




LE TAPPE VERSO IL CANCRO



QUANTE
ALTERAZIONI
SEQUENZIALI SONO
NECESSARIE PER
SVILUPPARE UNA
NEOPLASIA?

Mutazioni nel carcinoma del colon-retto

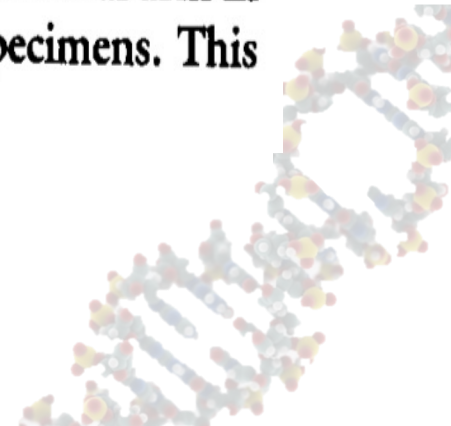
Allelotype of Colorectal Carcinomas

BERT VOGELSTEIN,* ERIC R. FEARON, SCOTT E. KERN,
STANLEY R. HAMILTON, ANN C. PREISINGER, YUSUKE NAKAMURA,
RAY WHITE



Uso di polimorfismi per cercare LOH nei tumore colon-rettali rispetto a tessuti normali e identificare geni mutati nel tumore

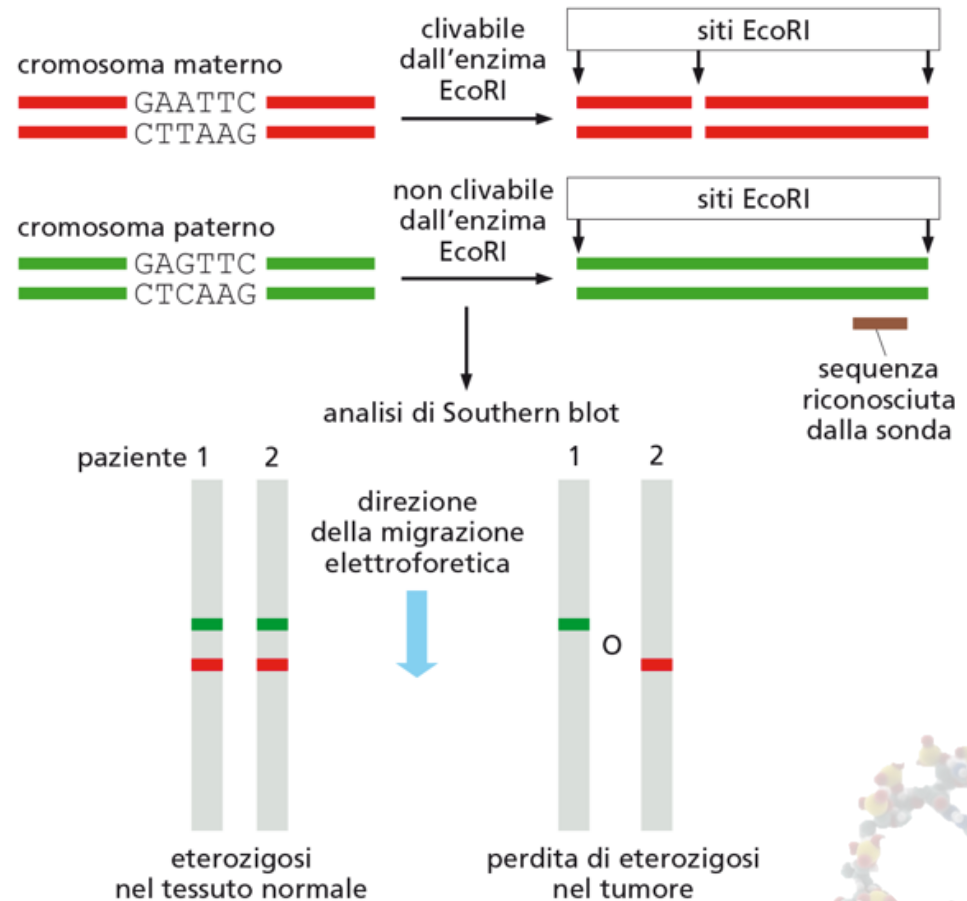
To examine the extent and variation of allelic loss in a common adult tumor, polymorphic DNA markers were studied from every nonacrocentric autosomal arm in 56 paired colorectal carcinoma and adjacent normal colonic mucosa specimens. This analysis was termed an allelotype, in analogy with a karyotype.



Mutazioni nel carcinoma del colon-retto

(A)

DNA was purified from cryostat sections of 56 primary colorectal carcinomas removed at surgery and compared to the DNA from normal colonic tissue of the same patients. Probes detecting RFLPs were used to determine whether one of the two parental alleles detected by each probe was specifically lost in the DNA from the tumor cells. All nonacrocentric autosomal arms were studied; the only genes known to be present on the acrocentric arms (13p, 14p, 15p, 21p, and 22p) are ribosomal. For each of these 39 chromosomal arms, enough probes were used to ensure that the two parental alleles could be distinguished in the normal tissue of at least 20 patients (that is, the informative patients).



Mutazioni nel carcinoma del colon-retto

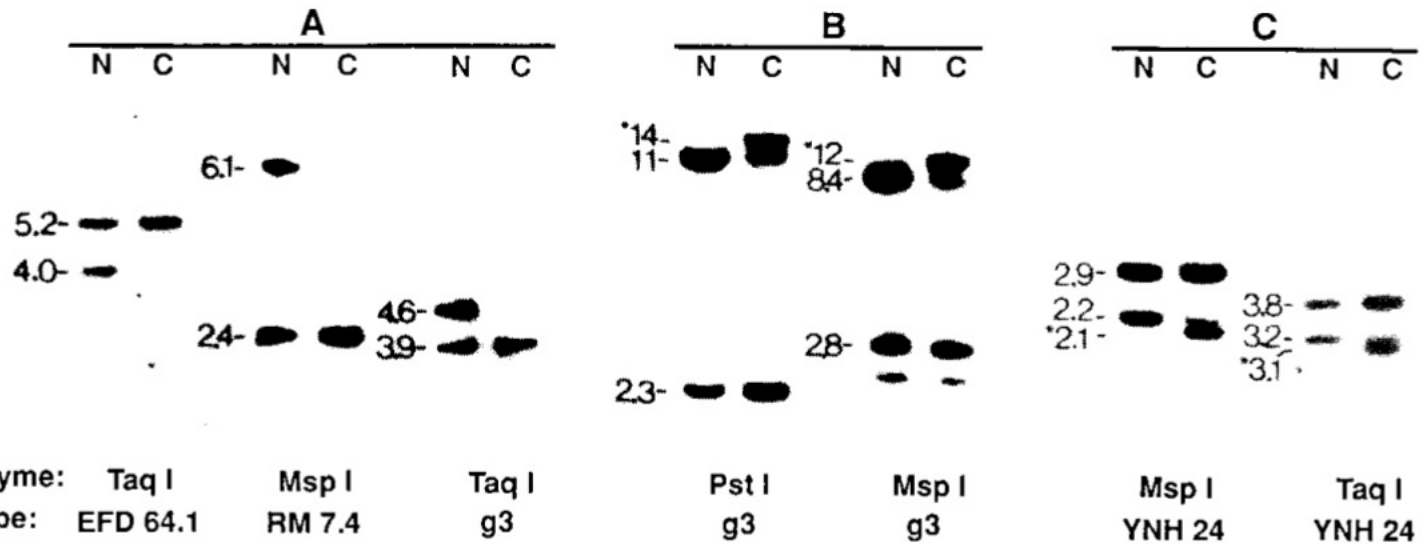


Fig. 2. (A) Examples of allelic deletions. DNA from normal (N) and carcinoma (C) tissues of patient S141 (tumor A) was cleaved with restriction endonucleases, and the fragments were separated by electrophoresis and transferred to nylon filters. The filters were incubated with the indicated radioactive probes. Sizes (in kilobases) of the polymorphic restriction fragments are shown on the left of each autoradiograph. With probes RM 7.4 and g3, the larger allele was lost from the tumor; with probe EFD 64.1, the smaller allele was lost. (B and C) New fragments detected in colorectal tumors with VNTR probes. Autoradiographs of DNA blots prepared as described in (A) are shown. For each N-C pair, the results of digestion with two different enzymes are shown, and the probe is indicated. (B) Patient S7 and (C) patient S191. Sizes (in kilobases) of the major polymorphic restriction fragments are shown on the left of each autoradiograph, and the new fragments in the tumor samples are marked with asterisks. Areas of tumors containing a high proportion of neoplastic cells were isolated as described, and 12- μ m-thick-cryostat sections of these areas were used to prepare DNA (12). Grossly normal colonic mucosa adjacent to the tumors was obtained from each patient and used to prepare control DNA. DNA purification, restriction endonuclease digestion, electrophoresis, DNA transfer, and DNA hybridization were performed as described (12, 24).

