; BUF rats are highly responsive, F344 and Brown–Norway rats are moderately sensitive, and ACI are relatively resistant to PhIP-induced formation of aberrant crypt foci.



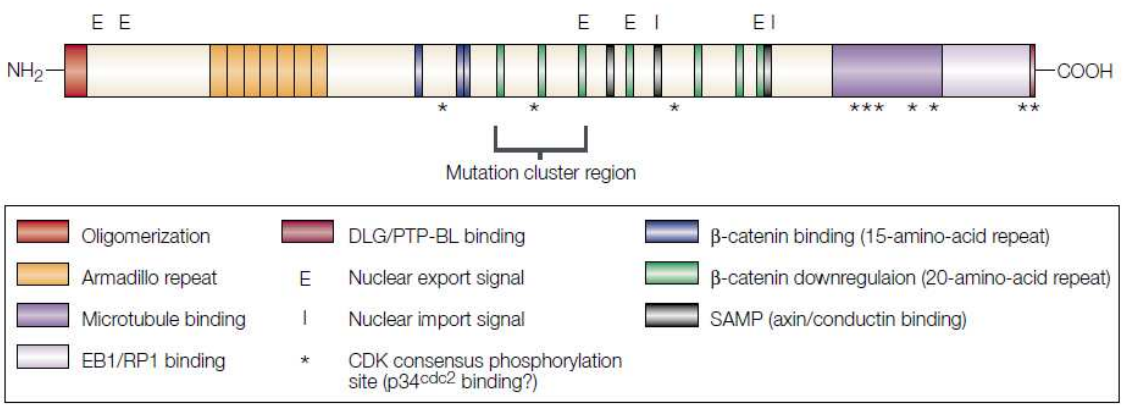
Chemical-induced models of colorectal cancer

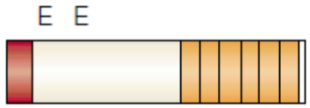
N-Methyl-N-nitro-N-nitrosoguanidine and N-methyl-Nnitrosourea N-Methyl-N-nitro-N-nitrosoguanidine (MNNG) and N-methyl-N-nitrosourea (abbreviated as both **MNU** and **NMU**) are **direct-acting carcinogens** that have been administered to mice and rats to induce neoplasia in a variety of organs. When **given orally**, **cancer** develops in the **stomach, small intestine, large intestine, kidney, skin, lung**, and **thymus**. Injection of MNU also induces prostate and breast cancer. Unfortunately, **the high incidence of extra-colonic neoplasia** induced by these nitroso compounds is a confounding variable in this model. When **administered via the rectum**, MNU reproducibly causes a **high incidence of colon cancer**, **but still induces thymic lymphoma and pulmonary cancers** that can cause mortality. This local route of administration of MNU has been shown to cause DNA adduct formation and aberrant crypt foci. Intrarectal administration of five weekly MNNG doses to rats induced formation of colonic adenoma and carcinoma (one to two per rat), and was modified by the level of dietary fat. When compared to DMH, 3- or 15-week courses of MNNG induced tumors with similar histopathologic features. MNU-induced colon cancer has been used to test a host of other potential preventive interventions against colorectal cancer (e.g., 1alphahydroxy- 24-ethylcholechalciferol vitamin D analogue, dietary restriction, dietary fatty acids, and ursodeoxycholic acid). The complete molecular profile of mutations induced by MNU and MNNG is unknown, but **15–30 %** of rat colonic tumors induced by MNU or MNNG have been found to contain **Kras mutations**. Endo et al. also found **Apc mutations in 6** % colon tumors induced by DMH or MNNG in rats. Interestingly, in aberrant crypt foci induced by MNNG in rats, the MUC5AC gene product, gastric M1 mucin, is expressed. This is also observed in human colonic aberrant crypt foci.

Mutagen-induced germline mutation models

The ApcMin mouse

The workhorse for preclinical colorectal cancer research over the past 30 years has been the ApcMin mouse. This mouse was identified in 1990 from an ethylnitrosourea (ENU) mutagenesis screen in **C57Bl/6J mice**. The phenotype of the first of these mutant mice was severe, sometimes fatal, regenerative anemia that was attributed to **multiple intestinal neoplasms or "Min."** The Min mutation is autosomal dominant, and homozygosity for the mutant allele is embryonic lethal. Tumors occurred in the small and large intestine, but **greater than tenfold more lesions were found in the small intestine**. The genetic basis for the intestinal phenotype is a **T-to-A transversion at nucleotide 2,549 of the mouse Apc gene that truncates the Apc protein at amino acid 850**. Similar to FAP, loss of heterozygosity of the remaining wild-type Apc allele was required for adenoma formation. Because of its molecular and pathologic similarity to human FAP, ApcMin mice have been used extensively to study the development, treatment, and prevention of colorectal cancers that contain somatic APC mutations.



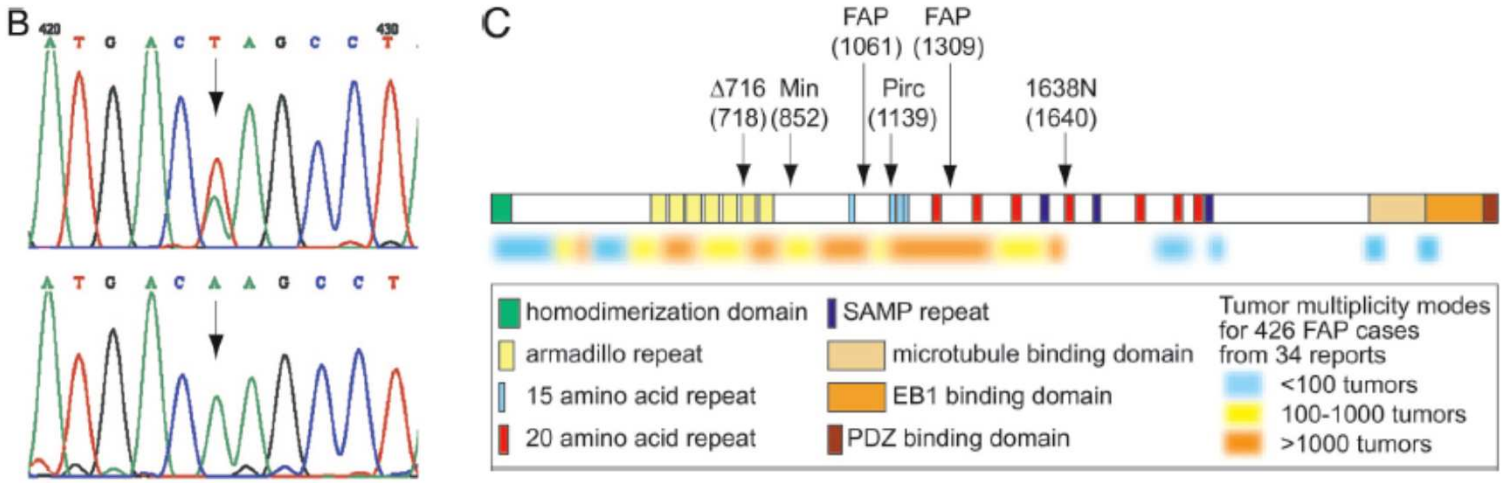


ApcMin

Mutagen-induced germline mutation models

The F344-Pirc rat

An ENU mutagenesis approach also led to the development of the Pirc (polyposis in the rat colon) rat. The rat FAP model harbors a mutation at nucleotide 3,409 of the Apc gene that results in **truncation of the Apc protein at codon 1,137**. On a F344 background, heterozygous Pirc rats developed adenomas throughout the intestine, with 100 % having at least one colonic tumor. There are several differences between the F344-Pirc rat and ApcMin mouse. Whereas ApcMin mice develop tumors with a small intestine-tocolon ratio of 40:1, the Pirc rat develops adenomas at a **ratio approaching 1:1**. As in the mouse, the adenomas of the Pirc rat mirror the morphology of human adenomas, including **progression to invasive adenocarcinoma**. The cancer incidence in Pirc rats is increased in males, while a gender effect has not been reported in the ApcMin mouse. The **increased size of the rat** over the mouse offers advantages for sample collection and use of advanced imaging techniques for longitudinal study.



Mutagen-induced germline mutation models

The F344-Pirc rat

A target-selected *Apc*-mutant rat kindred enhances the modeling of familial human colon cancer

James M. Amos-Landgraf*, Lawrence N. Kwong*, Christina M. Kendziorski†, Mark Reichelderfer‡, Jose Torrealba§, Jamey Weichert¶, Jill D. Haag*, Kai-Shun Chen*, Jordy L. Waller*, Michael N. Gould*, and William F. Dove*‖**

4036–4041 | PNAS | March 6, 2007 | vol. 104 | no. 10

Table 1. Tumor multiplicities in Pirc rats

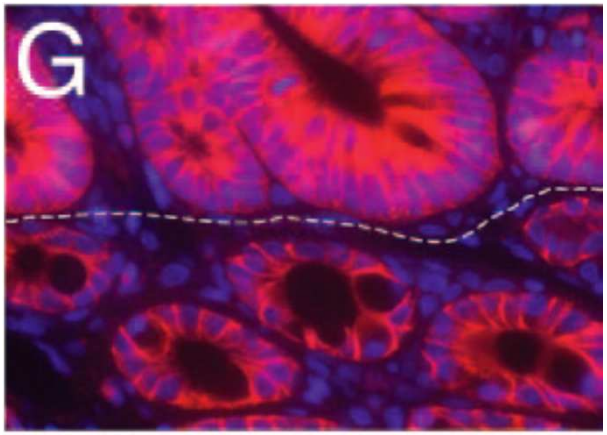
Background	Sex	Age, months	No.	Colonic polyps, mean ± SD	Lesions in small intestine, mean ± SD	
					Adenomas	Microadenomas
F344-Pirc	Male	3	5	2 ± 1	7 ± 9	21 ± 20
	Male	4–6	10	8 ± 3	14 ± 5	88 ± 64
	Male	7–13	17	14 ± 8	22 ± 9	178 ± 116
	Female	3	5	3 ± 2	0 ± 0	1 ± 2
	Female	4–6	11	5 ± 3	2 ± 2	19 ± 29
	Female	7–13	6	7 ± 5	4 ± 5	35 ± 44
F344-Pirc, ENU-treated	Male	7	3	79 ± 11	57 ± 13	665 ± 103
F344-Pirc, mock-treated*	Male	7	2	11 ± 12	18 ± 8.5	208 ± 223

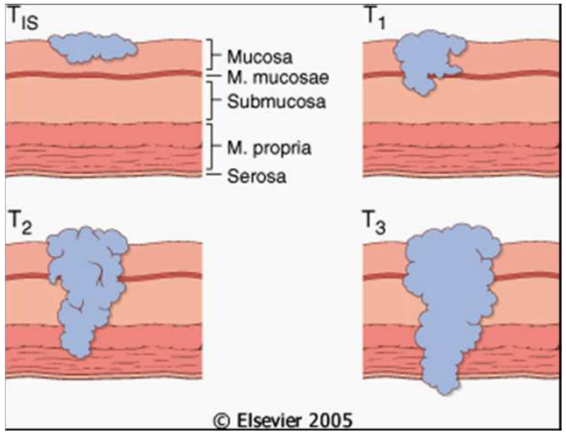
Colonic microadenoma multiplicities could not be accurately measured without histopathological confirmation and were excluded from these analyses. Neoplasms <0.5 mm in size were classified as microadenomas.
*Controls were injected with phosphocitrate buffer plus ethanol without ENU.

Mutagen-induced germline mutation models

The F344-Pirc rat

The histopathology and morphology of the tumors closely resembled that of human tumors, with **adenomatous changes evident, including dysplasia**, nuclear enlargement, an increased mitotic rate, and the **expansion of crypts** showing loss of the normal columnar architecture, …Immunofluorescent staining of tumors revealed **nuclear** and **cytoplasmic accumulation of β-catenin** within dysplastic cells….. in animals at 6 months of age or greater, **3 of 14** histologically examined colonic tumors were shown to have high-grade dysplasia accompanied by the local invasion of neoplastic cells into the stalk, classifying the tumors as adenocarcinomas with early signs of progression to a stage corresponding to **T1** in the human.





Mutagen-induced germline mutation models

The F344-Pirc rat

The Chemopreventive Action of Celecoxib. The Min mouse strain has enabled the analysis of the chemoprevention of intestinal adenomagenesis. The nonsteroidal antiinflammatory agents piroxicam, sulindac, and the clinically used celecoxib have been reported to show significant efficacy in the **Min mouse**. Statistically significant evidence for these effects depended on the **high multiplicity of adenomas in the small intestine**. By contrast, colonic tumor multiplicities in the range of two **compromised the power of tests for an effect in the colon**. Study designs involving large numbers of animals, enhanced colonic tumor multiplicities, or longitudinal analysis of individual imaged tumors would permit the analysis of response for colonic neoplasms.

Table 2. The effect of celecoxib on the multiplicity of intestinal tumors >1 mm in diameter in F344 Pirc rats

		Tumor multiplicity, mean ± SD (no. of rats)		
Sex	Tissue	Treated	Untreated	*P* value
Male	Small intestine	1.3 ± 1.2 (12)	7.6 ± 4.3 (11)	<0.005
Male	Colon	1.2 ± 0.9 (12)	3.6 ± 2.7 (11)	<0.01
Female	Small intestine	0.8 ± 0.9 (12)	0.6 ± 0.8 (15)	0.6
Female	Colon	0.3 ± 0.5 (12)	1.3 ± 0.7 (15)	<0.001

Animals were treated from 40 days of age with 1,200 ppm celecoxib in Teklad 8604 chow and euthanized at 6–7 months of age. Tumors were counted on freshly dissected tissue without using a dissecting microscope. *P* values were determined by using the Wilcoxon rank sum test.

Mutagen-induced germline mutation models

The F344-Pirc rat

The Rat Permits both Classical Endoscopy and Virtual Colonoscopy. To determine whether longitudinal in vivo studies of individual intestinal tumors can be carried out in trials with agents such as celecoxib, an 11-month-old F344-Pirc rat was anesthetized and its tumors visualized by endoscopy. As shown in Fig. 4B, a 6-mm diameter bronchoscope provided clear images of three tumors with diameters 5.3, 5.7, and 6.8 mm. The same tumors were identified in three-dimensional micro computed tomography (CT) images (Fig. 4A) and confirmed upon dissection (Fig. 4C).

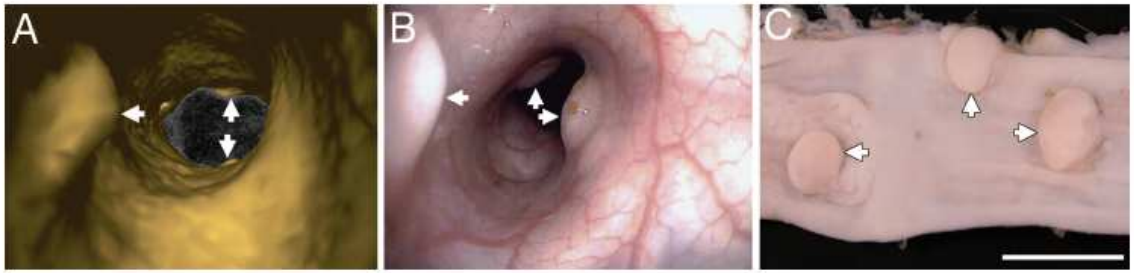
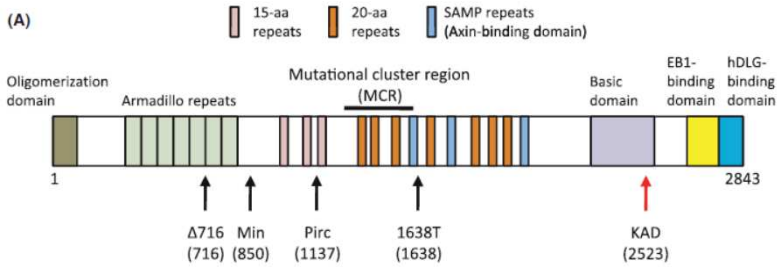


Fig. 4. *In vivo* imaging of Pirc tumors. MicroCT (*A*), endoscopic (*B*), and dissection (*C*) views of three colonic tumors in an 11-month-old F344 Pirc male. (Scale bar: 1 cm.)