Mutazioni nel carcinoma del colon-retto

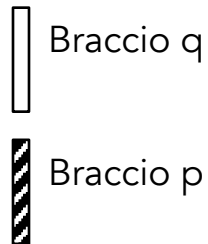
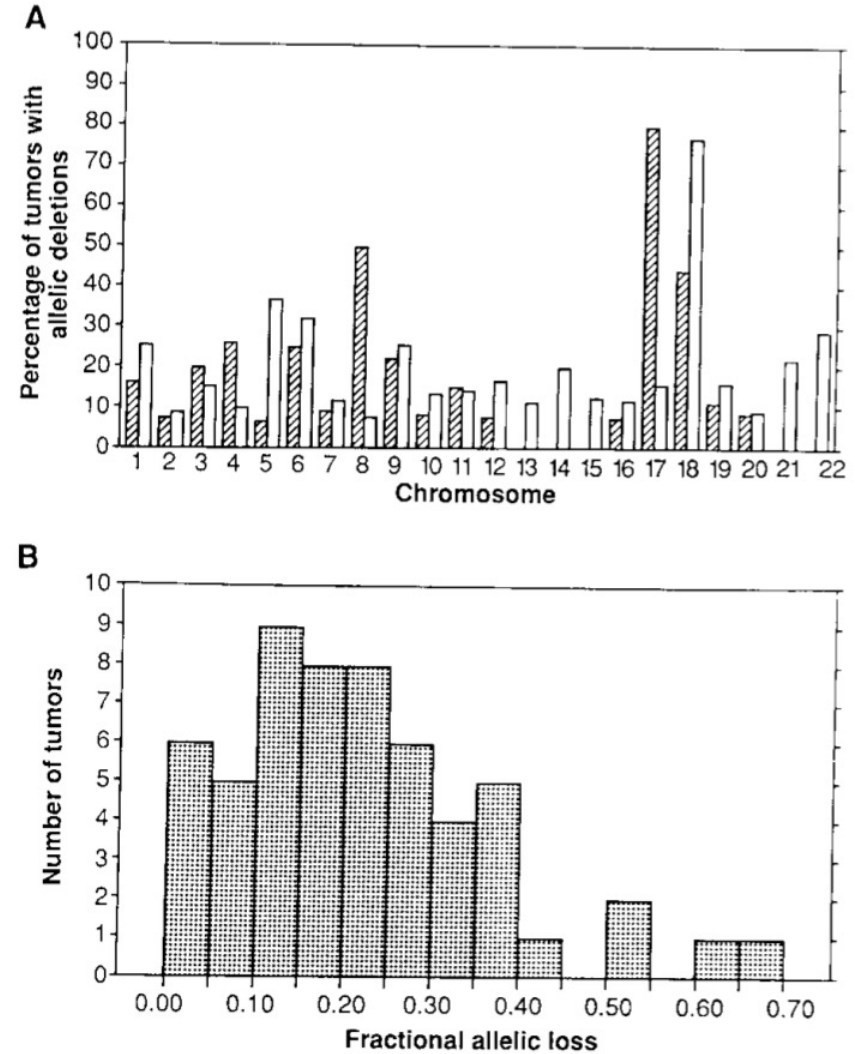


Fig. 1. (A) Frequency of allelic deletions in individual chromosomal arms. Allelic deletions were evaluated with RFLP analyses, examples of which are in Fig. 2. DNA from paired normal colonic mucosa and tumor tissues was cleaved with one of three enzymes (Taq I, Msp I, or Hind III), and evaluated with probes from each nonacrocentric autosomal arm. The probes used are listed (23), together with references describing their derivation and polymorphism patterns. Only informative tumors, that is, those in which DNA from the normal tissue exhibited a heterozygous pattern for one or more allelic markers from the indicated chromosomal arm, were used to determine allelic loss frequencies. The number of tumors informative for each chromosomal arm is listed in (23). An allelic loss was scored if an RFLP fragment present in normal DNA was lost in at least 80% of the neoplastic cells, as assessed by comparison of the autoradiographs with histologic evaluation of the cryostat sections from which the tumor DNA was purified. Open bars, q arm; hatched bars, p arm. **(B)** Frequency of allelic deletions in individual tumors. The FAI in each tumor was defined as the number of chromosomal arms on which allelic loss was observed divided by the number of chromosomal arms for which allelic markers were informative. The chromosomal arms on which the allelic deletions occurred in each tumor are listed in Table 1.



Mutazioni nel carcinoma del colon-retto

Table 2. Relation of FAL to clinical and histologic features. In comparing the means for group I with those of group II, age, follow-up period, tumor size, and Dukes' classification were nonsignificant on the basis of the *t* test. For the comparisons of percentages, RAS was nonsignificant and tumor recurrence and death were significant on the basis of the Fisher exact test ($P < 0.01$).

Group*	FAL (mean)	Number of patients†	Age (years)	Follow-up period‡ (months)	Tumor size (cm)	Dukes' class.§	RAS mutation (%)	Tumor recurrence¶ (%)	Death# (%)
I	0.11	27	67	38	5.3	2.3	52	30	26
II	0.32	25	67	38	5.6	2.4	52	68	64

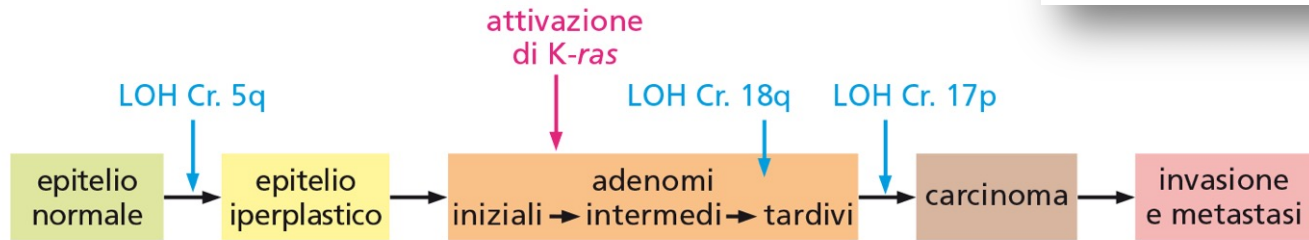
*Group I patients had tumors with an FAL less than the median value (0.2) of the 56 tumors listed in Table 1; group II patients had tumors with an FAL greater than 0.2. †All patients from Table 1 with a single carcinoma were included. ‡Mean follow-up period in patients who survive is listed. The mean follow-up period in all patients combined (that is, those who are still alive plus those who died) was 31 and 17.5 months for group I and II patients, respectively. §Dukes' classification scored as 1.0 for Dukes' A tumors (confined to muscularis propria), 2.0 for Dukes' B tumors (extension through muscularis propria), and 3.0 for Dukes' C tumors (metastatic to regional lymph nodes). ||RAS gene mutations in this group of tumors were reported in (8) and (13). ¶Distant metastases developed in all except one patient who developed tumor recurrence. #Death with or from carcinoma. An additional 6 and 12% of group I and II patients, respectively, died without definite evidence of recurrent carcinoma.

FAL : Fraction Allelic Loss

Mutazioni nel carcinoma del colon-retto

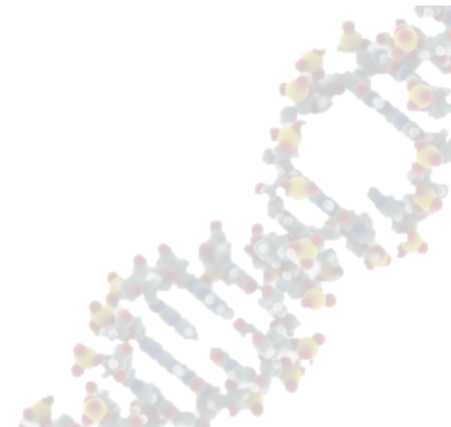
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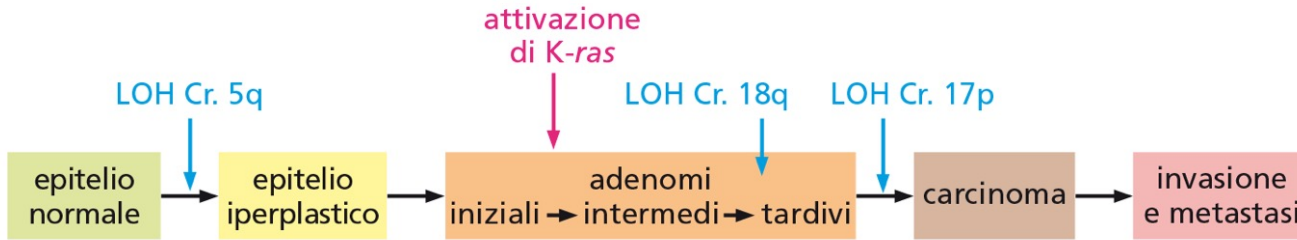


Mutazioni comuni nei tumori del colon-retto

Il numero dei geni oncosoppressori mutati è maggiore del numero di oncogeni attivati nei tumori umani

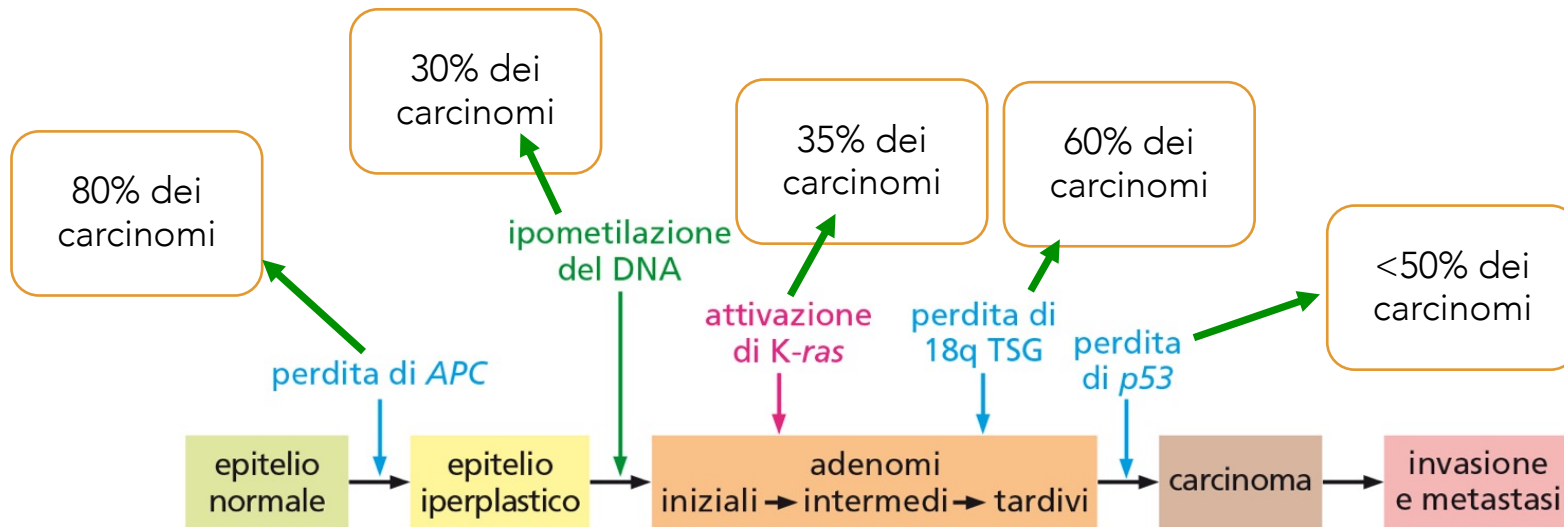


Mutazioni nel cancro del colon



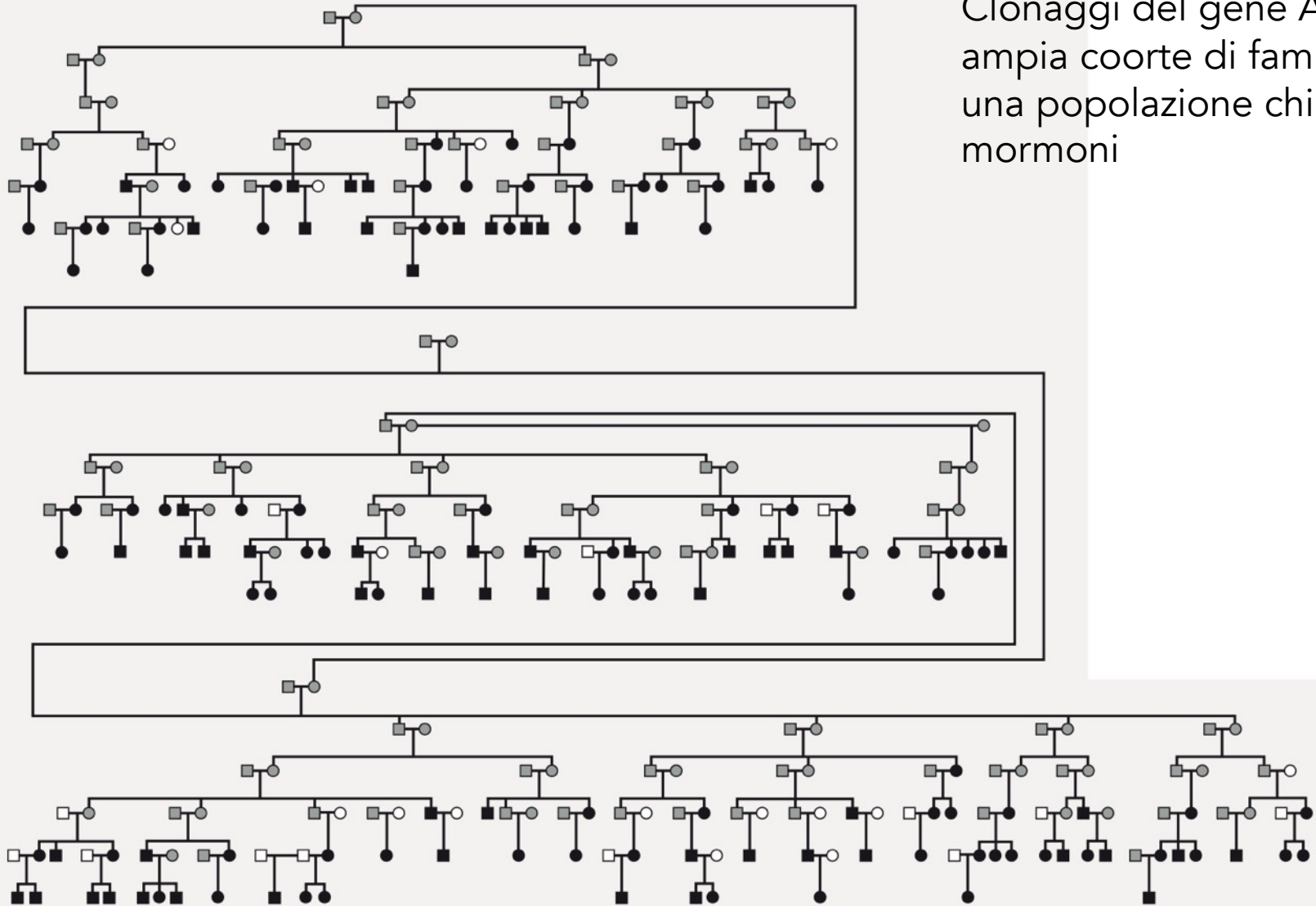
Mutazioni comuni nei tumori del colon-retto...

...e identificazione dei geni coinvolti nei diversi passaggi della trasformazione tumorale



Poliposi adenomatosa Famigliare (FAP)

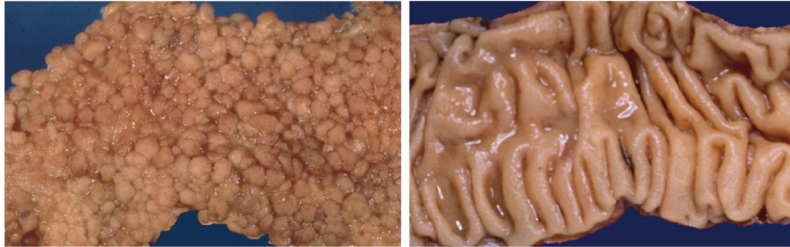
Clonaggi del gene APC :
ampia coorte di famiglie in
una popolazione chiusa, i
mormoni