Mutagen-induced germline mutation models

The KAD rat

To establish an efficient rat model for colitis-associated colorectal cancer, AOM/DSS-induced colon carcinogenesis was applied to a novel adenomatous polyposis coli (Apc) mutant, the Kyoto Apc Delta (KAD) rat. The KAD rat was derived from ENU mutagenesis and harbors a nonsense mutation in the Apc gene (S2523X). The truncated APC of the KAD rat was deduced to lack part of the basic domain, an EB1-binding domain, and a PDZ domain, but retained an intact beta-catenin binding region. KAD rats, homozygous for the Apc mutation on a genetic background of the F344 rat, showed no spontaneous tumors in the gastrointestinal tract. At 5 weeks of age, male KAD rats were given a single subcutaneous administration of AOM (20 mg/kg, bodyweight). One week later, they were given DSS (2% in drinking water) for 1 week. At week 15, the incidence and multiplicity of colon tumors developed in the KAD rat were remarkably severe compared with those in the F344 rat: 100 versus 50% in incidence and 10.7 +/- 3.5 versus 0.8 +/- 1.0 in multiplicity. KAD tumors were dominantly distributed in the rectum and distal colon, resembling human colorectal cancer. Accumulation of beta-catenin protein and frequent beta-catenin mutations were prominent features of KAD colon tumors. To our knowledge, AOM/DSS-induced colon carcinogenesis using the KAD rat is the most efficient to induce colon tumors in the rat, and therefore would be available as an excellent model for human colitis-associated CRC.



Mutagen-induced germline mutation models

The KAD rat

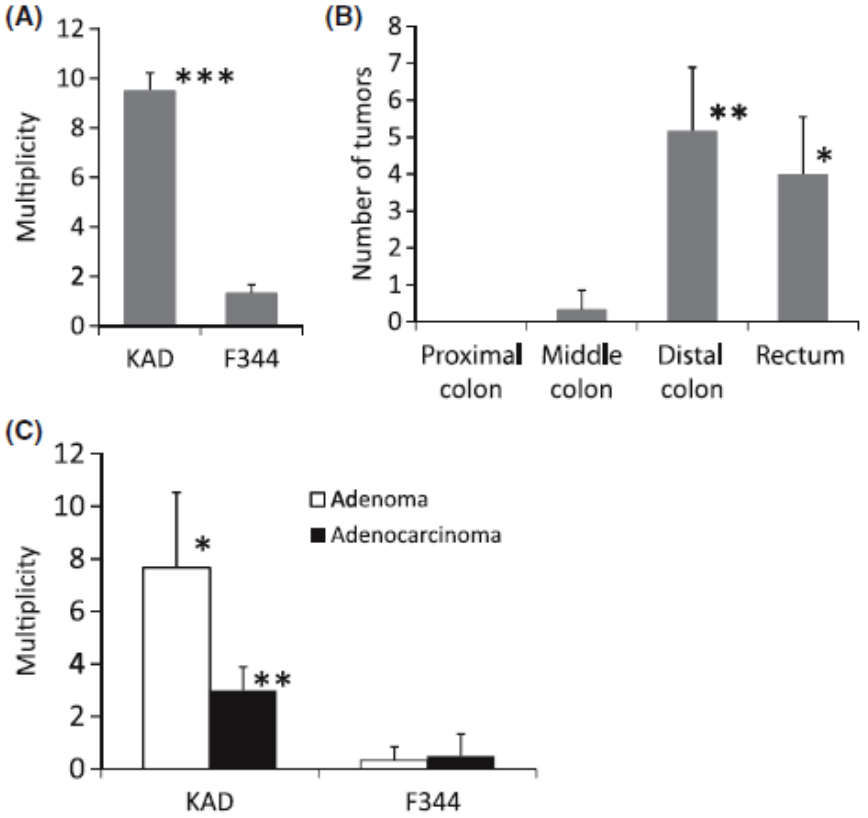


Fig. 4. Increased induction of colon tumors in azoxymethane (AOM)/dextran sodium sulfate (DSS)-treated Kyoto Apc Delta (KAD) rats. (A) Multiplicity of tumors observed macroscopically (mean ± SD) at week 15. ***$P < 0.0001$. (B) Distribution of colon tumors in AOM/DSS-treated KAD rats (mean ± SD) at week 15. **Distal colon versus middle colon, $P < 0.001$; *rectum versus middle colon, $P < 0.005$. (C) Multiplicities of adenoma and adenocarcinoma developed in KAD rats were significantly higher than in F344 rats at week 15. *$P < 0.005$, **$P < 0.001$.

Genetically modified mice

Apc mutation	Apc product length (amino acids)	Estimated No. tumors per mouse		Homozygous embryonic lethal
		Small intestine	Large intestine	
Apc^{WT}	2,843	0	0	No
Apc^{Min}	850	30	3	Yes
F344-Pirc rat	1,137	15	10	Yes
$Apc^{\Delta 716}$	716	300	3	Yes
$Apc^{\Delta ex14}$	580	40	4	Yes
$Apc^{\Delta 474}$	474	30	3	Yes
Apc^{1322T}	1,322	200	3	Yes
Apc^{1638N}	0	3	0	Yes
Apc^{1638T}	1,638	0	0	No
$Apc^{\Delta SAMP}$	1,322+(2,006–2,843)	200	3	Yes
$Apc^{\Delta 15}$	650	175	8	Yes
Apc^{1309}	1,309	30	3	Yes
Apc^{mNLS}	Full length, mutant nuclear localization signals	0	0	No

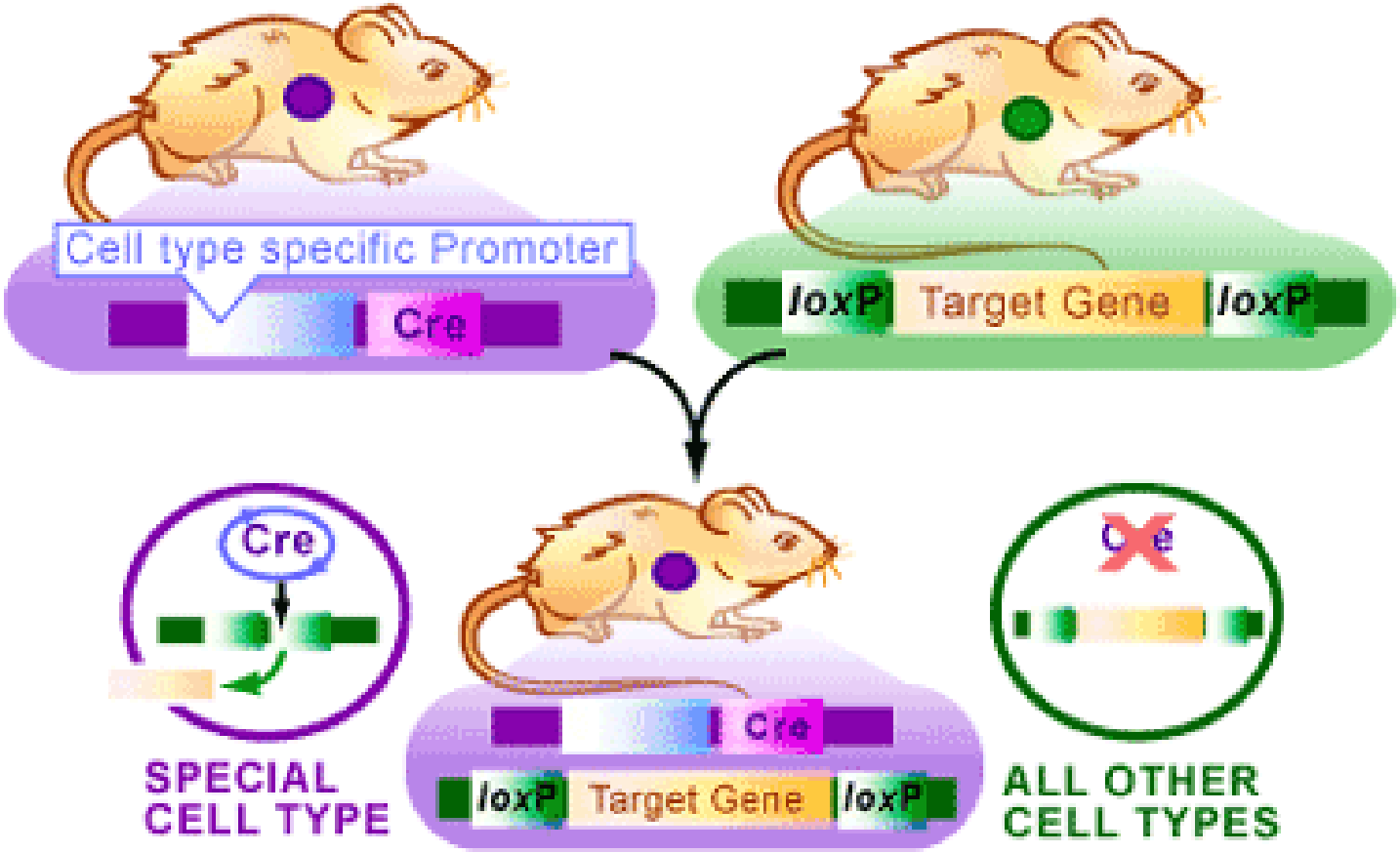
Genetically modified mice

Tissue-specific and inducible genetic modifications

Genetically modified mice offer the potential to precisely recapitulate specific molecular etiologies relevant to human disease. When considering the features that would make an optimal mouse model of sporadic colon cancer, several features are desirable. First, a researcher should be able to **increase or reduce the expression of genes that are hypothesized, or known, to influence human colorectal cancer**. Chemically induced cancer may lead to mutations in relevant human cancer genes, but they do not have the ability to target them, per se. Second, a researcher should be able to **control the timing of the cancer-inducing molecular event**. Like familial human cancers, traditional transgenic and knockout mice have germline modifications and express that modification embryonically. In contrast, most human cancers develop in adults (even if initiating events occur earlier in life). Later onset of controlled genetic modifications in animal models, rather than during the hormonal milieu of adolescent growth, could improve our ability to translate animal data to humans. Controlling the **timing of induction** is an advantage of chemically induced cancers, but advances in inducible transgenic mouse models have made this feasible for genetically modified mice as well. Finally, the **molecular event should be limited to the colon and/or rectum**. Most genetically modified mouse models were generated to have a single gene defect in all cells in the body. While we have learned a lot about cancer from these models, they are often confounded by the existence of precancerous or cancerous lesions in other tissues.