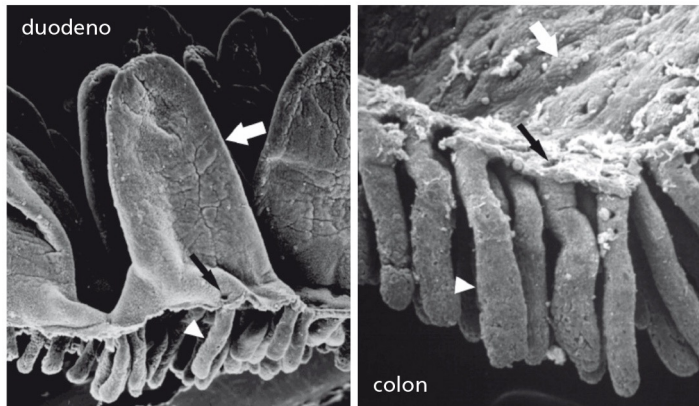
Biologia del cancro del colon

Polipi in FAP

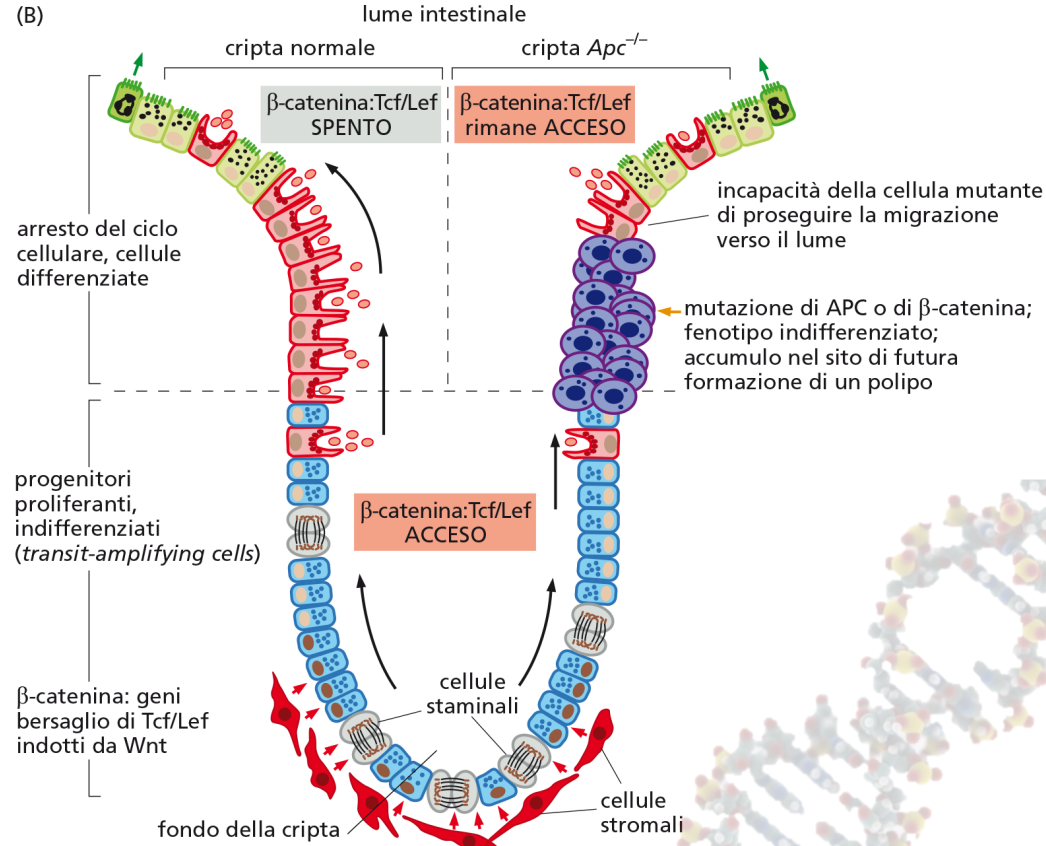
Normale



Migrazione cellulare è fondamentale per maturazione cellule epiteliali

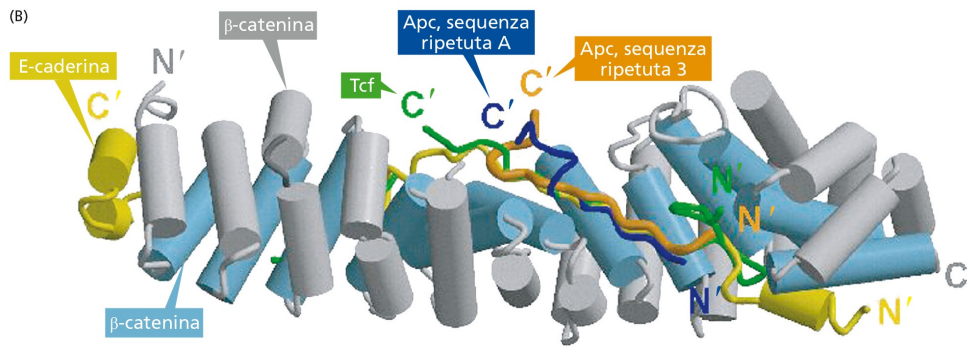
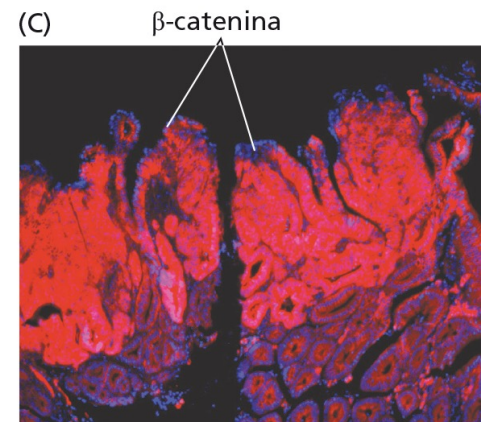
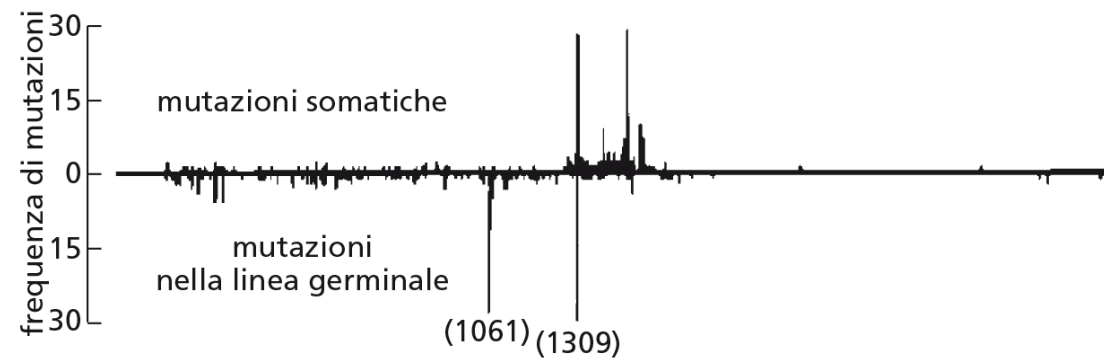
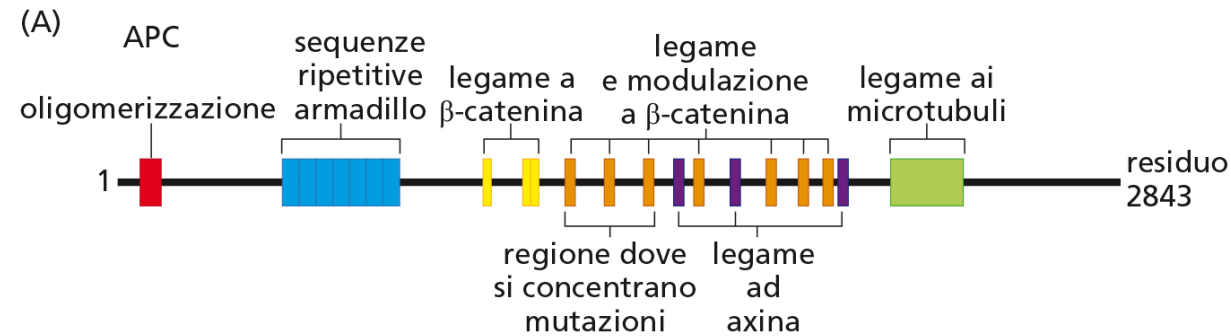


Organizzazione precisa dei diversi tipi di cellule epiteliali nelle cripte del colon. Cellule staminali alla base delle cripte per continuo ricambio epitelio.



Mutazioni APC

Mutazioni ricorrenti in APC causano stop prematuro e eliminazione domini di legame con β -catenina e axina

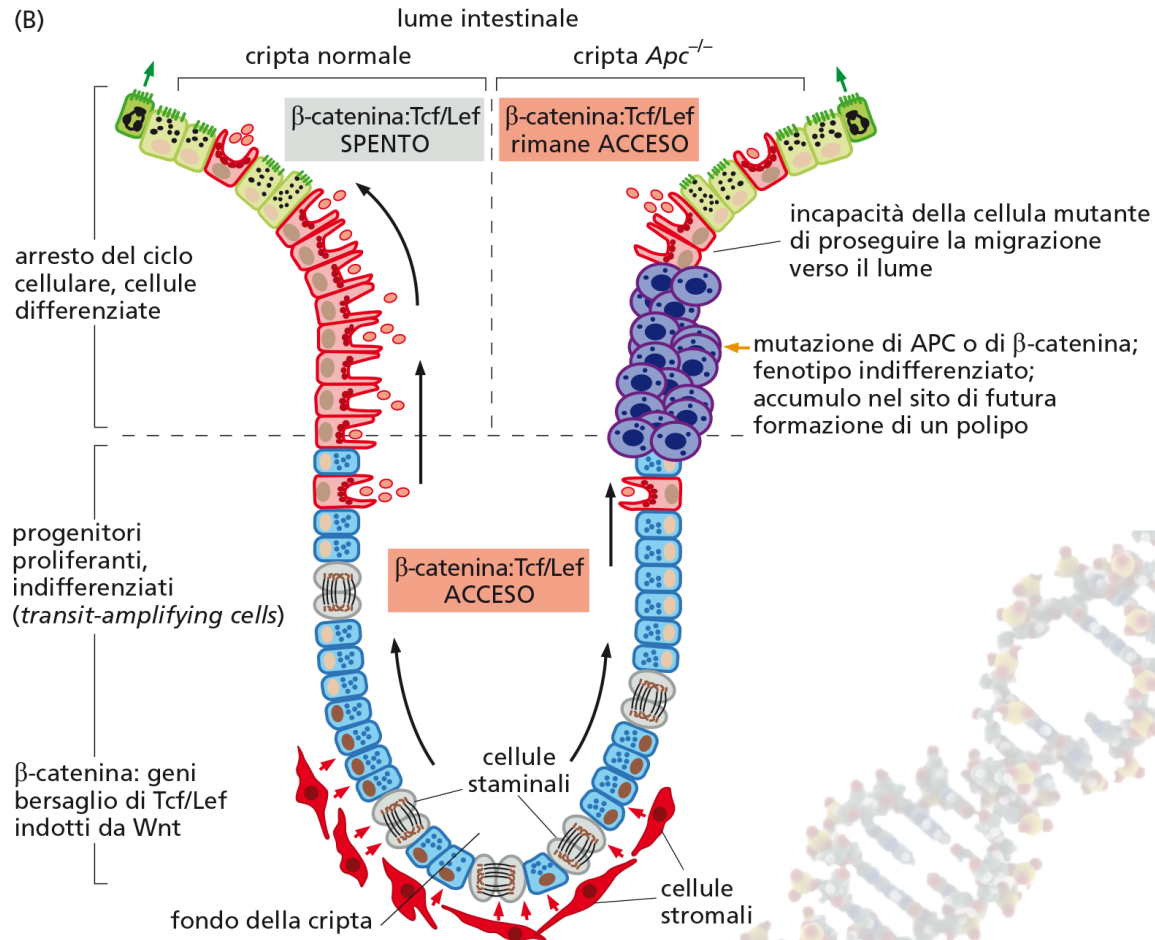


Mutazioni APC non sono sufficienti per sviluppo carcinoma

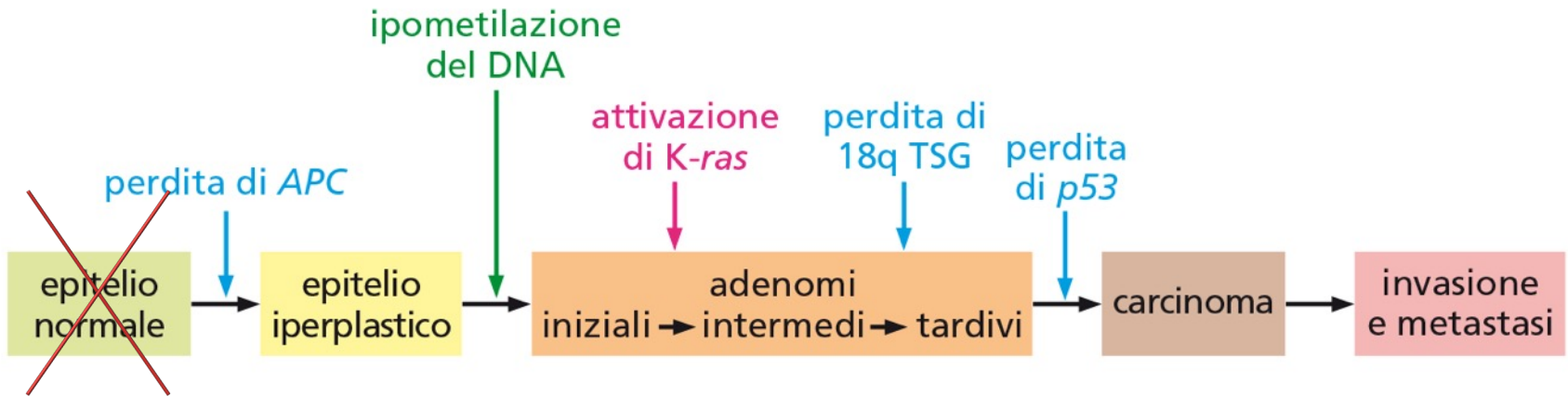
Mutazioni APC

- ❖ Migrazione cellule epiteliali verso l'esterno delle cripte durante il differenziamento e morte per apoptosi → cellule mutate perse rapidamente
- ❖ Mutazioni APC o altri geni che controllano β -catenina → cellule non migrano
- ❖ Altre mutazioni → polipo nella cripta

Migrazione cellulare è fondamentale per maturazione cellule epiteliali

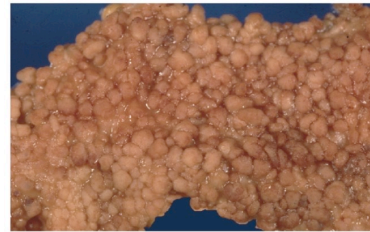
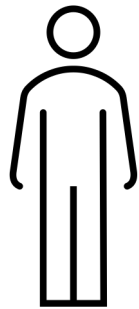