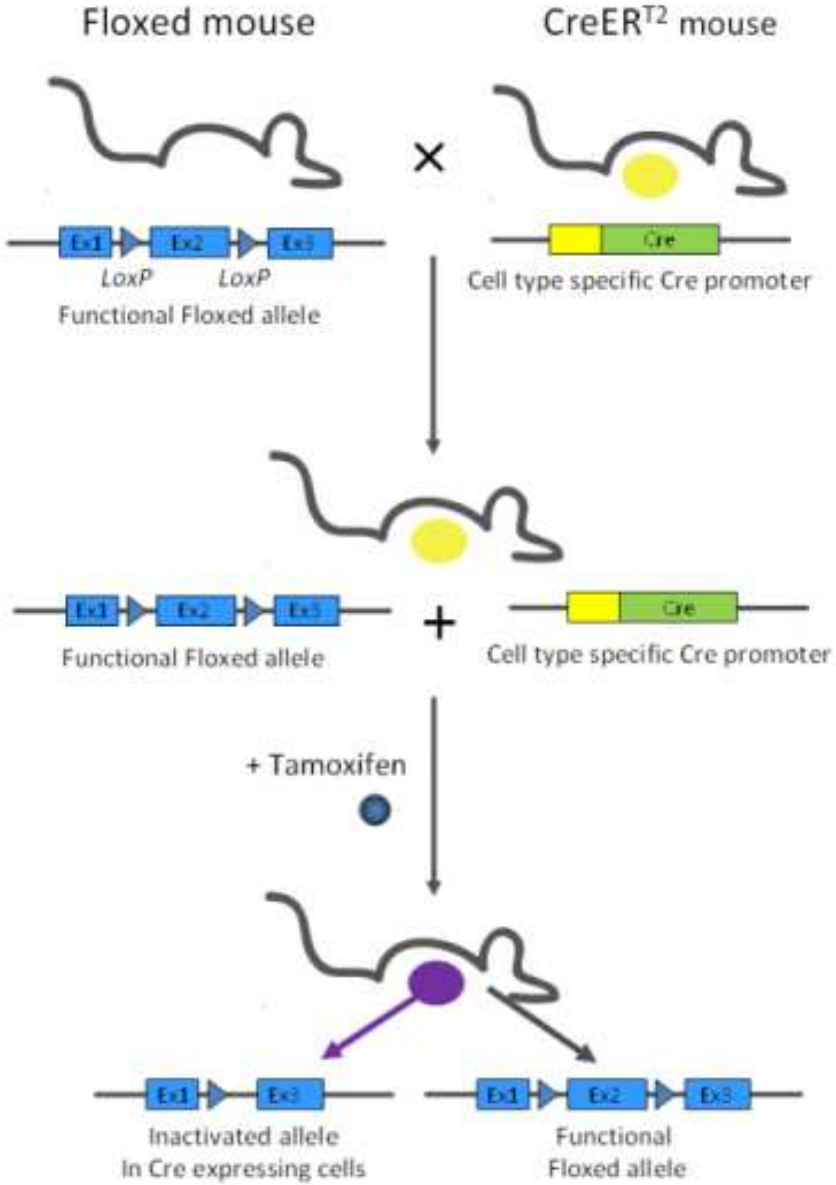
Genetically modified mice

Tissue-specific and inducible genetic modifications



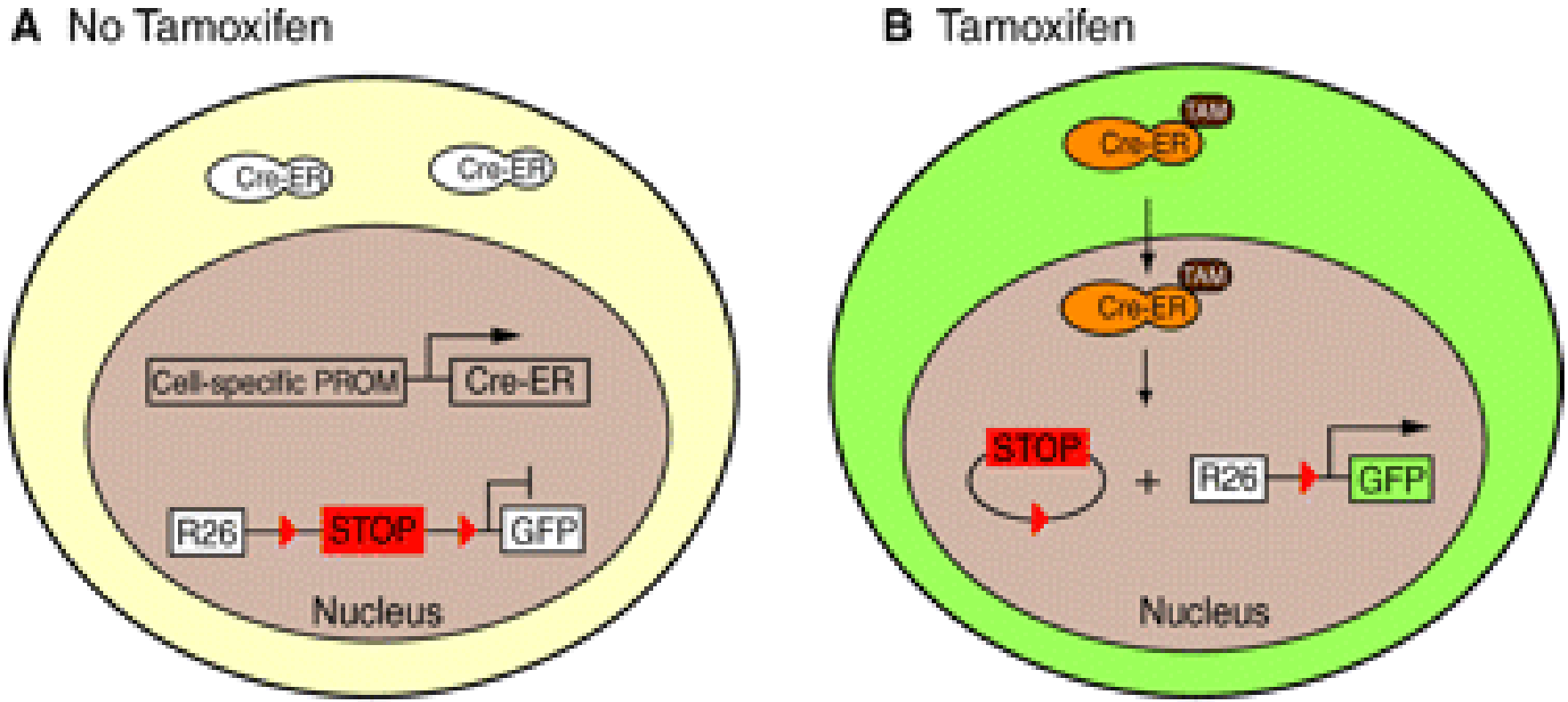
Genetically modified mice

Tissue-specific and inducible genetic modifications



Genetically modified mice

Tissue-specific and inducible genetic modifications



Genetically modified mice

Tissue-specific and inducible genetic modifications

Table 3 Promoters and methods for inducible or intestine-specific gene expression

Promoter	Small intestine			Large intestine			Extra intestinal expression	Transgene(s) Available	Inducible	Citation
	Dd	Je	Il	Ce	ACo	DCo				
Epithelial cell expression										
Villin	X	x	x	x	x	x	Stomach, kidney	Cre Cre-ER^{T2}	Yes[a]	[117]
CK-19	x	x	x	x	x	x	Pancreatic ducts, hepatic ducts, stomach	Cre-ER^{T2}	Yes[a]	[122]
Lgr5	x	x	x	x	x	x	Stem cells	Cre-ER^{T2}	Yes[a]	[123]
Cyp1A1	x	x	x	x	x	x	Broadly	Cre	Yes[b]	[135]
Fabpl		x	x	x	x	x	Renal calyces, pelvis, ureter, bladder	Cre tetO-PhCMV-Cre	Yes[c]	[119, 120]
CDX2P9.5			x	x	x	x	Embryo (kidney, spleen, hind limbs, skin)	Cre	No	[125]
CDX2P9.5-G22			x	x	x			Cre	No	[126]
Car1				x	x	x	Liver	Cre	No	[127]
Secretory cell expression										
ITF	xx	xx	x	x		x	Stomach	SV40 T Ag	No	[128]
Muc2	xx	xx	x				Stomach, spleen, lymph node	SV40 T Ag	No	[129]

Dd duodenum, *Je* jejunum, *Il* Ileum, *Ce* cecum, *Co* colon

[a] Inducible by tamoxifen

[b] Inducible by β-naphthoflavone

[c] Inducible by tetracycline

Cancer Genes and Genomics

Generation of a Transgenic Mouse for Colorectal Cancer Research with Intestinal Cre Expression Limited to the Large Intestine

Yingben Xue[1], Robert Johnson[2], Marsha DeSmet[3], Paul W. Snyder[2], and James C. Fleet[1,3]

Abstract

Genetically modified mice have been used for colon cancer research, but findings from these models are confounded by expression of cancer in multiple organs. We sought to create a transgenic mouse with Cre recombinase (Cre) expression limited to the epithelial cells of the large intestine and used this model to study colon cancer driven by adenomatosis polyposis coli (*APC*) gene inactivation. A promoter/enhancer from the mouse carbonic anhydrase I gene was used to generate a Cre-expressing transgenic mouse (*CAC*). After characterizing transgene expression and distribution, *CAC* mice were crossed to APC^{580S} mice to generate mice with *APC* inactivation at one ($CAC;APC^{580S/+}$) or both alleles ($CAC;APC^{580S/580S}$). Transgene expression was limited to the epithelial cells of the cecum and colon, extended from the crypt base to the luminal surface, and was expressed in approximately 15% of the crypts. No abnormal gross phenotype was seen in 3- or 6-week-old $CAC;APC^{580S/+}$ mice, but $CAC;APC^{580S/580S}$ mice had significant mucosal hyperplasia in the colon at 3 weeks, which developed into tumors by 6 weeks. By 10 weeks, 20% of $CAC;APC^{580S/+}$ mice developed adenomatous lesions in the distal colon (3.0 ± 0.4 mm; 1.1 per mouse). Dextran sulfate sodium treatment increased the incidence and number of tumors, and this occurred predominantly in distal colon. Our new model has improved features for colon cancer research, that is, transgene expression is limited to the epithelium of the large bowel with normal cells found next to genetically modified cells. *Mol Cancer Res; 8(8); 1095–104.* ©*2010 AACR.*

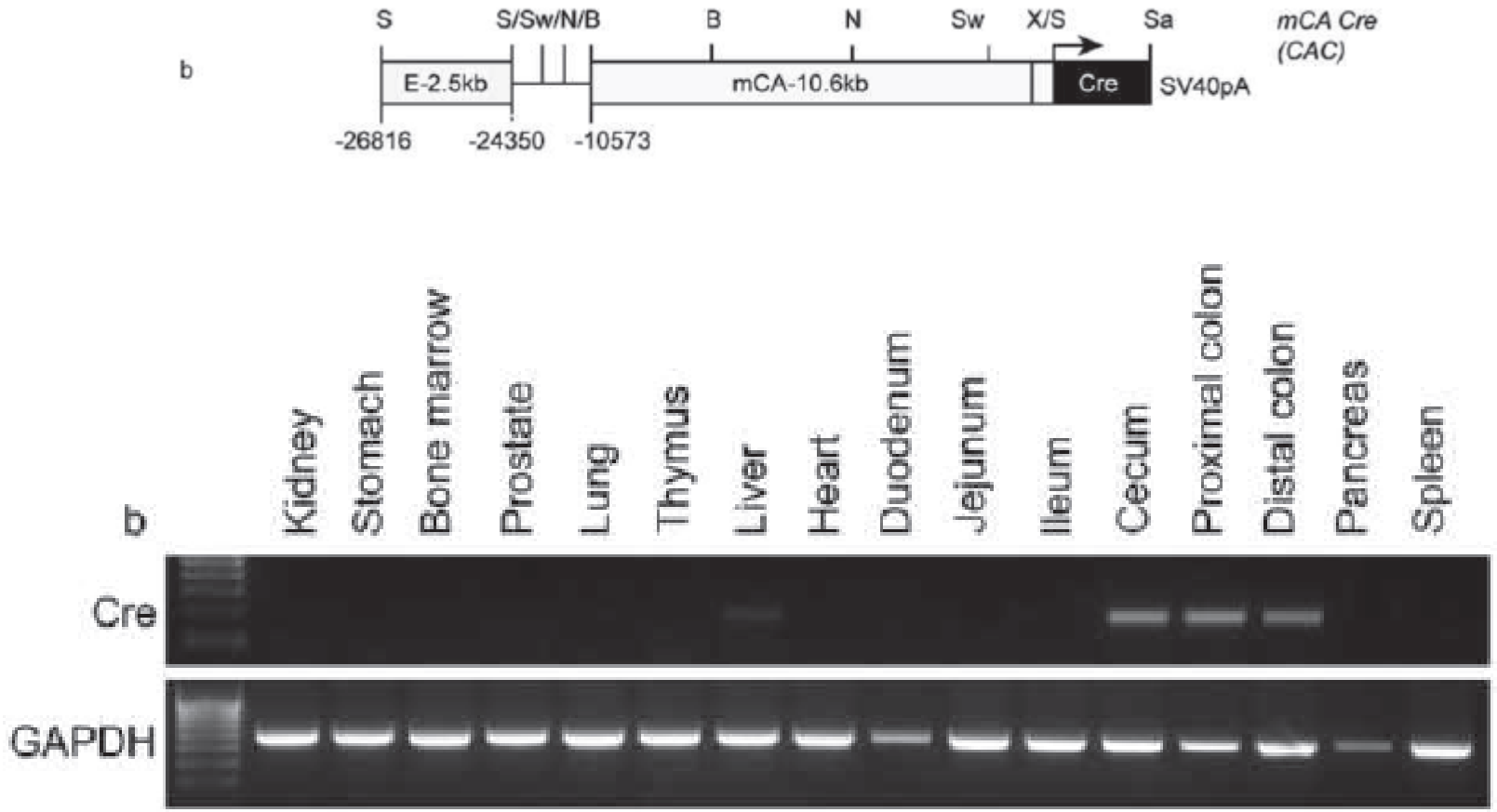

b
S
S/Sw/N/B
B
N
Sw
X/S
Sa
mCA Cre
(CAC)
E-2.5kb
mCA-10.6kb
Cre
SV40pA
-26816
-24350
-10573
b
Kidney
Stomach
Bone marrow
Prostate
Lung
Thymus
Liver
Heart
Duodenum
Jejunum
Ileum
Cecum
Proximal colon
Distal colon
Pancreas
Spleen
Cre
GAPDH

Table 1. Tumor characteristics in *CAC;APC*$^{580S/+}$ mice

Group	No. mice	No. with gross tumors	Incidence (%)	No. gross tumors	Tumor size (mm)	Tumor location*
No DSS	50	10	20	12	3.0 ± 0.4	2.4 ± 0.5
2% DSS, 7 d	18	12	66.7	70	3.2 ± 0.2	3.1 ± 0.3
2% DSS, 5 d	8	4	50	12	2.3 ± 0.4	2.0 ± 0.3

Microadenomas†	No. mice	No. with microlesions	Incidence (%)	No. micro lesions
No DSS	50	5	10	10
2% DSS, 7 d	18	11	61.1	41
2% DSS, 5 d	8	3	37.5	5

Total lesions	No. mice	No. mice with lesions	Incidence (%)	No. lesions
No DSS	50	13	26	22
2% DSS, 7 d	18	13	72.2	111
2% DSS, 5 d	8	4	50	17

*Distance (in centimeters) from rectum.

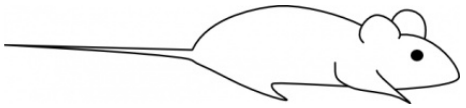
Genetically modified mice

Tissue-specific and inducible genetic modifications

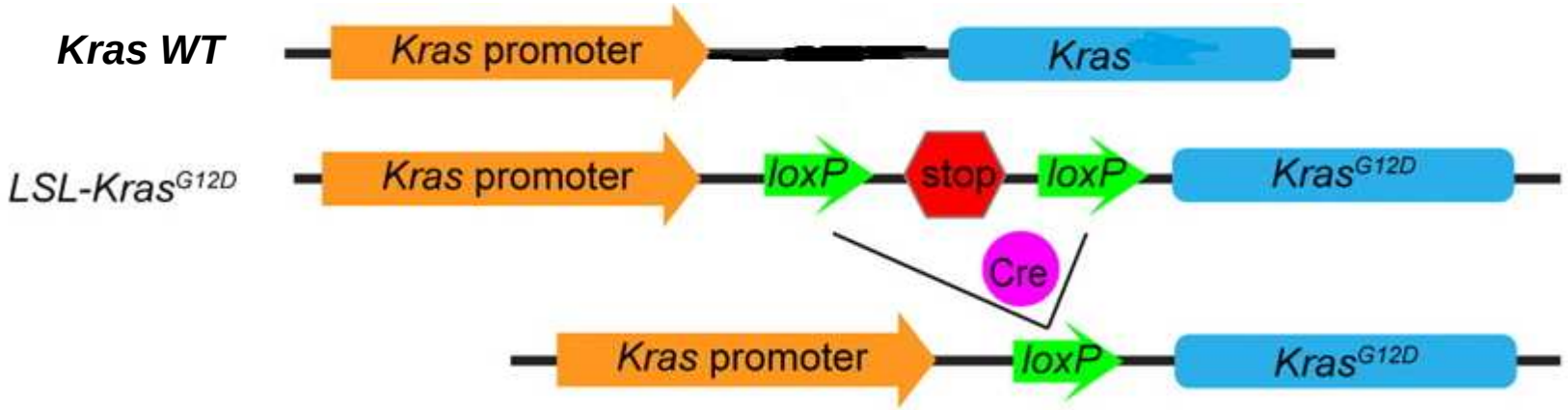
Table 4 Mouse models with floxed alleles

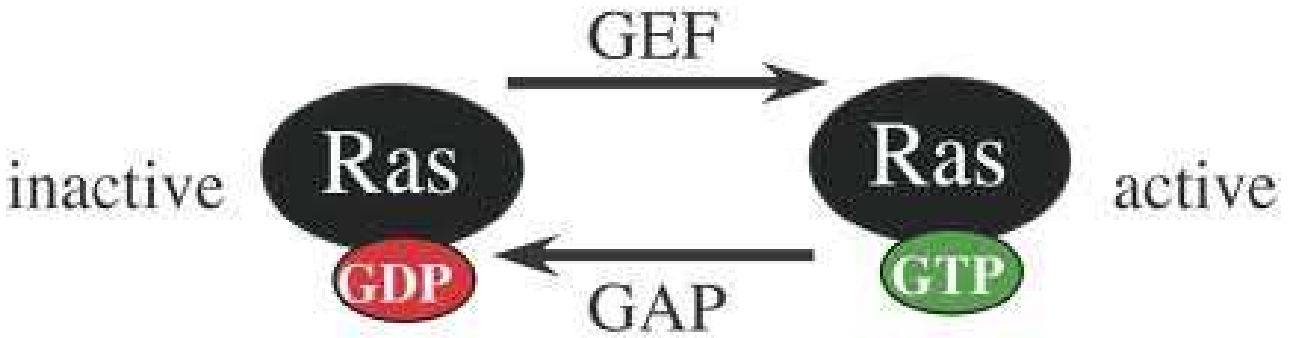
Gene	Floxed allele	Published Cre promoters	Observed tumor phenotype
Apc	Exon 14	Numerous intestinal-specific promoters and intrarectal adenovirus-Cre	Tubular adenoma formation in regions matching promoter expression
	Exon 15	*Fabpl*	Severe Apc^{Min}-like phenotype
Ctnnb1	Exon 3	*CK-19* *Fabpl*	Tubular adenoma formation consistent with promoter expression
Kras	LSL-$Kras^{G12D}$	*Fabpl* *CDX2P9.5-G22* *Villin*	Epithelial hyperplasia, but no tumor formation unless combined with carcinogens or *Apc* mutations
	LSL-$Kras^{G12V}$	*AhCre*	Same as $Kras^{G12D}$
Msh2	Exon 12	*Villin*	Small intestinal tumors only
TGFβR2	Exon 2	*Villin*	No tumors unless combined with other relevant mutations
Fbxw7	Exon 5	*Villin*	Small polyps and increased crypt fission

LSL LoxP-STOP-LoxP



Mouse Kras $^{+/LSL-G12D}$



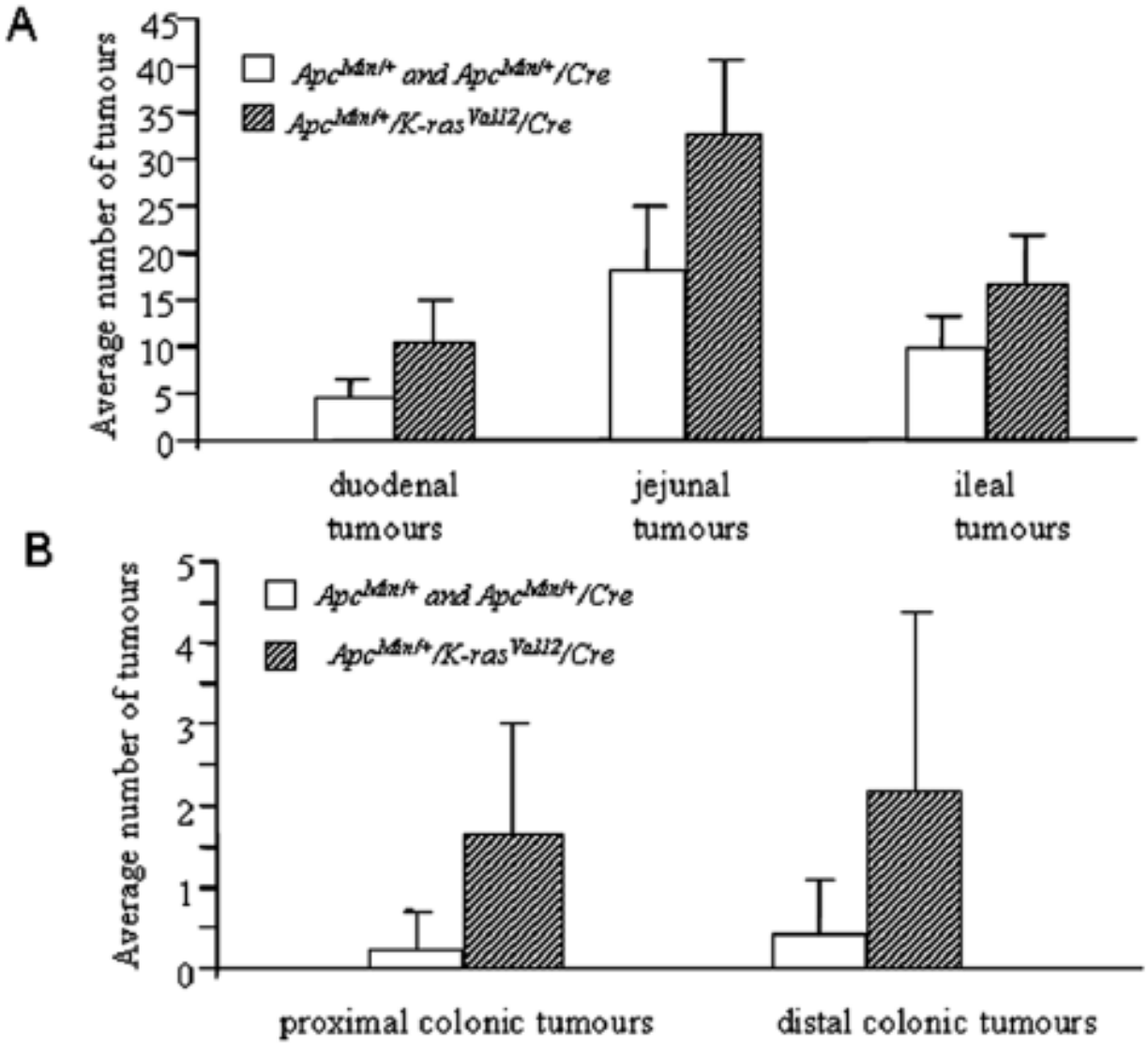


Synergism between K-*ras*^{Val12} and mutant *Apc* accelerates murine large intestinal tumourigenesis

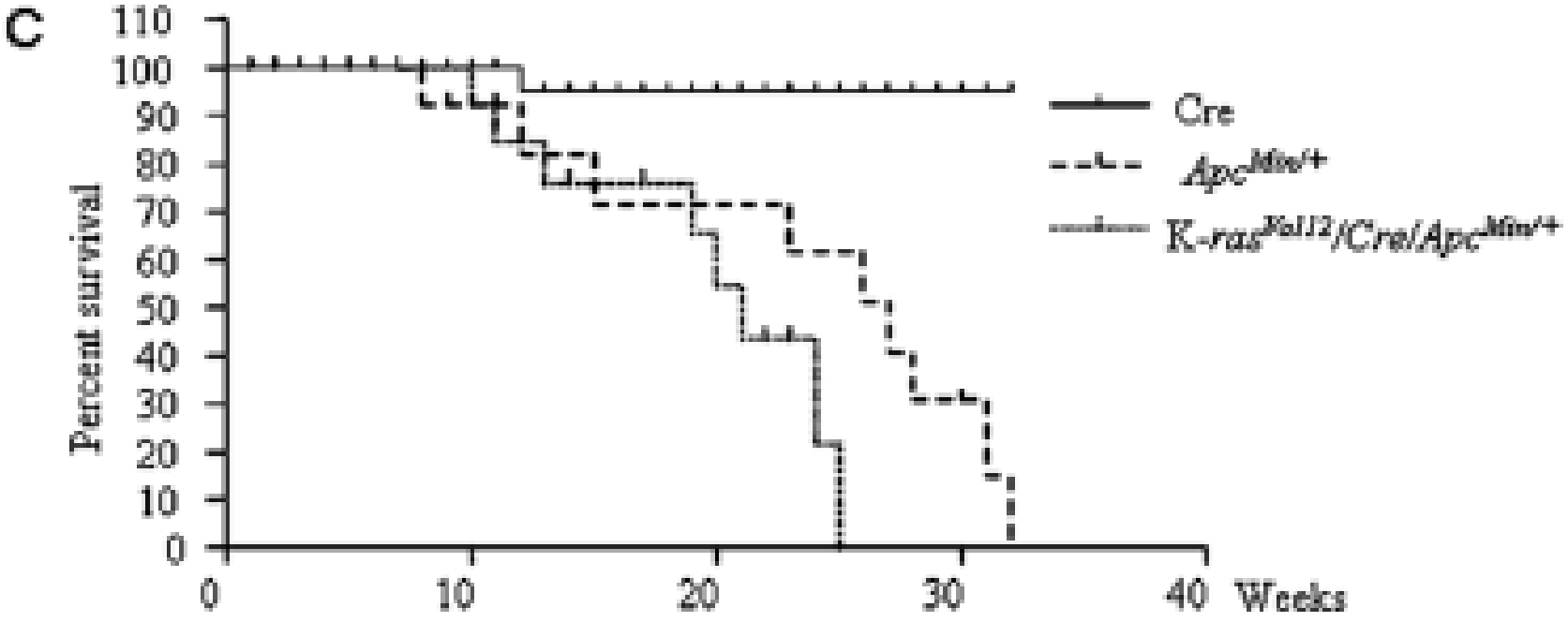
FEIJUN LUO[1,2], GEORGE POULOGIANNIS[1], HONGTAO YE[1], RIFAT HAMOUDI[1] and MARK J. ARENDS[1]

Abstract. K-*ras* (*KRAS*) is mutated in 40-50% of human colorectal adenomas and carcinomas and plays key roles in cell proliferation, apoptosis, motility and differentiation, but its functional contribution to intestinal tumourigenesis *in vivo* remains incompletely understood. We have previously crossed K-*ras*^{Val12} transgenic mice with Ah-*Cre* mice to produce K-*ras*^{Val12}/*Cre* offspring that inducibly express K-*ras*^{Val12} 4A and 4B in the intestines, but this alone showed no significant effect on intestinal adenoma formation. Here, we crossed these mice with Min mice to evaluate the effect of K-*ras*^{Val12} and *Apc* mutation on intestinal tumourigenesis *in vivo*. The double mutant K-*ras*^{Val12}/*Cre*/*Apc*$^{Min/+}$ mice showed a moderate (1.86-fold) increase in adenomas in the small intestines, but a striking acceleration (6-fold increase) of large intestinal adenoma formation ($P<0.01$) and significantly reduced survival (by ~5 weeks) compared with control *Apc*$^{Min/+}$ mice ($P<0.01$). There was recombination of the mutant K-*ras*^{Val12} transgene in 80% of large intestinal adenomas with expression of both K-*ras*^{Val12} 4A and 4B isoform transcripts and expression of K-Ras^{Val12} protein. The large intestinal adenomas showed immunohistochemical evidence of activation of MapK, Akt and Wnt signaling pathways and this was confirmed by quantitative RT-PCR analysis of relative transcript expression levels of target genes using a panel of 23 selected genes evaluated in both adenomas and non-tumour-bearing intestines. Several genes including *Tiam1*, *Gastrin*, *CD44*, *uPA*, *Igfbp4*, *VEGF*

Ah-Cre beta-naftoflavone inducible
Kras +/LSL-G12V
ApcMin



Ah-Cre beta-naftoflavone inducible
Kras +/LSL-G12V
ApcMin



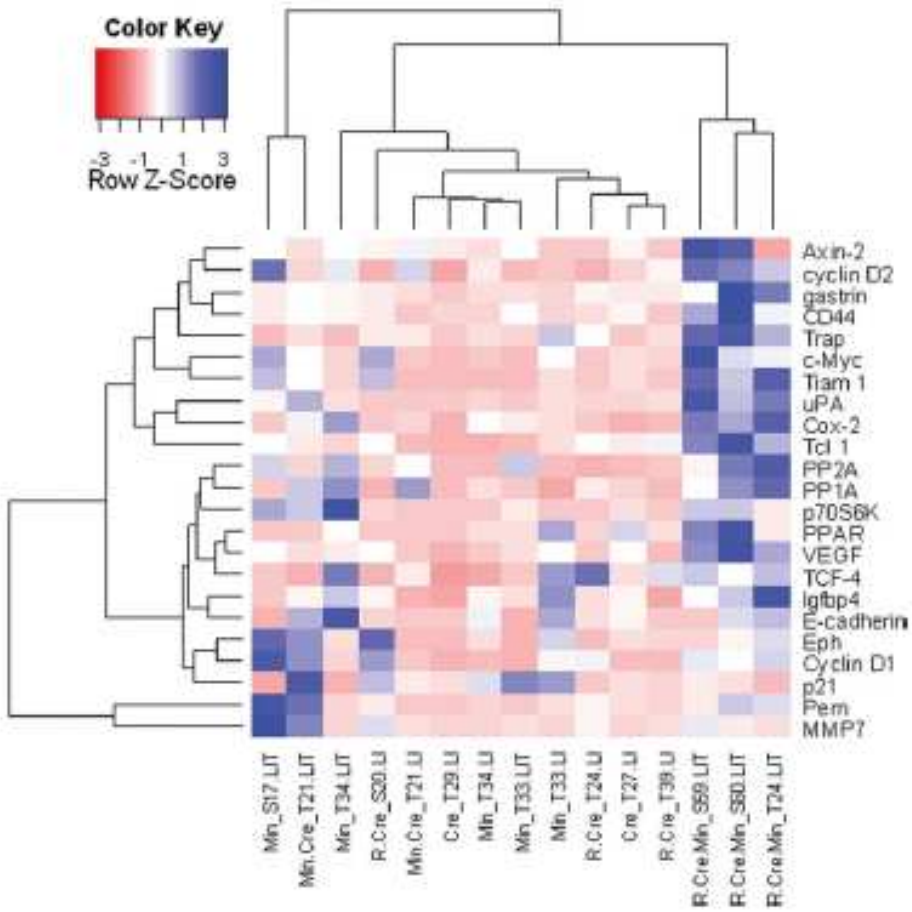


Figure 5. Unsupervised hierarchical clustering analysis of differentially expressed genes in non-neoplastic colonic tissues and large intestinal tumours. Relative RNA expression levels determined by quantitative RT-PCR amplification of 23 selected genes (right) from either morphologically normal large intestinal tissues (LI) or large intestinal tumours (LIT) (bottom), were analysed by unsupervised hierarchical clustering to show grouping of LIT from K-ras^{Val12}/Cre/$Apc^{Min/+}$ mice (R.Cre.Min) on the right of the heatmap and LIT from $Apc^{Min/+}$ mice (Min) or Cre/$Apc^{Min/+}$ mice (Min.Cre) mostly on the left of the heatmap, with grouping of normal large intestine (LI) tissues from all cohorts in the middle of the heatmap. Red and blue cytobands indicate underexpressed and overexpressed genes respectively (key provided top left).

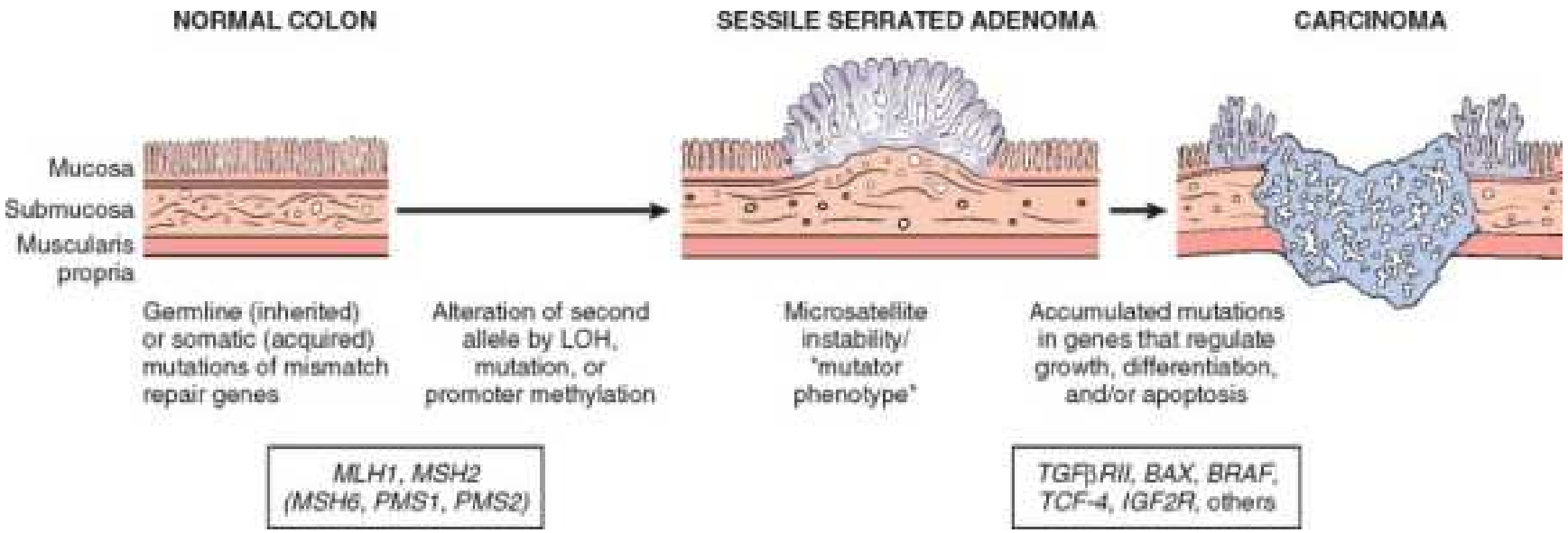

NORMAL COLON
SESSILE SERRATED ADENOMA
CARCINOMA
Mucosa
Submucosa
Muscularis propria
Germline (inherited) or somatic (acquired) mutations of mismatch repair genes
Alteration of second allele by LOH, mutation, or promoter methylation
Microsatellite instability/ "mutator phenotype"
Accumulated mutations in genes that regulate growth, differentiation, and/or apoptosis
MLH1, MSH2 (MSH6, PMS1, PMS2)
TGFβRII, BAX, BRAF, TCF-4, IGF2R, others