APC e la poliposi adenomatosa familiare

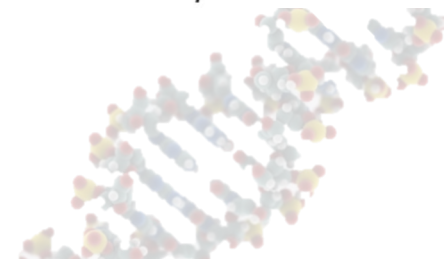
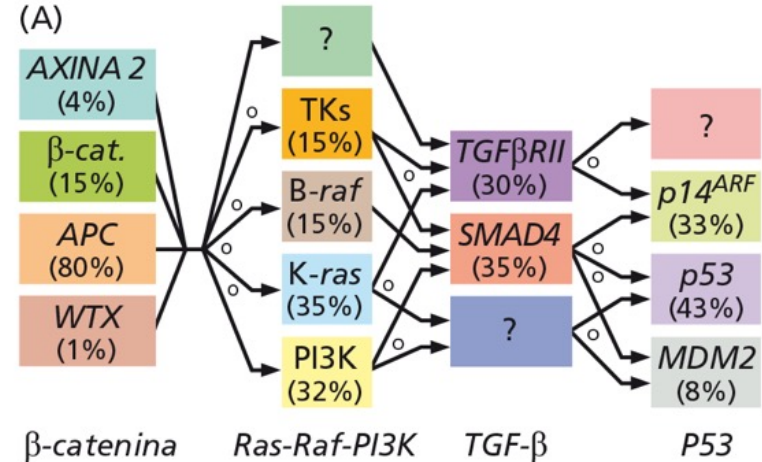


APC^{+/-}

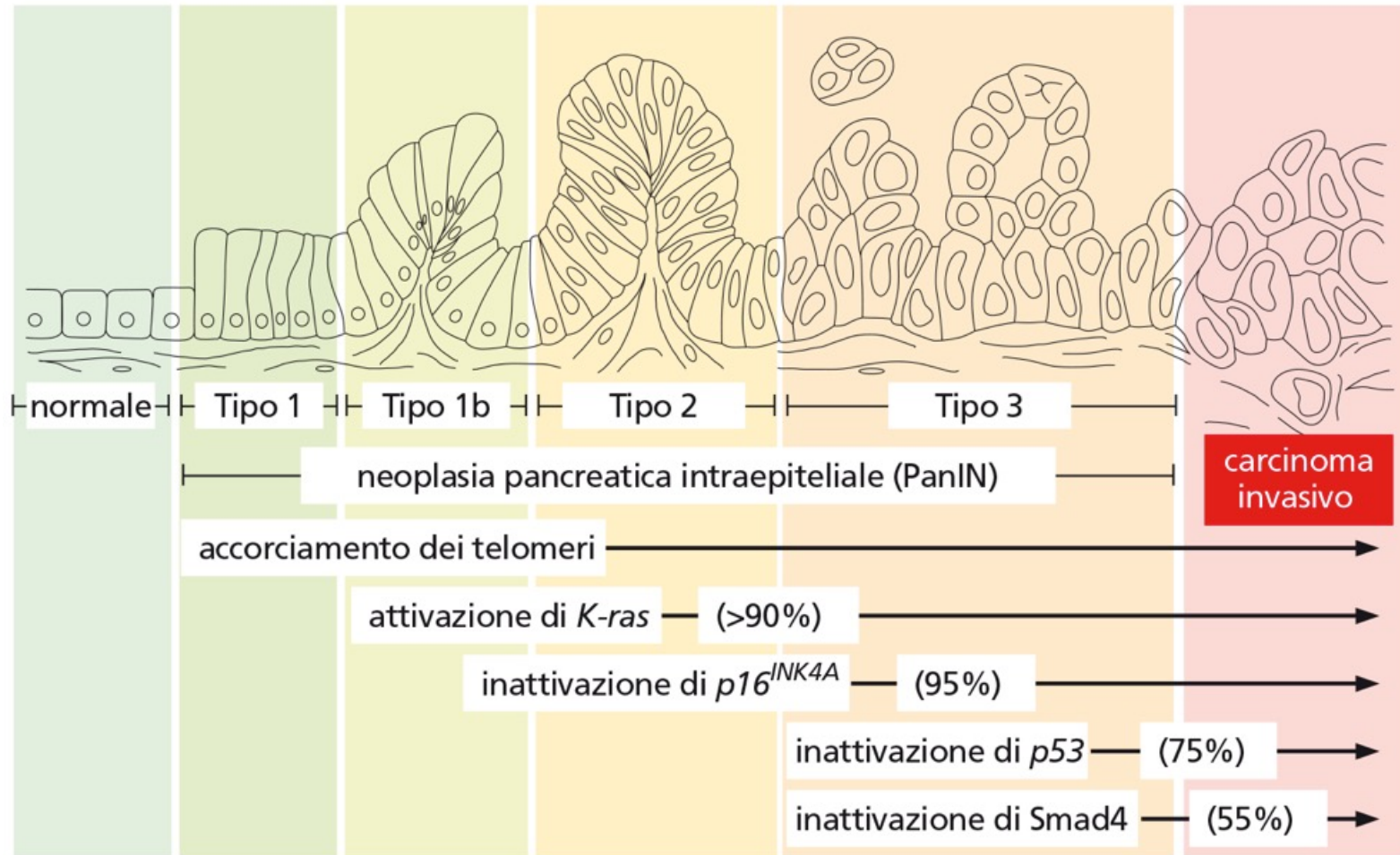
APC^{-/-}



Accelerazione della progressione multi-step perché il primo evento non dipende più da un evento casuale

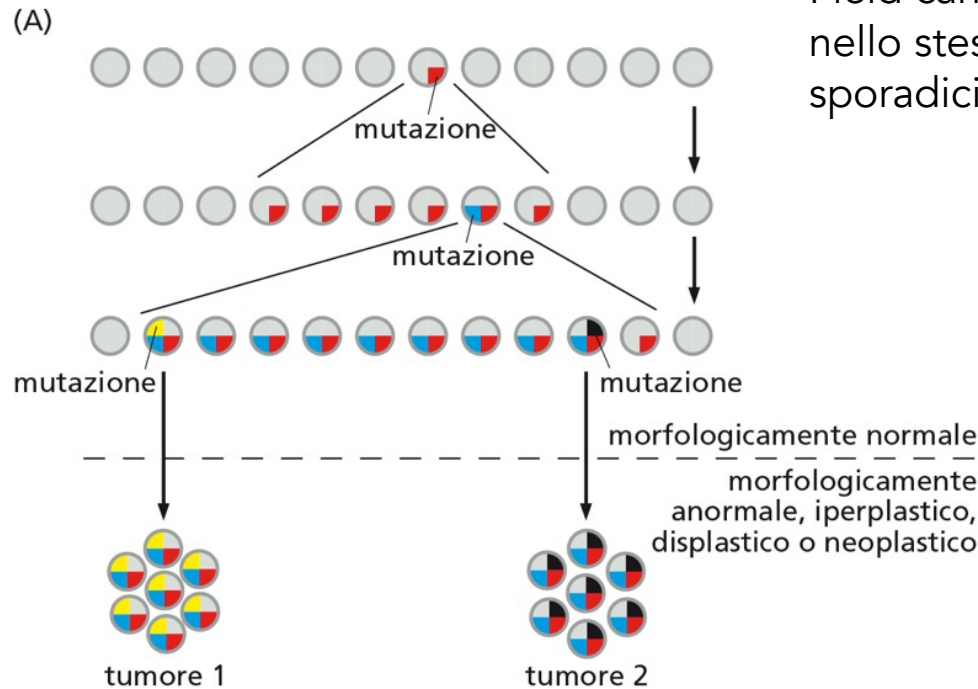


Evoluzione del cancro del colon

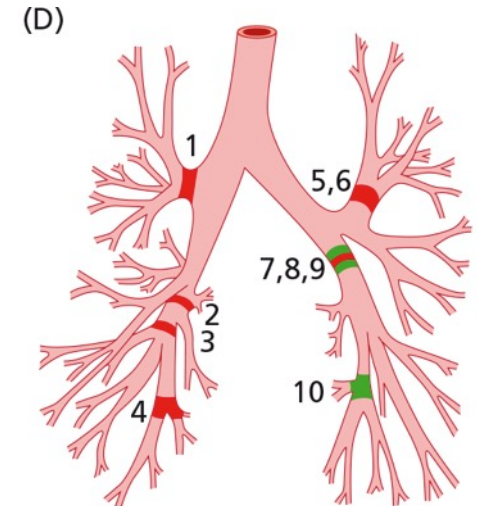
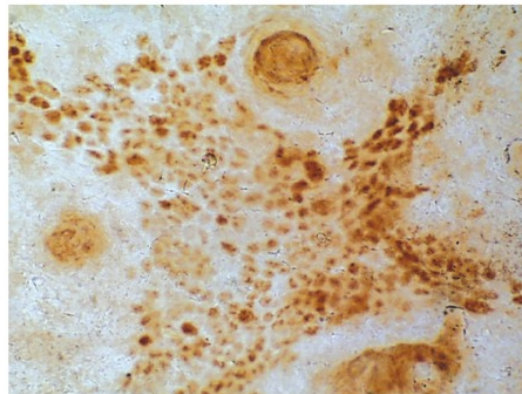


Cancerizzazione di area

Field cancerization: formazione di tumori multipli nello stesso organo (caratteristica dei tumori sporadici)