Table 2. *MMR* Mutant Mouse Lines

Mouse line	Tumor incidence	Tumor spectrum[a]				Repair defect (MSI)		DNA damage response[b]
		Gastrointestinal	Lymphoma	Skin	Other	Mononucleotide	Dinucleotide	
Knockout mouse lines								
MutS homologues								
Msh2$^{-/-}$	High	√	√	√	√	High	High	Defective
Msh3$^{-/-}$	Low	√	—	—	√	Moderate	High	Normal
Msh6$^{-/-}$	High	√	√	√	√	None	Low	Defective
Msh3$^{-/-}$ *Msh6*$^{-/-}$	High	√	√	√	√	High	High	Defective
Msh4$^{-/-}$	None	—	—	—	—	N/A	N/A	N/A
Msh5$^{-/-}$	None	—	—	—	—	N/A	N/A	N/A
Msh2$^{loxP/loxP}$; *Vill-cre*	High	√	—	—	—	High	High	Defective
MutL homologues								
Mlh1$^{-/-}$	High	√	√	√	√	High	High	Defective
Pms1$^{-/-}$	None	—	—	—	—	Low	Low	N/A
Pms2$^{-/-}$	High	—	√	√	√	High	High	Defective
Mlh3$^{-/-}$	High	√	√	√	√	Moderate	N/A	Defective
Pms2$^{-/-}$ *Mlh3*$^{-/-}$	High	√	√	—	—	High	N/A	Defective
Exonuclease								
Exo1$^{-/-}$	Moderate	—	√	—	—	High	Low	Defective
Knock-in mouse lines								
Msh2$^{G674A/G674A}$	High	√	√	√	√	High	High	Normal
Msh6$^{T1217D/T1217D}$	High	√	√	√	√	High	High	Normal
Mlh1$^{G67R/G67R}$	High	√	√	√	√	High	High	Normal

IBD-related CRC models

Inflammation-related colorectal cancer	DSS-induced mouse models	Easy and reproducible. Tumor incidence is low. AOM/DSS combination produces more tumors at earlier time point.
	IL10$^{-/-}$, *IL2*$^{-/-}$, T-cell receptor$^{-/-}$/*p53*$^{-/-}$ or *TGF-1*$^{-/-}$/*Rag-2*$^{-/-}$	Tumor incidence is low. Requires the involvement of enteric microflora.
	Muc2$^{-/-}$	High incidence of colon and rectal tumors. Early development of rectal prolapse reduces life span.

Human disease	Mouse model	Advantages and disadvantages
FAP	*Apc* mutants or β-catenin transgenic mice	Mimic APC mutation in human. However, most tumors located in the small intestine. Tumors are not metastatic.
HNPCC	*Msh2*$^{-/-}$, *Msh6*$^{-/-}$, and *Mlh1*$^{-/-}$ mice	Mimic MMR deficiency in human. However, MMR-deficient mice develop tumors in other organs. The colonic tumors are not metastatic.

Development of a mouse model for sporadic and metastatic colon tumors and its use in assessing drug treatment

Kenneth E. Hung[a,1], Marco A. Maricevich[b], Larissa Georgeon Richard[c], Wei Y. Chen[a], Michael P. Richardson[a], Alexandra Kunin[b], Roderick T. Bronson[d], Umar Mahmood[b], and Raju Kucherlapati[c]

PNAS | January 26, 2010 | vol. 107 | no. 4 | 1565–1570

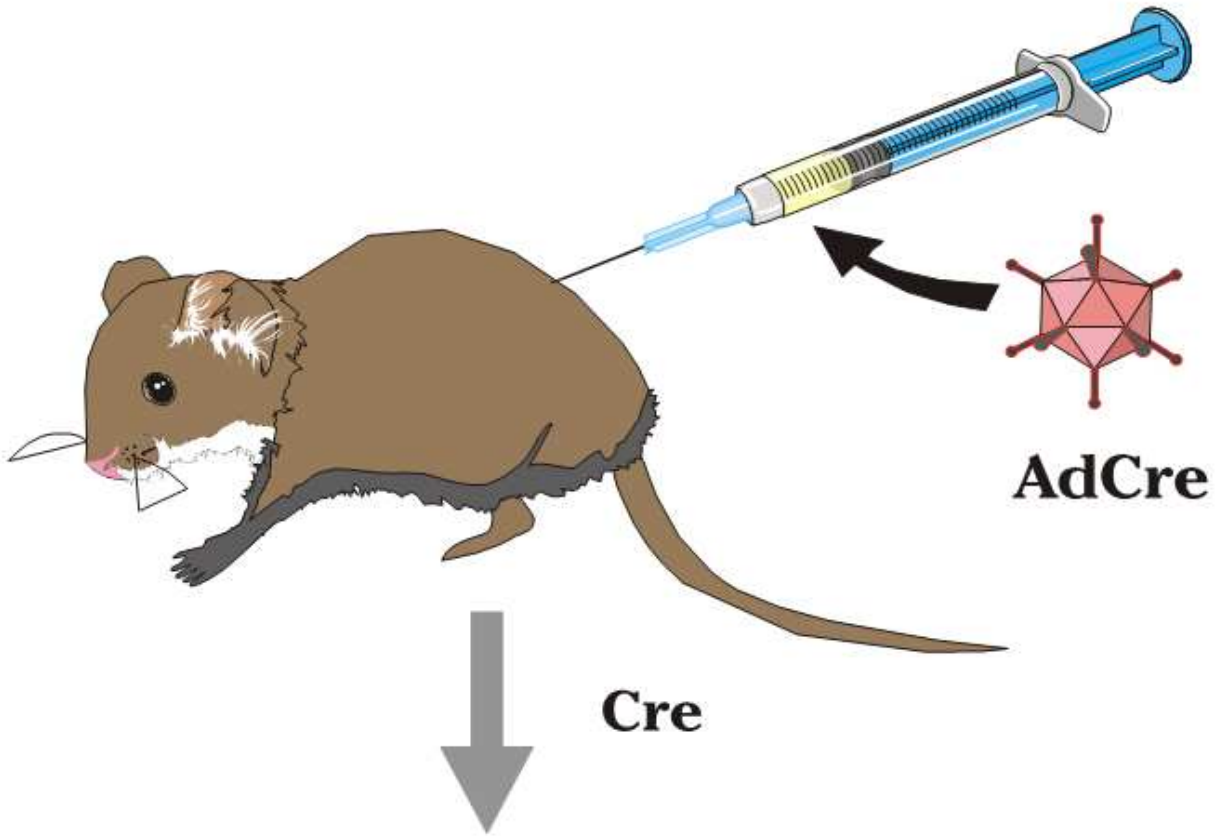
Most genetically engineered mouse (GEM) models for colon cancer are based on tissuewide or germline gene modification, resulting in tumors predominantly of the small intestine. Several of these models involve modification of the adenomatous polyposis coli (*Apc*) gene and are excellent models for familial cancer predisposition syndromes. We have developed a stochastic somatic mutation model for sporadic colon cancer that presents with isolated primary tumors in the distal colon and recapitulates the entire adenoma–carcinoma–metastasis axis seen in human colon cancer. Using this model, we have analyzed tumors that are either solely mutant in the *Apc* gene or in combination with another colon cancer-associated mutant gene, the *Kras* G12D allele. Because of the restricted location in the distal colon, the natural history of the tumors can be analyzed by serial colonoscopy. As the mammalian target of rapamycin (mTOR) pathway is a critical component of the complex signaling network in colon cancer, we used this model to assess the efficacy of mTOR blockade through rapamycin treatment of mice with established tumors. After treatment, *Apc* mutant tumors were more than 80% smaller than control tumors. However, tumors that possessed both *Apc* and *Kras* mutations did not respond to rapamycin treatment. These studies suggest that mTOR inhibitors should be further explored as potential colorectal cancer therapies in patients whose tumors do not have activating mutations in KRAS.

A true sporadic model for colon cancer should have the following features: (*i*) the model develops one or two tumors in the colon, (*ii*) the tumors derive from somatic modification of genes known to be involved in human colorectal cancer, (*iii*) the somatic mutations involve the colonic epithelium, and (*iv*) the tumors present along the entire adenoma–carcinoma–metastasis axis. Mice with conditional mutations in *Apc* are an excellent starting point for developing such models.

Delivery of adenovirus expressing cre recombinase (adeno-cre) to conditional knockout mice is an attractive approach, as the spatial and temporal sequence of gene modification(s) can be controlled (6). This approach has been used to focally modify critical carcinogenic genes in lung, liver, ovarian, and other mouse cancer models (7–12). Colon tumorigenesis using rectal adeno-cre enemas in mice carrying floxed *Apc* alleles has been described, but we and other groups have found that the incidence, multiplicity, and location of the intestinal tumors can be highly variable in this model (13).

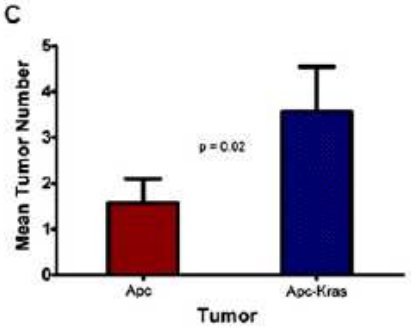
In this report, we describe a unique surgical procedure to limit adeno-cre infection to the most distal colon, resulting in highly penetrant tumor formation (14). These tumors present with the full spectrum of adenomas, invasive carcinoma, and metastases.

TRANSGENICS CONTAINING GENES CONTROLLED BY A MOLECULAR SWITCH



Stocastic AdCre-infected cell Apc KO RAS mut

Adeno-Cre Treatment of Apc CKO/LSL-Kras Mice Results in Advanced Primary and Metastatic Colon Tumors. To assess whether the incorporation of an activated *Kras* gene would alter tumor progression in our mouse model, we generated mice that were homozygous for the Apc CKO allele and heterozygous for a latent activated allele of *Kras* (*Kras*$^{tm4tyj/+}$) (Apc CKO/LSL-Kras) (7). The distal colons of these mice were treated with 10^9 pfu of adeno-cre in 100 μL PBS. As with the Apc CKO mice, the induced Apc CKO/LSL-Kras mice presented with primary tumors exclusively in the distal colon. Of the 55 mice that were infused with adeno-cre, we were able to detect tumors, by colonoscopy (see below), in 53 (96%) of the mice in as little as 3 weeks after viral administration, with an average tumor burden of 3.6 lesions per mouse. Tumor histology was assessed using the same criteria as was used for tumors from the Apc CKO mice. Of the 42 tumors examined, 27 (64%) were adenomas and the remaining 15 (36%) were carcinomas. However, carcinomas first presented 20 weeks after adeno-cre injection. Of the 30 tumors that were examined after this time, 15 (50%) were carcinomas (Fig. 2 *A–C*). Furthermore, spontaneous gross liver metastases were noted starting 24 weeks after adeno-cre injection (Fig. 2*D*). Of the 25 mice that were examined after this time, 5 (20%) mice showed these lesions. Upon histological examination, these lesions were classified as adenocarcinomas (Fig. 2*E*). To confirm that the metastatic tumors were of intestinal origin, both primary and metastatic tumors were stained with the intestinal-specific transcription factor cdx-2 (Fig. 2 *F* and *G*). Wnt activation was noted in metastatic tumors by nuclear β-catenin staining. (Fig. 2*H*). These results suggest that the addition of an activated *Kras* allele can accelerate tumor progression and lead to eventual metastasis.



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Smad3 Mutant Mice Develop Metastatic Colorectal Cancer

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Summary

TGFβ-related growth factors have been implicated in a variety of developmental and physiological processes in organisms ranging from nematodes to mammals. TGFβ transduces its signal to the interior of the cell via Smad2, Smad3, and Smad4. We report the cloning and targeted disruption of the mouse *Smad3* gene. *Smad3* mutant mice are viable and fertile. Between 4 and 6 months of age, the *Smad3* mutant mice become moribund with colorectal adenocarcinomas. The neoplasms penetrate through the intestinal wall and metastasize to lymph nodes. These results directly implicate TGFβ signaling in the pathogenesis of colorectal cancer and provide a compelling animal model for the study of human colorectal cancer.

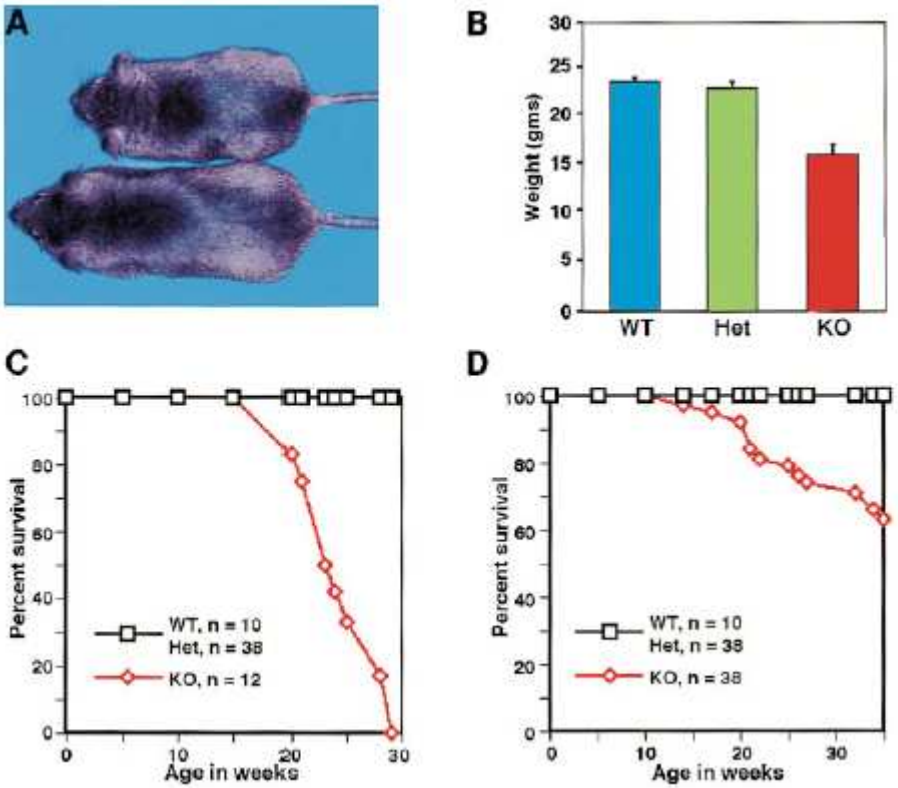


Figure 4. *Smad3* Homozygous Null Mice Are Small and Have Decreased Survival

(A) Photograph of a heterozygous and a representative inbred homozygous (top) *Smad3* mutant at 5 months of age.

(B) Weight plots of 2-month-old male 129/Sv mice. Wild-type (WT, n = 10), heterozygous (Het, n = 9), and homozygous (KO, n = 6). gms, grams.

(C and D) Survival curves of inbred (C) and hybrid (D) *Smad3* mice.