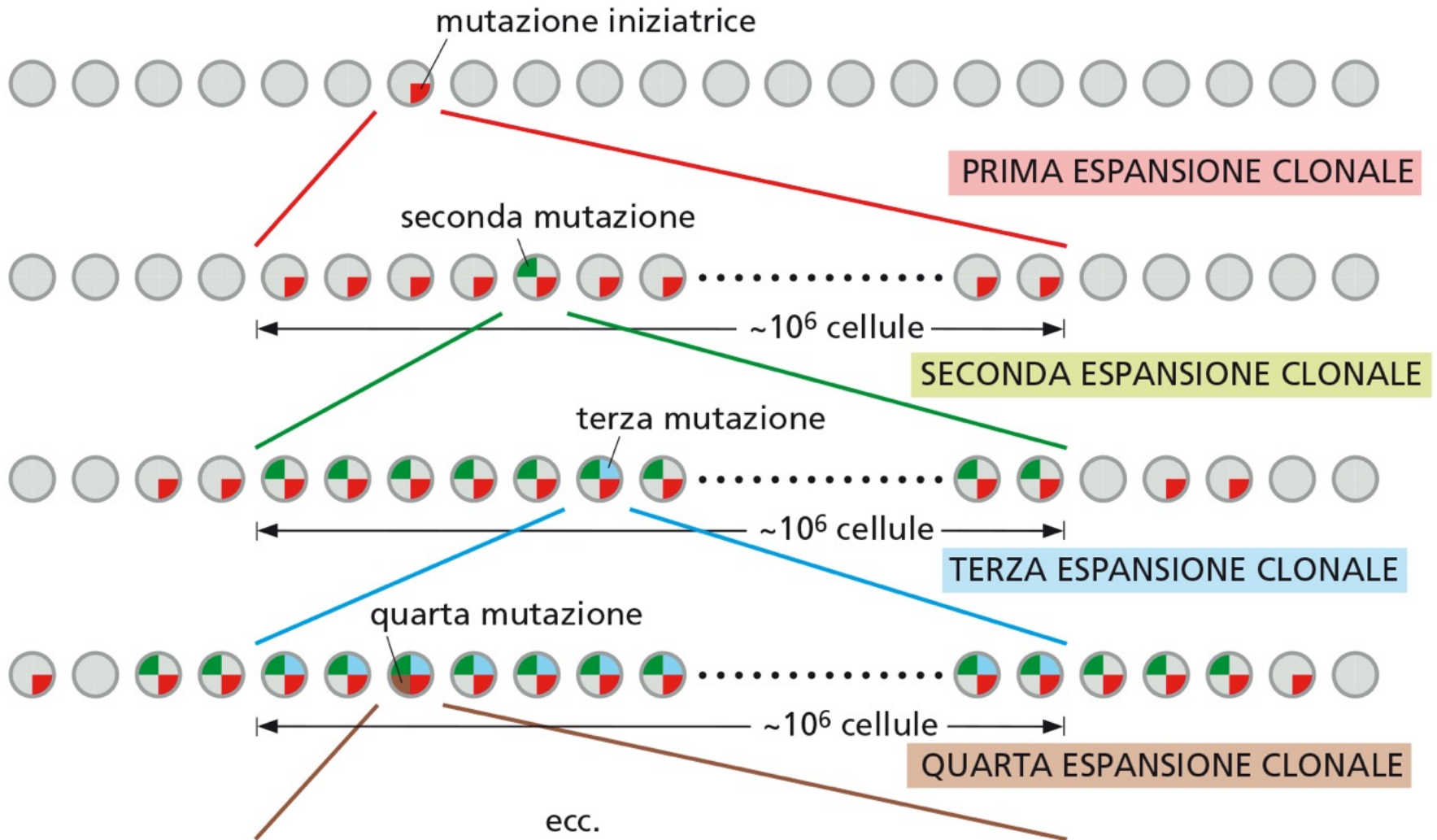
(C)



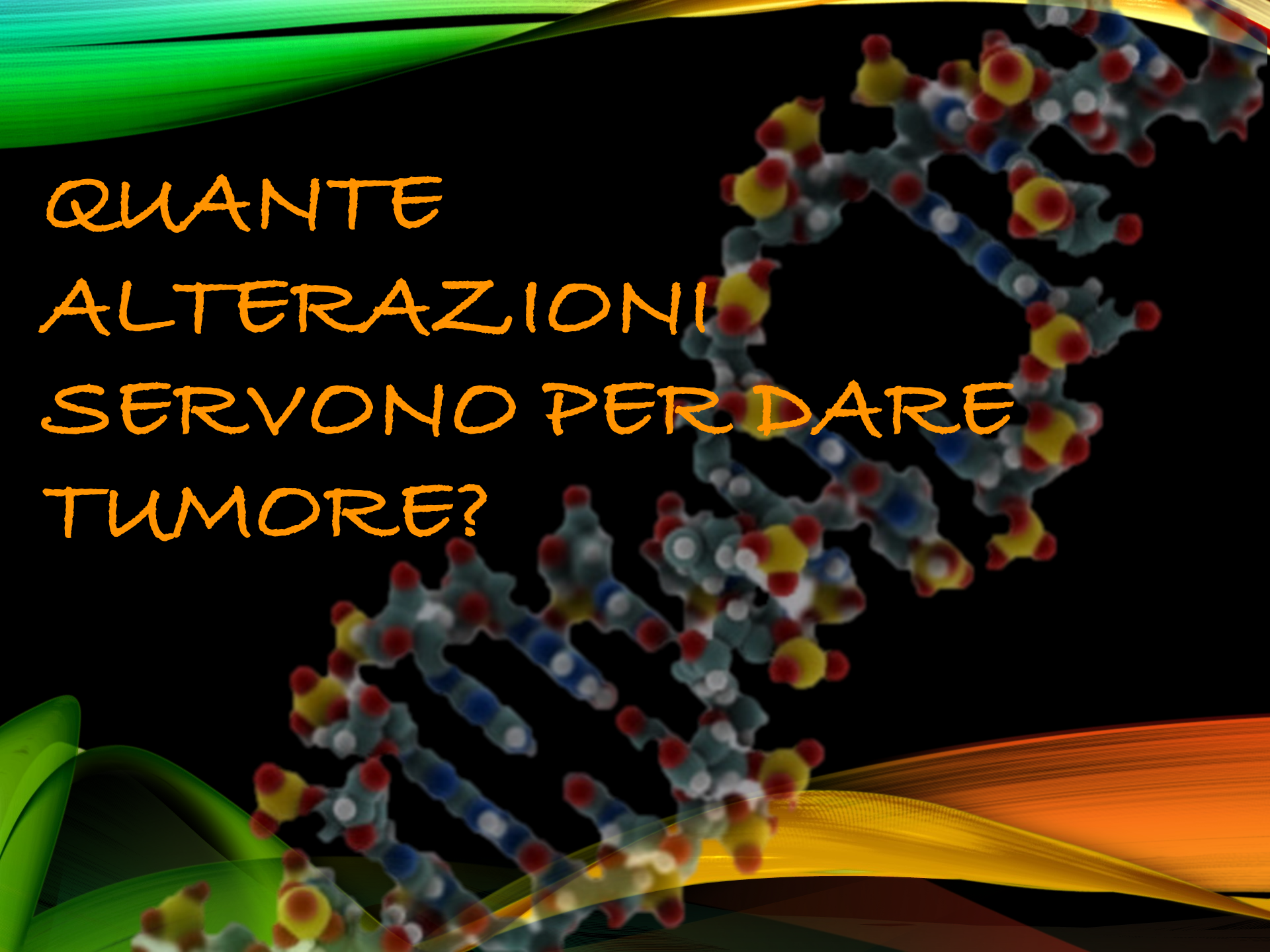
■ solo *p53* non mutata

■ $G \rightarrow T^{245} + p53$ non mutata

Evoluzione darwiniana del tumore



QUANTE
ALTERAZIONI
SERVONO PER DARE
TUMORE?



Tappe della tumorigenesi nell'uomo

REVIEW ARTICLES | OCTOBER 1, 2003

Leukemia in twins: lessons in natural history

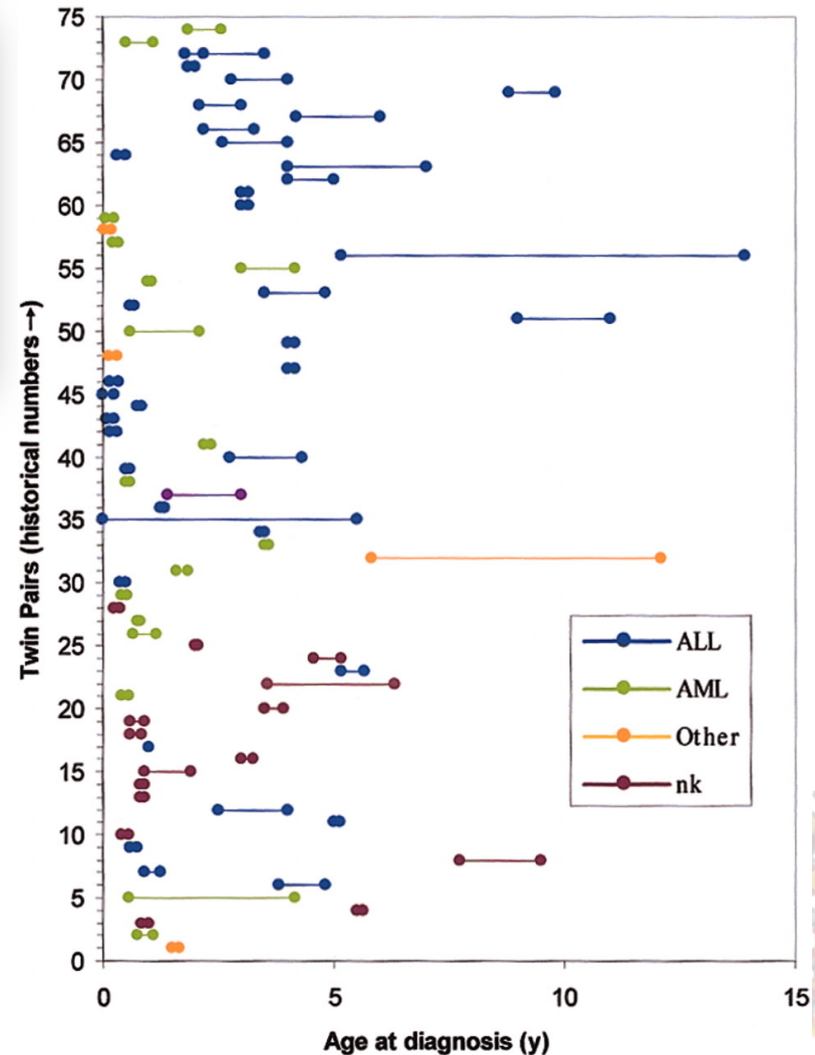
Mel F. Greaves, Ana Teresa Maia, Joseph L. Wiemels, Anthony M. Ford



Blood (2003) 102 (7): 2321-2333.