Helicobacter Infection Is Required for Inflammation and Colon Cancer in Smad3-Deficient Mice

Lillian Maggio-Price,[1] Piper Treuting,[1] Weiping Zeng,[1] Mark Tsang,[1] Helle Bielefeldt-Ohmann,[2] and Brian M. Iritani[1,3]

Table 1. Tumor incidence in the *Helicobacter*-infected and uninfected broth SMAD3$^{-/-}$ mice

Study	SMAD3$^{-/-}$ plus broth	% Tumor incidence	SMAD3$^{-/-}$ plus *Helicobacter*	Bacteria*	% Tumor incidence	Time to tumor development
1	0/8	0	5/9	Hb; Hh	56	10-22 wk
2a	0/6	0	4/6	Hb; Hb	66	7-26 wk
2b	—	0	4/6	Hb; Hh	66	23-30 wk
3	0/10	0	5/10	Hb; Hn	50†	5-14 wk
4	0/9	0	2/9	Hb	22†	8-12 wk
5	NA	0	NA	none	0	Aging
6 Set 1	0/5	0	0/5	Hb; Hh	0	Inflammation evaluation
6 Set 2	0/5	0	3/5	Hb; Hh	60†	12-14 wk

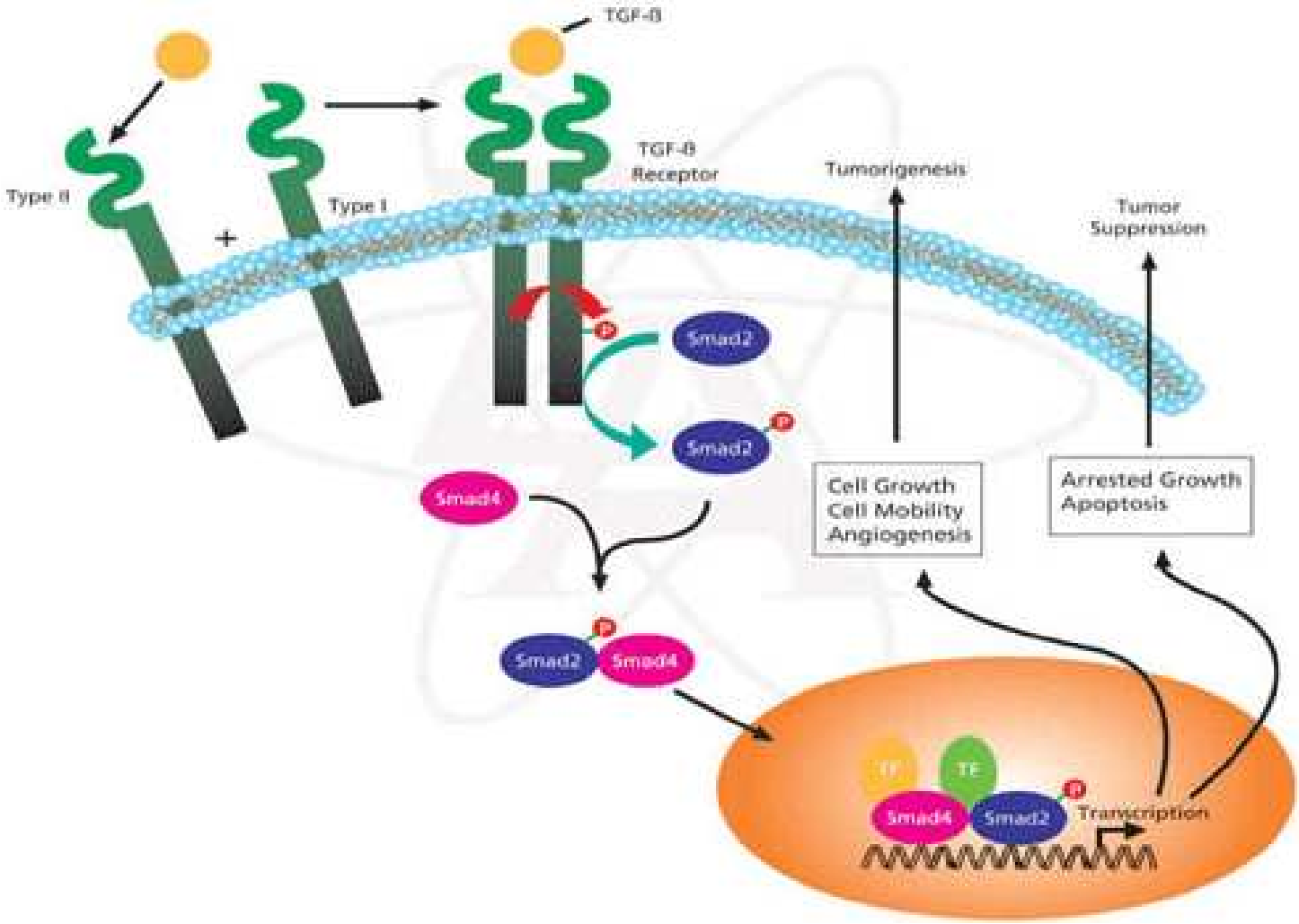
NOTE: Studies 1 to 4 contained 11 to 19 SMAD3$^{+/-}$ and SMAD3$^{+/+}$ mice; no tumors were noted in *Helicobacter*-infected and broth SMAD3$^{+/-}$ and SMAD3$^{+/+}$ mice. Study 5 contained 11 SMAD3$^{-/-}$, 19 SMAD3$^{+/-}$, and 11 SMAD3$^{+/+}$ mice. Study 6 contained 30 SMAD3$^{-/-}$, 31 SMAD3$^{+/-}$, and 7 SMAD3$^{+/+}$ mice. Study 7 contained 6 broth *Apc* $^{Min/+}$ mice and 6 Hb/Hh-infected *Apc* $^{Min/+}$ mice (data not shown).

Abbreviations: Hb, *H. bilis*; Hh, *H. hepaticus*; Hn, novel *Helicobacter* spp.; NA, not available.

*Mice were gavaged with 2×10^7 organisms twice within 1 week (study 4) and again 1 week later (studies 1, 3, and 6), and the infection regimen was repeated again 1 month later (studies 2a and 2b).

†Does not reflect true incidence because animals were terminated at early time points to obtain tissues.

-<u>perdita di SMADs</u>: una perdita allelica comune nel cancro CR è sul cromosoma 18q21. SMAD2 e SMAD4 sono coinvolti nel segnale del TGF-β, la loro perdita aumenta la tumorigenesi gastrointestinale.



GASTROENTEROLOGY 2009;136:1680–1688

TGF-β Receptor Inactivation and Mutant *Kras* Induce Intestinal Neoplasms in Mice via a β-Catenin-Independent Pathway

PATTY TROBRIDGE,[*] SUE KNOBLAUGH,[‡] M. KAY WASHINGTON,[§] NINA M. MUNOZ,[*] KAREN D. TSUCHIYA,[*,‖,¶] ANDRES ROJAS,[*,#] XIAOLING SONG,[**] CORNELIA M. ULRICH,[**] TAKEHIKO SASAZUKI,[‡‡] SENJI SHIRASAWA,[‡‡] and WILLIAM M. GRADY[*,§§,‖‖]

Methods: We analyzed intestinal tumors that arose in mice that express an oncogenic (active) form of *Kras* and that have *Tgfbr2* inactivations—2 common molecular events observed in human colorectal tumors. *LSL-KrasG12D* mice were crossed with *Villin*-Cre;*Tgfbr2E2flx/E2flx* mice, which do not express Tgfbr2 in the intestinal epithelium. ***Results:*** Neither inactivation of *Tgfbr2* nor expression of oncogenic *Kras* alone was sufficient to induce formation of intestinal neoplasms. Histologic abnormalities arose in mice that expressed *Kras*, but only the combination of Tgfbr2 inactivation and Kras activation led to intestinal neoplasms and metastases. The cancers arose via a β-catenin-independent mechanism; the epidermal growth factor-signaling pathway was also activated. Cells in the resulting tumors proliferated at higher rates, expressed decreased levels of p15, and expressed increased levels of cyclin D1 and cdk4, compared with control cells.

Conclusions: A combination of inactivation of the TGF-β-signaling pathway and expression of oncogenic Kras leads to formation of invasive intestinal neoplasms through a β-catenin-independent pathway; these adenocarcinomas have the capacity to metastasize.

Table 1. Tumor Incidence in KVcT$^{wt/wt}$ and KVcTT Mice

Genotype	Number of mice with tumors	Average number of tumors per mouse[a]	Total number of adenomas (small intestine:colon)	Total number of adenocarcinomas (small intestine:colon)[b]	Total number of tumors[b]
Villin-Cre; LSL-Kras^{G12D};Tgfbr2$^{wt/wt}$ (KVcT$^{wt/wt}$) (n = 20)	0	0	0:0	0:0	0
LSL-Kras^{G12D}; Tgfbr2 ^{IEKO} (KVcTT) (n = 21)	15	2.4	3:8	8:17	36

[a]Only mice with tumors included in this calculation.
[b]$P = .0005$, Student *t* test.

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REVIEWS: CURRENT TOPICS

Mouse models for unraveling the importance of diet in colon cancer prevention

Alexandra E. Tammariello[a], John A. Milner[b,*]

Abstract

Diet and genetics are both considered important risk determinants for colorectal cancer, a leading cause of death worldwide. Several genetically engineered mouse models have been created, including the Apc^{Min} mouse, to aid in the identification of key cancer related processes and to assist with the characterization of environmental factors, including the diet, which influence risk. Current research using these models provides evidence that several bioactive food components can inhibit genetically predisposed colorectal cancer, while others increase risk. Specifically, calorie restriction or increased exposure to n-3 fatty acids, sulforaphane, chafuroside, curcumin and dibenzoylmethane were reported protective. Total fat, calories and all-trans retinoic acid are associated with an increased risk. Unraveling the importance of specific dietary components in these models is complicated by the basal diet used, the quantity of test components provided and interactions among food components. Newer models are increasingly available to evaluate fundamental cellular processes, including DNA mismatch repair, immune function and inflammation as markers for colon cancer risk. Unfortunately, these models have been used infrequently to examine the influence of specific dietary components. The enhanced use of these models can shed mechanistic insights about the involvement of specific bioactive food and components and energy as determinants of colon cancer risk. However, the use of available mouse models to exactly represent processes important to human gastrointestinal cancers will remain a continued scientific challenge.
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Table 1
Dietary components with inhibitory and stimulatory effects on small intestinal tumors in the Apc^{Min} mouse

I. Inhibitory			
Food component	Basal diet[a]	Mean difference	Reference
Bilberry (10%)	AIN-93G	40%	Misikangas et al. [28]
Bowman-Birk Inhibitor	AIN-76A		Kennedy et al. [29]
(.1%)		44%	
(.5%)		39%	
Caffeic-acid phenethyl ester	AIN-76A	63%[b]	Mahmoud et al. [30]
Calorie restriction (60% calories of control)	AIN-76A	60%	Mai et al. [31]
Cellulose	AIN-93G with 20% soybean oil+no fiber		Yu et al. [32]
(5%)		77%	
(10%)		42%	
Chafuroside (10 ppm)	AIN-76A	44%	Niho et al. [33]
Cloudberry (10%)	AIN-93G	34%	Misikangas et al. [28]
Copper (6 ppm)	AIN-93G with 1 ppm copper	48%[c]	Davis et al. [34]
Curcumin			
(.01%)	AIN-76A	64%[b]	Mahmoud et al. [30]
(.2%)	RM3	42%[b]	Perkins et al. [35]
Cyanidin-3-glucoside (.3%)	AIN-93G	51%[d]	Cooke et al. [36]

Dibenzoylmethane (1%)	AIN-76A	49%[c]	Shen et al. [37]
EGCG (.16%)+fish oil (12%)	AIN-76A	53%	Bose et al. [38]
Eicosapentaenoic acid (31 g/kg)	US-17	48%	Petrik et al. [39]
Flaxseed (15%)	AIN-93G	31%	Oikarinen et al. [40]
Guar gum fiber	AIN-93G with 20% soybean oil+no fiber		Yu et al. [32]
(5%)		57%	
(10%)		30%	
Hydroxsymatairesinol (.02%)	Modified AIN-936+2.5% inulin	32%	Oikarinen et al. [41]
Ligonberry (10%)	AIN-93G	42%	Misikangas et al. [28]
Mirtoselect (.3%)	AIN-93G	37%[d]	Cooke et al. [36]
Selenium-enriched broccoli (2.2 g/kg)	AIN-93G+2.2 g/kg low selenium broccoli	29%[c]	Davis et al.[42]
Stearidonic acid (31 g/kg)	US-17	45%	Petrik et al. [39]
Sulforaphane			
(300 ppm)	AIN-76A	25.3%	Hu et al. [43]
(600 ppm)	AIN-76A	47%	Shen et al. [37]
(600 ppm)		47%[c]	
Tricin (.2%)	AIN-93G	36%[c]	Cai et al. [44]

Table 1 (*continued*)

I. Inhibitory

Food component	Basal diet[a]	Mean difference	Reference
Wheat Bran Fiber	AIN-93G with 20% soybean oil+no fiber		Yu et al. [32]
(5%)		53%	
(10%)		43%	
Wheat Bran oil (2%)	AIN-93G	35%	Sang et al. [45]
White Currant (10%)	AIN-93G	37%	Rajakangas et al. [46]

II. Stimulatory

Food component	Basal diet	Mean difference	Reference
Apple pomace (20%)	RM1	32%	Mandir et al. [47]
Fat	R20		Wasan et al. [48]
(10%)		28%	
(15%)		47%	
Retinoic-acid (all trans) (10 g/kg)	AIN-76A	133%	Mollersen et al. [49]

[a] More information about the composition of the AIN-93G, AIN-76A, RM3, US17, RM1 and R20 diets is available [35,39, 41,48,50–52].

[b] Total tumors only, predominantly in the small intestine.

[c] Extrapolated from manuscript figure.

[d] Extrapolated from manuscript figure, total tumors only.

Table 2
Dietary components with inhibitory and stimulatory effects on colonic tumors in the Apc^{Min} mouse

I. Inhibitory

Food component	Basal diet[a]	Mean difference	Reference
Steridonic acid (31 g/kg)	US17	85%	Petrik et al. [39]
Sulforaphane (600 ppm)	AIN-76A	80%	Shen et al. [37]

II. Simtulatory

Food component	Basal diet	Mean difference	Reference
Fat	R20		Wasan et al. [48]
(10%)		207%	
(15%)		225%	
Retinoic acid (all-trans) (10 g/kg)	AIN-76A	500%	Mollersen et al. [49]
White currant (10%)	AIN-93G	268%	Rajakangas et al. [46]

[a] More information about the composition of the US-17, AIN-76A, R20 and AIN-93G diets is available at [39,48,50,51].

Table 3
Food components that have been reported to Influence intestinal tumors

Food component	Basal diet[a]	Small intestine Δ no.	Colon Δ no.	Reference
Alpha Linolenic acid (31 g/kg)	US17	+8%	−38%	Petrik et al. [39]
Anthocyanin (800 mg/l)	Modified AIN-93G	+24%	−15%	Kang et al. [53]
Apple Pomace (20%)	RM1		−10%	Mandir et al. [47]
Arachidonic Acid (15 g/kg)	AIN-93G+ 15 g/kg oleic acid	−29%	−50%	Petrik et al. [39]
Beef	AIN-93G	+50%	+80%	Mutanen et al. [54]
Bilberry (10%)	AIN-93G		+83%	Misikangas et al. [28]
Bovine lactoferrin	AIN-93G			Ushida et al. [55]
(.2%)		−15%	−11%	
(2%)		−20%	−23%	
Bowman-birk inhibitor	AIN-76A			Kennedy et al. [29]
(.1%)			−36%	
(.5%)			−38%	
Calcium	AIN-93G+ 1000 IU/kg Vitamin D			Huerta et al. [56]
(2.5 g/kg)		−8%	+10%	
(10 g/kg)		+22%	+15%	
Calorie restriction (60% calories of control)	AIN-76A		+40%	Mai et al. [31]
Chafuroside	AIN-76A			Niho et al. [33]
(2.5 ppm)		−17%	+10%	
(5 ppm)		−27%	−28%	
(10 ppm)			−4%	
Cherries (200 g/kg)	Modified AIN-93G	−27%	−10%	Kang et al. [53]
Cloudberry (10%)	AIN-93G		+50%	Misikangas et al. [28]
Conjugated linoleic acid (31 g/kg)	US17	+21%	−23%	Petrik et al. [39]
Conjugated linoleic acid isomer	RM1			Mandir et al. [57]
C9t11		+28%[b]	−51%[b]	
T10c12		−1%[b]	−61%[b]	
C9t11+t10c12		+12%[b]	−66%[b]	
Copper (6 ppm)	AIN-93G+ 1 ppm copper		+73%[b]	Davis et al. [34]
Curcumin				
(.1%)	RM3		+25%[b]	Perkins et al. [35]
(.2%)			−13%[b]	
Cyanidin-3-glucoside	AIN-93G			Cooke et al. [36]
(.03%)		−9.5%[c]		
(.1%)		−25%[c]		
Dibenzoylmethane (1%)	AIN-76A		−58%[c]	Shen et al. [37]
Docosahaenoic acid (31 g/kg)	US17	−38%	+8%	Petrik et al. [39]
EGCG				
(.08%)	AIN-93G	−57% (females)	−10% (males)	Ju et al. [58]
(.16%)	AIN-76A	−45% (males)		Bose et al. [38]
(.16%)		−18%		
Eicosapentaenoic acid (31 g/kg)	US17		−30%	Petrik et al. [39]
Fish oil (12%)	AIN-76A	+3%		Bose et al. [38]
Fish oil concentrate K85	AIN-76A			Paulsen et al. [59]
(.4%)		−39%	+5%	
(1.25%)		−26%	−40%	
(2.5%)		−37%	−55%	
Flaxseed (defatted) (.5%)	AIN-93G	−10%	−35%	Oikarinen et al. [60]
Folate	Amino acid defined diet with 2 mg/kg folate			Song et al. [61]
(0 mg/kg)		−68%		
(8 mg/kg)		−12%		
(20 mg/kg)		−67%		
Fruit and vegetable mixture (19.5%)	Muracon-SSP/tox	−18%	+48%	Van Kranen et al. [62]
Gamma-linolenic acid (31 g/kg)	US17	+25%	−15%	Petrik et al. [39]
Hydroxsymatairesinol (.02%)	Modified AIN-936+ 2.5% inulin		+55%	Oikarinen et al. [41]
Indole-3-carbinol	AIN-76A			Kim et al. [51]
(100 ppm)		−8%	−40%	
(300 ppm)		−5%	−6%	
Ligonberry (10%)	AIN-93G		−28%	Misikangas et al. [28]
Mirtoselect	AIN-93G			Cooke et al. [36]
(.03%)		−8%[c]		
(.1%)		−20%[c]		
Oat bran (10%)	AIN-93G	+33%		Mutanen et al. [54]
Resveratrol	AIN-93G			Zeigler et al. [63]
(4 mg/kg)		+6%[b]		
(20 mg/kg)		+13%[b]		
(90 mg/kg)		+18%[b]		
Rye bran				
(10%)	AIN-93G	−25%	−20%	Mutanen et al. [54]
(10%)	Modified AIN-936+ 2.5% inulin	−9%	+7%	Oikarinen et al. [60]
Tricin (.2%)	AIN-93G		+50%[b]	Cai et al. [44]
Wheat bran (10%)	AIN-93G	−1%	+20%	Mutanen et al. [54]
Wheat bran oil (2%)	AIN-93G	−35%	−38%	Sang et al. [45]
White currant (10%)	AIN-93G	−37%		Rajakangas et al. [46]

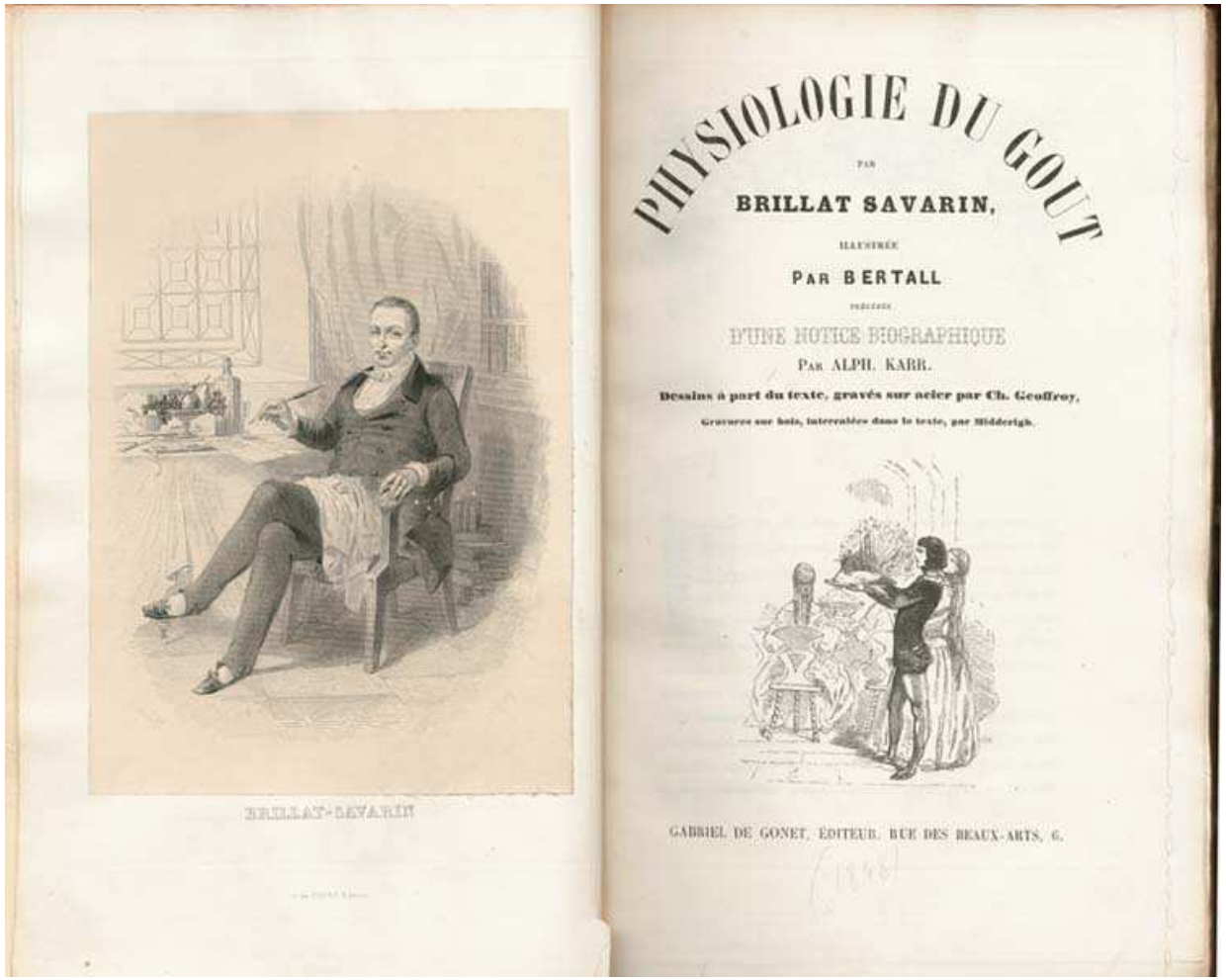
[a] More information about the composition of the US17, AIN-93G, RM1, AIN-76A, RM3, amino-acid defined and Muracon-SSP/tox diets is available [35,39,50–52,62,64].

[b] Extrapolated from manuscript figure.

[c] Extrapolated from manuscript figure, total tumors only predominantly in small intestine.

Stearidonic acid (SDA) is an ω-3 fatty acid

Sulforaphane is a molecule within the isothiocyanate group of organosulfur compounds. which is obtained from cruciferous vegetables such as broccoli, Brussels sprouts or cabbages



Dis-moi ce que tu manges, je te dirai ce que tu es

A partire da questi modelli si è verificato l'effetto di alcuni componenti della dieta
ESEMPI

1)Una dieta con un contenuto di calorie inferiore del 60% provoca una 60% di riduzione del numero di polipi del tenue (la riduzione delle calorie riduce la proliferazione cellulare, l'infiammazione e induce apoptosi)

2) Oltre alla restrizione calorica, il consumo di acidi grassi sembra ridurre il rischio di CRC (il ruolo chiave è giocato dal rapporto tra gli acidi grassi n-6 e n-3; quando il rapporto è basso i prodotti proinfiammatori che derivano da n-6 diminuisce e diminuisce pertanto la proliferazione cellulare. Inoltre un incremento di n-3 inibisce l'attività della COX2 e quindi diminuisce la produzione di agenti proinfiammatori)
La somministrazione di acido steraridonico e di acido eicosapentaenico (EPA) provoca una riduzione nel numero e nella grandezza dei tumori. La somministrazione di EPA riduce il numero dei tumori del tenue del 48% e del colon del 30%

3) Il solforano, componente delle crucifere, quando addizionato alla dieta, provoca una diminuzione del numero medio dei polipi intestinali (provoca una diminuzione dose-dipendente della proliferazione cellulare e induce apoptosi)

4) cafuroside: una flavone che deriva dal te provoca una significativa inibizione dei tumori intestinali poiché rimuove I radicali liberi, riduce l'infiammazione e induce apoptosi

5) Il curcumino e il CAPE (caffeic-acid phenethyl ester), fenoli di alcune piante, addizionati alla dieta diminuiscono di circa il 63% l'incidenza di adenomi intestinali

La combinazione di piu elementi inoltre è piu efficace della somministrazione indipendente:

1) Combinazione di sulforano e di dibenzoilmetano (DBM) derivato dalla liquerizia e di beta-diketone (analogo del curcumino), la combinazione è due volte piu efficace rispetto alla singola somministrazione

Al contrario:

1)Aumentando il contenuto di grassi del 15% si osserva un aumento del 47% del numero dei tumori

2) Il tipo di grasso ha un ruolo fondamentale in topi geneticamente predisposti: gli acidi grassi saturi promuovono il rischio di sviluppo di CRC, mentre quelli da fonti vegetali hanno effetto Inverso

3)l'acido retinoico all-trans, derivato dalla vitamina A sembra determinare un aumento nella formazione di tumori intestinali. I cibi che contengono una quantità esagerata di retinolo tendono ad avere anche un livello elevato di grassi (pertanto non si sa quale effetto sia prevalente)

Modelli immunocompetenti:

I modelli IL-2 e Il-10 knockout mostrano un'infiammazione spontanea che porta alla formazione di adenocarcinomi (simili a IBD che progredisce in CRC).

1) I folati e il ferro sono stati esaminati nei modelli IL-2 KO mice: contenuti scarsi o troppo elevati di questi due elementi sono associati a sviluppo di CRC

2) Il topo IL-10 KO è stato utilizzato per studiare l'impatto degli acidi grassi (olio di mais, di pesce e di oliva) sul CRC. Una diminuzione del rischio è stata osservata con ingestione di olio di oliva e di pesce

Vegetali

J. Nutr. 135:1879-1888 2005

Vegetables Affect the Expression of Genes Involved in Anticarcinogenic Processes in the Colonic Mucosa of C57Bl/6 Female Mice[1]

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Ruolo della dieta

Fino al 90% dei casi di CRC può essere prevenuto cambiando la dieta, in particolare somministrando una dieta con elevato contenuto di fibre vegetali. La via molecolare resta però sconosciuta.

Questa ricerca studia l'effetto di 4 tipologie vegetali, scelte perché rappresentano ciascuna una sottoclasse coinvolta nel meccanismo anticarcinogenico in diversi modi:

-piselli: (e fagioli) riduzione del tempo di transito nell'intestino, aumento della massa fecale e inibizione della proliferazione cellulare per diluizione degli acidi biliari che vengono comunque prodotti in quantità minore.

-cipolle: (e aglio) contengono composti organosolforici come dallyl-sulfide che può modificare l'attivazione dei carcinogeni per inibizione di alcuni enzimi di biotrasformazione e per induzione di enzimi detossificanti

-cavolfiori: (e crucifere in generale) inibiscono l'attivazione metabolica di procarcinogeni tramite isotiocianati

-carote: (e zucca) ricchi in antiossidanti come β- e α-carotene, hanno effetto *scavenger* nei confronti dei radicali liberi dell'ossigeno. Inoltre questi agenti inibiscono la proliferazione cellulare tramite up-regolazione della connexina 43, gene responsabile delle connessioni giunzionali intercellulari.

Modello animale

Topo C57B1/6 frequentemente usato negli studi di modulazione della dieta a livello molecolare (tra cui l'espressione genica), fornisce la base di numerosi modelli di topi transgenici che potrebbero essere usati in futuro.

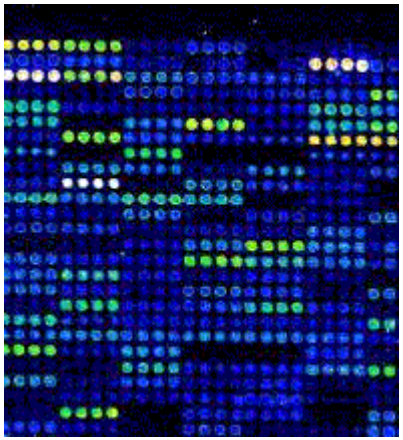
Somministrazione delle diete per 2 settimane:

Dieta 1: dieta di controllo senza vegetali (casein control diet)
diete contenenti miscela di: cavolfiori (30%), carote (30%), piselli (30%), cipolle (10%)
-Dieta 2 mistura di vegetali: contenente 100 g/kg (10% della dose)
-Dieta 3 mistura di vegetali: contenente 200 g/kg (20% della dose)
-Dieta 4 mistura di vegetali: contenente 400 g/kg (40% della dose)

-Dieta 5: contenente 70 g/kg di cavolfiori
-Dieta 6: contenente 73 g/kg di carote
-Dieta 7: contenente 226 g/kg di piselli
-Dieta 8: contenente 31 g/kg di cipolle

Eutanasia e asportazione del crasso. L'intestino viene poi posto in un contenitore apposito a 4° C. Dopo la rimozione del retto il colon è stato aperto longitudinalmente lungo tutta la lunghezza e sono state rimosse le feci e il muco. Le cellule della mucosa sono state incubate e poi rimosse con il bordo di un vetrino.
Le cellule sono poi state trasferite, omogeneizzate e conservate a -80° C.

microarray



Risultati:
La prima linea di difesa contro l'inizio di CRC è l'abilità dell'epitelio del colon di intercettare e di detossificare xenobiotici potenzialmente dannosi per il DNA o sostanze endogene.
I geni coinvolti nel metabolismo degli xenobiotici sono stati attivati dalla somministrazione delle diete contenenti le misture di vegetali:
-SULT1A1 (sulfotrasferasi 1A1)
-GSTA2 (glutatione S-trasferasi, α2)
-ALDH1A1 (aldeide deidrogenasi 1A1)
SULT1A1 e GSTA2 codificano entrambi per gli enzimi di biotrasformazione della fase II, il primo è coinvolto nel metabolismo delle amine aromatiche eterocicliche (HCA) e il secondo si combina con il glutatione.
Inoltre i vegetali attivano un altro gene detossificante ALDH1A1 che ossida l'acetaldeide in acido acetico e quindi protegge le cellule dagli effetti collaterali dell'acetaldeide (che è altamente tossica, mutagena e carcinogenica).