<https://doi.org/10.1182/blood-2002-12-3817>

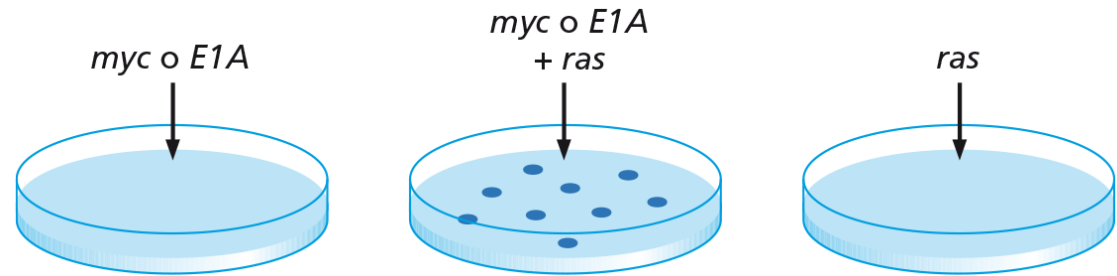
- ❖ Coppie di gemelli monozigoti che sviluppano lo stesso tipo di leucemia
- ❖ A volte a distanza di tempo ma con stesso marcatore genetico o mutazione
- ❖ Origine comune di traslocazione o mutazione, ma servono altri eventi per sviluppare il tumore



Ricostruire la tumorigenesi in laboratorio

In vitro: *H-ras* da tumore della vescica umana capace di trasformare cellule NIH3T3

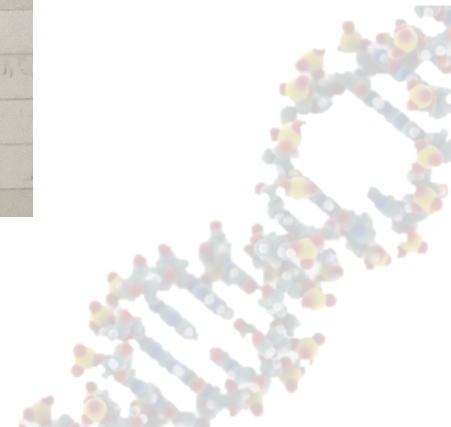
Anni '80: cellule primarie da embrioni di topo, ratto, coniglio



Sinergia o cooperazione tra oncogeni di gruppi funzionali diversi

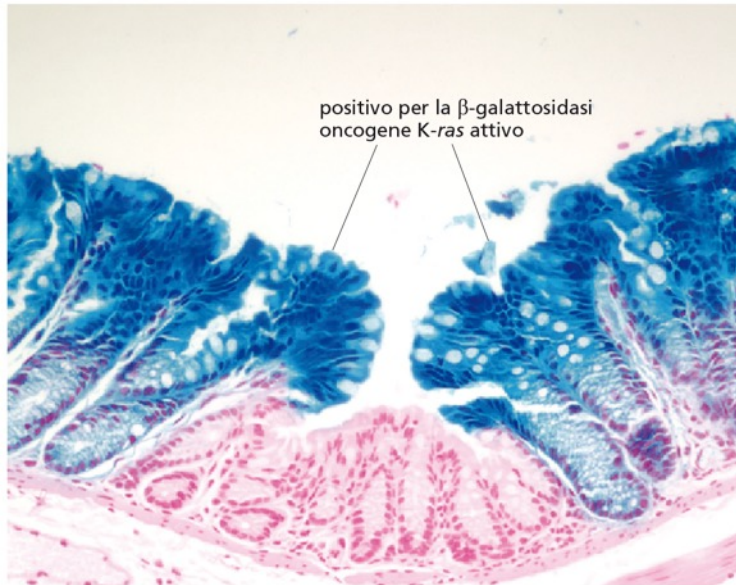
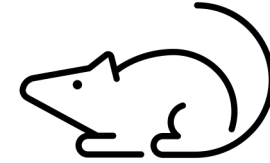
TABELLA 11.1
Esempi di collaborazione tra oncogeni *in vitro* e *in vivo*

Oncogeni di tipo <i>ras</i> ^a	Oncogeni di tipo <i>myc</i>	Cellula o organo bersaglio
Trasformazione <i>in vitro</i>		
<i>ras</i>	<i>myc</i>	fibroblasti embrionali di ratto trasfettati (REF)
<i>ras</i>	E1A	cellule renali di ratto trasfettate
<i>ras</i>	<i>large T</i> di SV40	REF trasfettati
Notch-1	E1A	cellule renali di ratto trasfettate

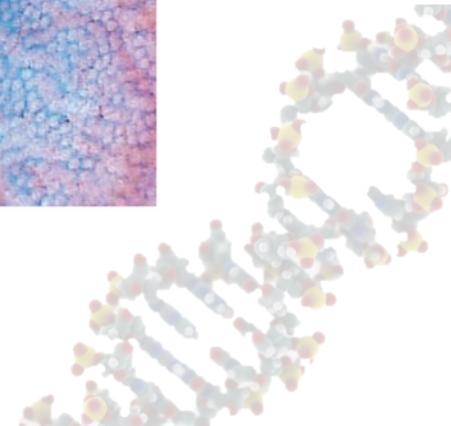
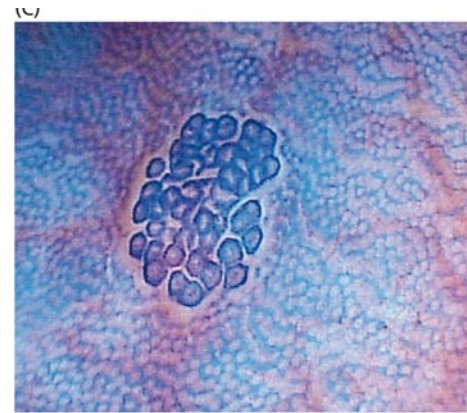


Ricostruire la tumorigenesi in laboratorio

In vivo: oncogene *K-ras* + reporter β -galattosidasi in cellule germinali di topo. Attivazione condizionale oncogene \rightarrow iperplasia con cellule normali

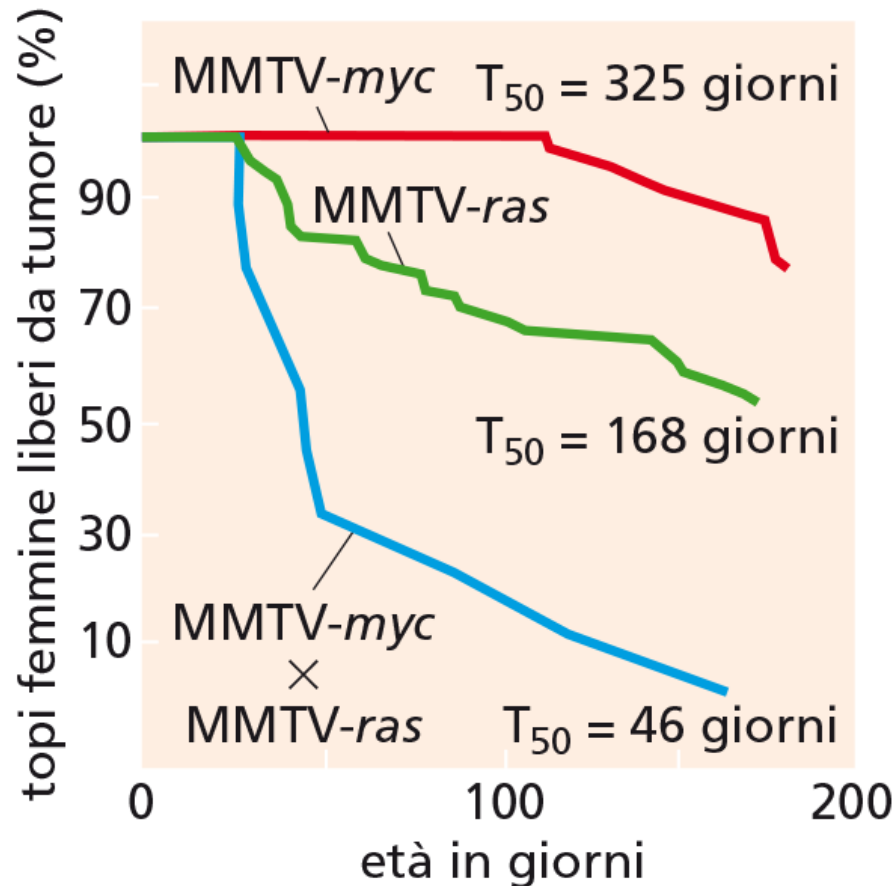
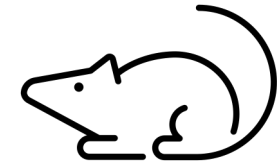


In vivo: oncogene *K-ras* + inattivazione APC.
Polipo adenomatoso ma non carcinoma

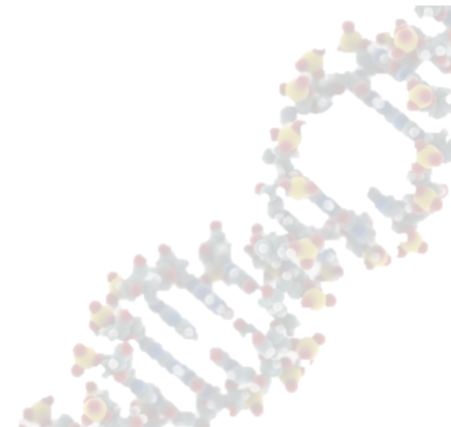


Ricostruire la tumorigenesi in laboratorio

In vivo: topi transgenici con MMTVK-*ras* e/o MMTV-*myc*

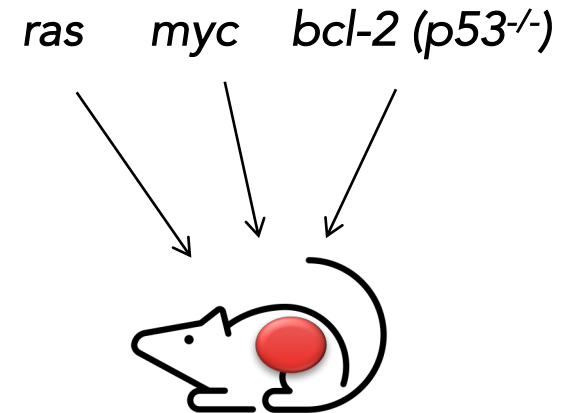


Anche nei topi doppi transgenici MMTVK-*ras* MMTV-*myc* non si sviluppano tumori alla nascita