Un altro gene coinvolto è HPGD, espresso nel gruppo con la miscela maggiore di vegetali, che codifica per un enzima che metabolizza prostaglandine e composti non-prostanoidi i cui prodotti sono altamente reattivi (aldeidi e chetoni) e possono determinare carcinogenesi.

Si è osservata inoltre up-regolazione del gene RAD51, coinvolto nella riparazione del danno cellulare e anche di 7 geni (TNFRSF6, CASP4, CASP7, CASP3, CTSB, TMSB10, STAT1) coinvolti nell'apoptosi (cellule delle cripte danneggiate vengono rimosse prima che possano proliferare e dare origine a cellule neoplastiche).

SAT1: spermidine/spermine N1-acetyl transferasi 1 gene coinvolto nel metabolismo delle poliamine che sono correlate con un rischio elevato di CRC. La dieta 4 ha indotto un'up-regolazione di SAT1.

Conclusioni:

-La miscela vegetale induce l’espressione genica della mucosa del colon del topo con caratteristiche dose-dipendenti.

-L’espressione di un solo gene (CASP4), che potrebbe essere coinvolto nella prevenzione del CRC, è stata modulata dalla dieta con cavolfiori.

-La dieta ad elevato contenuto di miscela vegetale ha modulato geni coinvolti nei meccanismi protettivi e di riparazione con inibizione dei carcinogeni, riduzione della crescita cellulare e dell’invasione tumorale

Flora Microbica

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Sporadic colorectal cancer – role of the commensal microbiota

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Flora microbica del colon e normale funzione intestinale

-Il colon umano dopo la nascita è costituito per il 99% da anaerobi obbligati che colonizzano l'intestino in base all'interazione con l'ambiente.

-La flora batterica rimane più o meno costante per tutta l'età adulta e le perturbazioni sono essenzialmente dovute al cambiamento di dieta, all'uso di antibiotici e all'età.

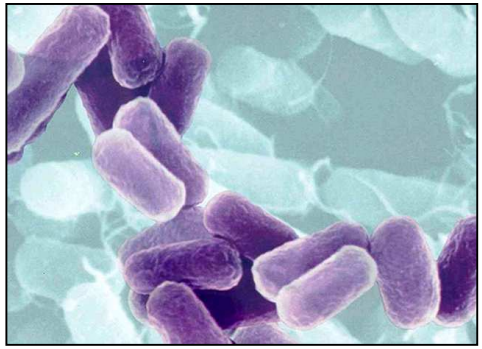
-I polisaccaridi che rimangono indigeriti ed entrano nel colon sono metabolizzati in acidi grassi a catena corta (SCFA): butirrato, acetato, propionato, dai batteri commensali e poi assorbiti per diffusione passiva

La produzione di SCFA dipende dal substrato disponibile: ad es. l'amido determina la formazione di butirrato.

Le concentrazioni di SCFA sono più elevate nel colon destro per la maggior presenza di carboidrati.

SCFA inoltre mantengono l'integrità dell'epitelio perché le cellule hanno a disposizione un'elevata quantità di energia. Nonostante che sia stato dimostrato in modelli animali che il butirrato induce apoptosi, arresto del ciclo cellulare e differenziazione cellulare neoplastica, è stato osservato che il CRC si riscontra più frequentemente a sinistra probabilmente perché, anche se i livelli di butirrato sono più bassi, il transito del materiale fecale è più ridotto.

-La flora batterica inoltre ha la capacità di resistere alla colonizzazione da parte di nuovi ceppi batterici (patogeni e non): *colonisation resistance* per competizione per il substrato e/o dei siti di adesione, tramite alterazione delle condizioni fisiologiche (potenziale redox, pH) e tramite produzione di sostanze tra cui le batteriocine, che creano un ambiente che inibisce gli altri batteri.



Disattivazione dei metaboliti attivi

Organismi come i lattobacilli, bifidobatteri e streptococchi appartengono al gruppo del LAB (lactic acid-producing bacteria) che portano benefici come stimolazione del sistema immunitario e inibizione della colonizzazione da parte di specie potenzialmente dannose. Nei modelli animali l'ingestione di LAB previene le lesioni pre-neoplastiche o i tumori e inoltre sono coinvolti nella detossificazione di numerosi carcinogeni con i PAH (idrocarburi policiclici aromatici) e le amine eterocicliche aromatiche. I meccanismi dell'inattivazione sono ancora sconosciuti. E' possibile che i LAB si leghino direttamente ai carcinogeni e li degradino, catalizzino le reazioni di detossificazione e producano metaboliti che portano alla detossificazione dei carcinogeni. I benefici sono stati dimostrati solo quando i LAB sono presenti in elevate quantità.

Infiammazione e flora batterica

Nell'infiammazione cronica del colon è coinvolta la flora batterica intestinale. Alcuni batteri sono in grado di indurre un'attivazione continua di linfociti T e B per cui si dice che il colon sia in uno stato di "infiammazione fisiologica". L'infiammazione cronica è caratterizzata da infiltrazione del tessuto danneggiato da parte di cellule mononucleate come macrofagi, linfociti e plasmacellule. I macrofagi giocano il ruolo più importante in quanto producono citochine, chemochine e ossido nitrico: questi mediatori sono la principale via di difesa contro il danno e l'invasione. Tuttavia un'attivazione macrofagica persistente può determinare un danno continuo. Gli individui con IBD sono a rischio elevato di sviluppare IBD-related cancer. I meccanismi di sviluppo dell' IBD-related cancer e CRC sono simili: mutazioni multiple, perdita allelica e instabilità cromosomica

ROI e carcinogenesi

I metaboliti attivi della flora batterica del colon sono responsabili della produzione di ROI (derivati della molecola dell'ossigeno: superossidi, perossido di idrogeno, acido ipocloridrico, radicale ossigeno). I ROI sono prodotti da tutte le cellule attraverso il normale metabolismo cellulare e possono reagire con lipidi e protidi producendo prodotti intermedi che reagiscono con il DNA. Possono inoltre indurre alterazioni del DNA come modificazioni delle basi. Questi effetti, associati ad un lento processo riparativo, possono portare ad instabilità cromosomica (mutazioni, delezioni etc…).

Modelli animali: *Knock-out mice e germ-free mice*

T-cell receptor beta-chain and p53 double-knockout, IL-10 knockout, SMAD4 con APC, TGFβ-1 e RAG2: molti di questi modelli hanno dimostrato che, sotto specifiche condizioni germ-free, la probabilità di sviluppare colite e tumore sono pari a 0.

Il più delle volte però gli studi sono stati condotti su monospecie batteriche e non tengono in considerazione le interazioni tra le varie specie di batteri e tra i batteri e l'ospite.

Gli studi condotti hanno dimostrato che ci sono colonie batteriche che agiscono come carcinogeniche e altre che hanno un'azione protettiva. Per es. *Streptococcus bovis* è in grado di indurre la formazione di ACF (aberrant crypt foci) nei ratti.

Indizi dagli studi clinici

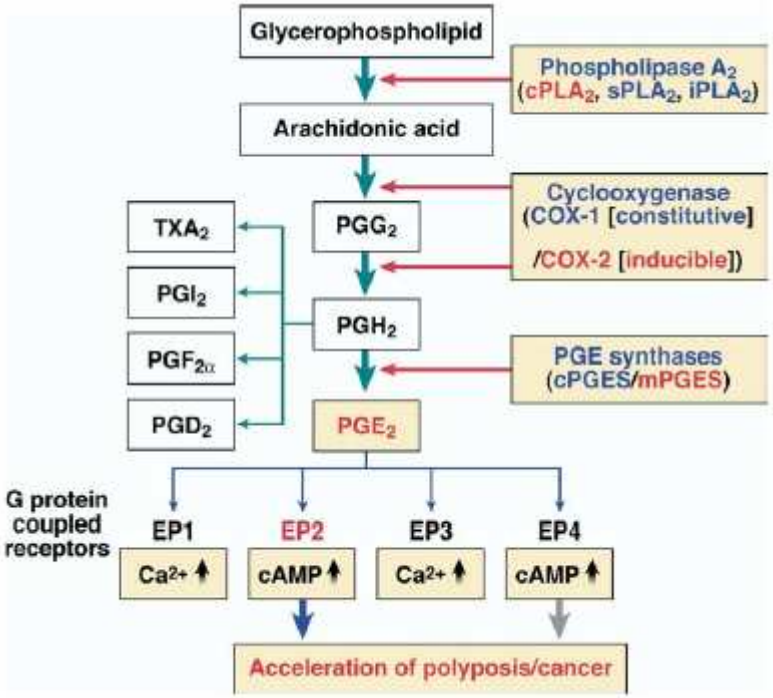
E' stata osservata la presenza di batteri intracellulari nella maggior parte di biopsie provenienti da adenoma e da CRC (soprattutto E. coli), ma non è stato dimostrato se la presenza di batteri è dovuta alla patologia o se è la causa della patologia.

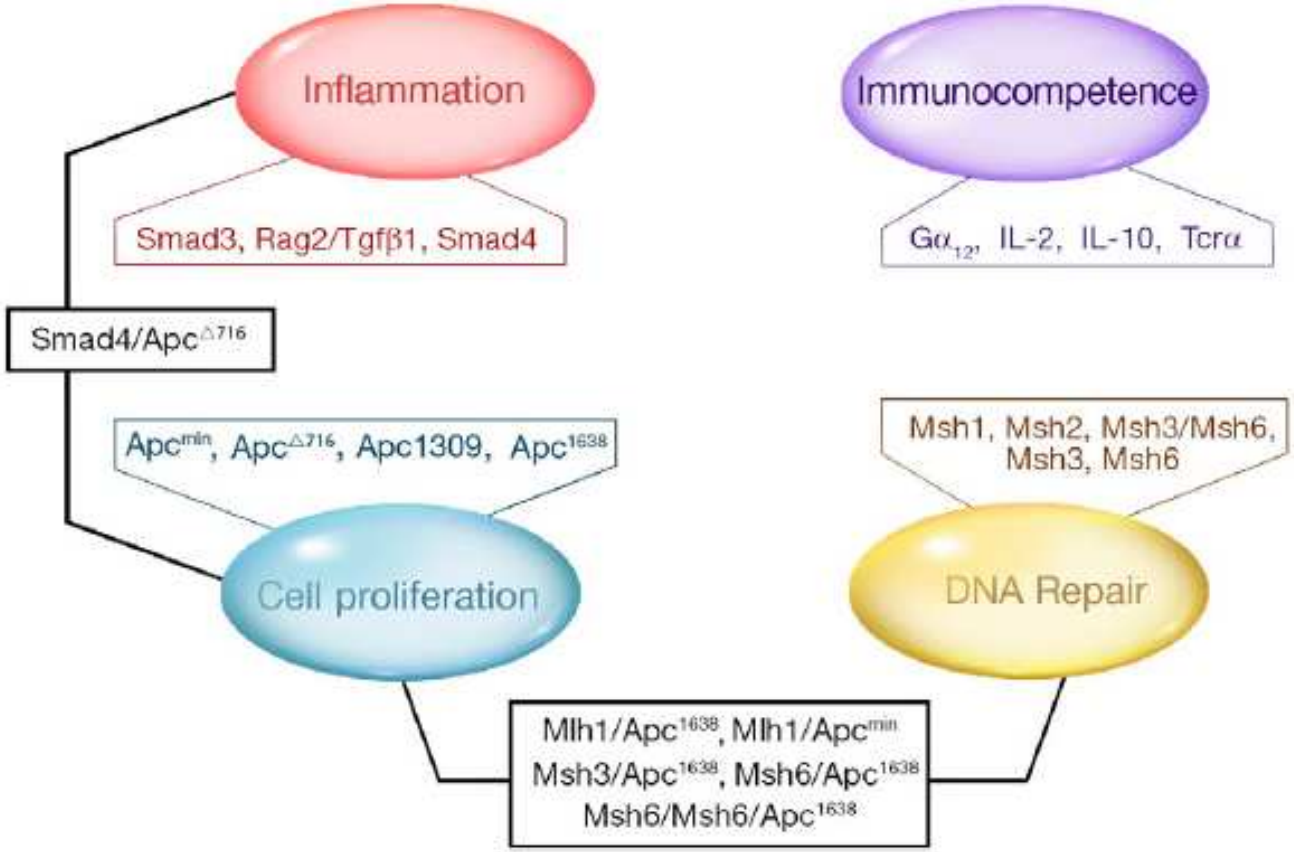
Alcuni studi hanno dimostrato che gli SRB (sulphate-reducing bacteria), normali residenti della mucosa del colon, potrebbero essere implicati nella carcinogenesi in quanto producono H_2S che può danneggiare la barriera epiteliale.

Effetti dei batteri intestinali sull'espressione di carboidrati da parte dell'ospite

La flora batterica influisce sulla quantità di muco, spessore e composizione dello strato di muco nel colon, inoltre i batteri, cambiando la distribuzione cellulare e subcellulare dei glicani, agiscono sui pattern di glicosilazione. Alterazioni della glicosilazione sulle cellule superficiali nel tessuto neoplastico sono ben documentati (topi, geneticamente deficienti per Muc2, la mucina più abbondante a livello GI, sono particolarmente predisposti per lo sviluppo di tumori GI)

Mutations that affect intestinal adenomas of *Apc*$^{+/-}$ mice		
Genes in the arachidonic acid pathway (gene product)		
Ptgs2 (COX-2)	(+/−) 34% and (−/−) 14%	Smaller adenomas
Ptgs1 (COX-1)	(+/−) 57% and (−/−) 23%	Smaller adenomas
Ptgerep2 (EP2)	(−/−) 58%	Smaller adenomas
Pla2g4a (cPLA2)	(+/−) 100% and (−/−) 85%	Smaller adenomas
Ptges (mPGES-1)	(−/−) 34%	Smaller adenomas




Inflammation
Smad3, Rag2/Tgfβ1, Smad4
Immunocompetence
Gα12, IL-2, IL-10, Tcrα
Smad4/Apc△716
Apcmin, Apc△716, Apc1309, Apc1638
Msh1, Msh2, Msh3/Msh6,
Msh3, Msh6
Cell proliferation
DNA Repair
Mlh1/Apc1638, Mlh1/Apcmin
Msh3/Apc1638, Msh6/Apc1638
Msh6/Msh6/Apc1638

Ruolo della dieta

-basso contenuto di fibre vegetali: diminuita massa fecale, aumentato tempo di transito nell'intestino e flora batterica alterata

-eccesso di *intake* calorico rispetto ai fabbisogni:

-elevato contenuto di carboidrati: i prodotti dell'ossidazione dei carboidrati, potenzialmente tossici, derivanti dall'azione dei batteri, sono presenti in quantità elevate nelle feci e rimangono a lungo a contatto con la mucosa del colon

-carni rosse: un'elevata quantità di colesterolo derivante dalla carne rossa aumenta la sintesi di acidi biliari da parte del fegato, che vengono a loro volta convertiti in potenziali carcinogeni da parte della flora batterica intestinale

-diminuito *intake* di micronutrienti protettivi: basso contenuto in vitamina A, C e E, che agiscono come *scavengers* dei radicali dell'ossigeno

Alcuni studi epidemiologici hanno dimostrato che l'aspirina e altri NSAIDs hanno un ruolo protettivo contro il cancro al colon, probabilmente per inibizione della ciclossigenasi -2, enzima sovraespresso nell'epitelio neoplastico che sembra essere coinvolto nell'apoptosi e nella neoangiogenesi.
Per questo motivo la FDA ha approvato l'uso preventivo di inibitori della COX-2 nei pazienti con sindrome familiare adenomatosa-poliposa.