Ricostruire la tumorigenesi in laboratorio

- ❖ Cellule *ras* + *myc* trasformate in vitro avevano mutazioni in p53 o metilazione del suo promotore
- ❖ Cooperazione *bcl-2* + *myc*



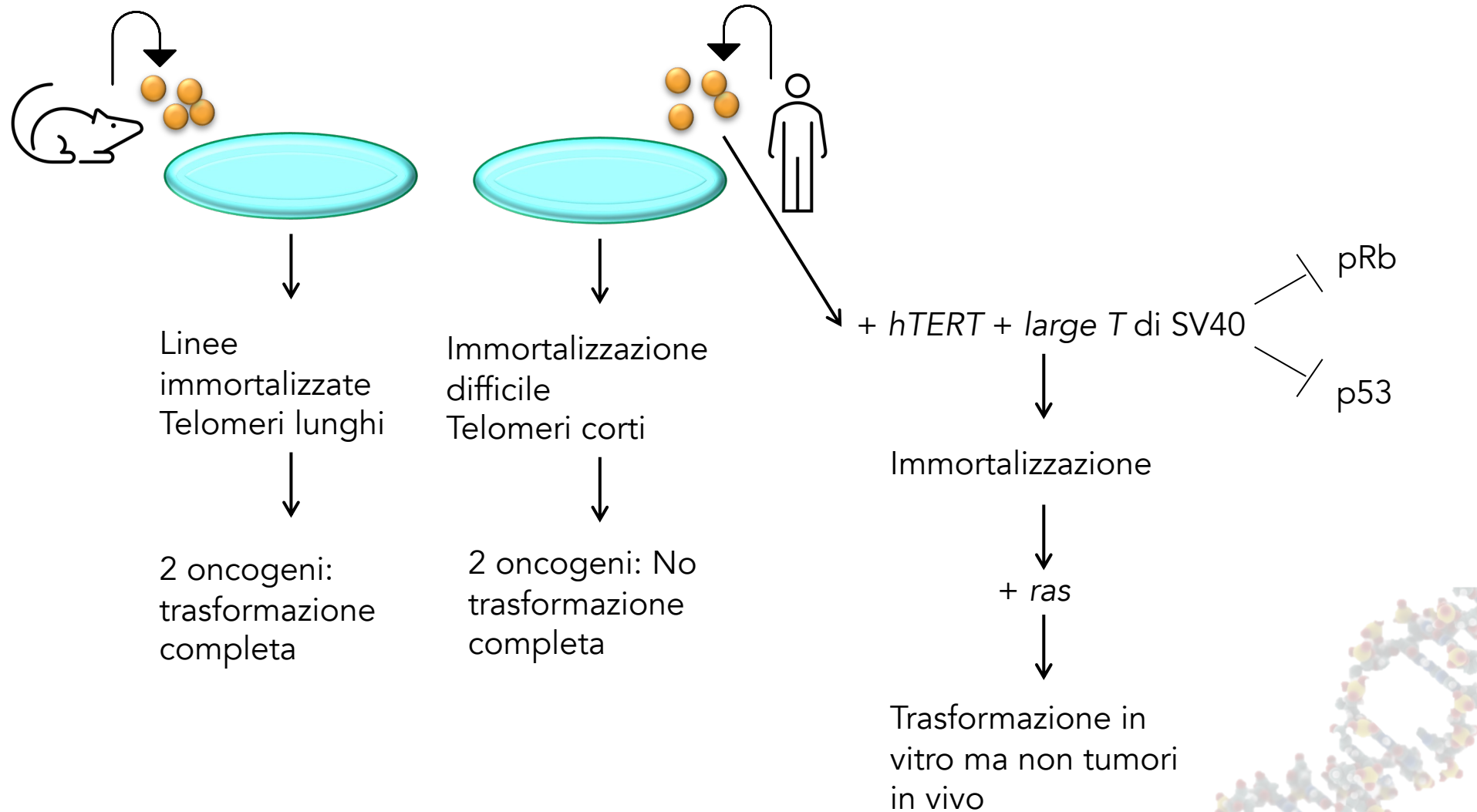
Bastano 3 eventi?

TABELLA 11.1
Esempi di collaborazione tra oncogeni *in vitro* e *in vivo*

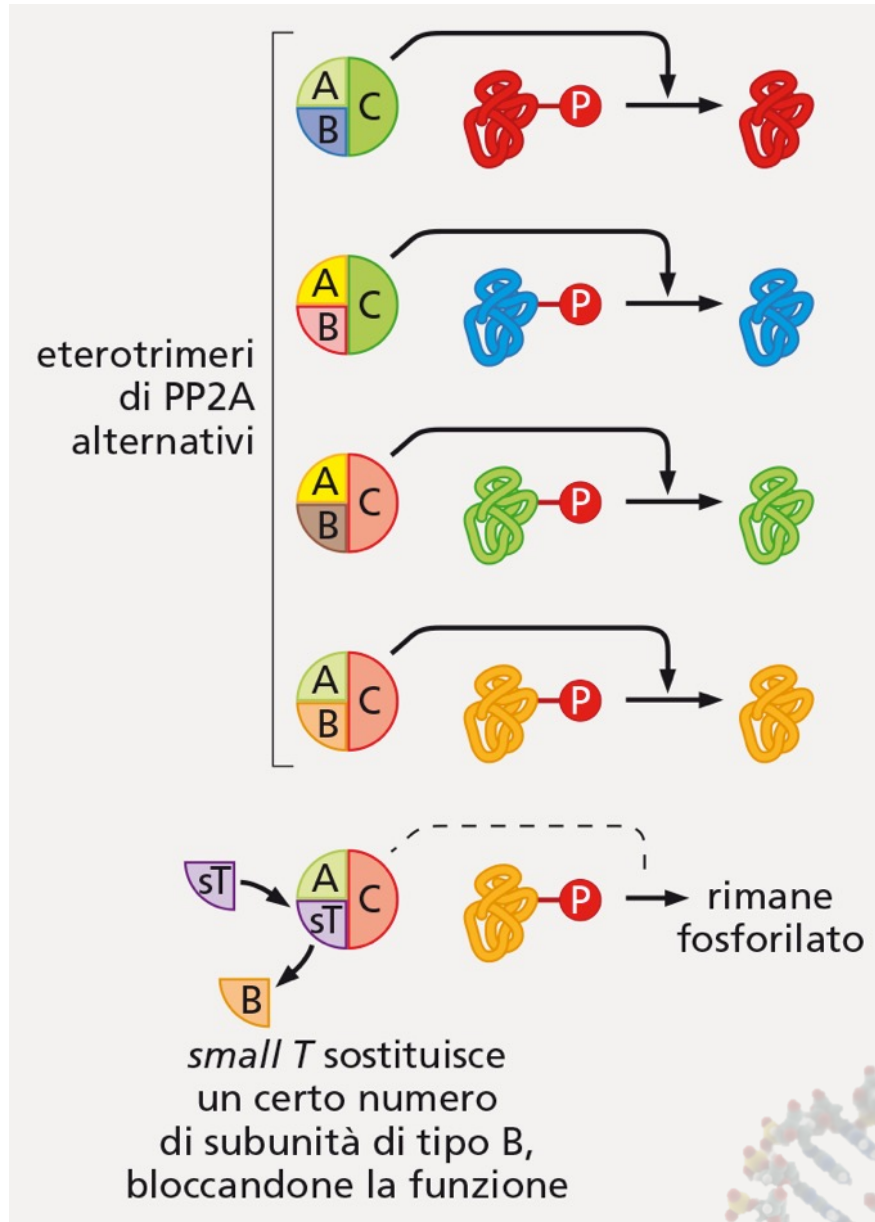
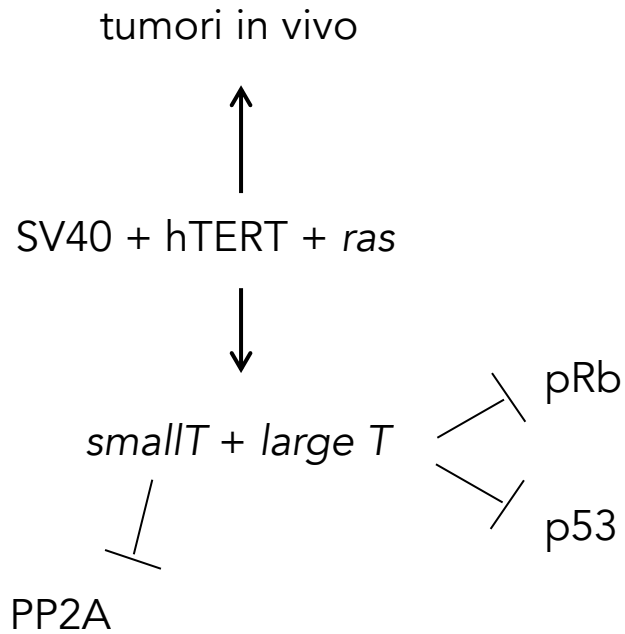
Oncogeni di tipo <i>ras</i> ^a	Oncogeni di tipo <i>myc</i>	Cellula o organo bersaglio
Trasformazione <i>in vitro</i>		
<i>ras</i>	<i>myc</i>	fibroblasti embrionali di ratto trasfettati (REF)
<i>ras</i>	E1A	cellule renali di ratto trasfettate
<i>ras</i>	<i>large T</i> di SV40	REF trasfettati
Notch-1	E1A	cellule renali di ratto trasfettate
Tumorigenesi <i>in vivo</i>		
<i>middle T</i>	<i>large T</i>	tumori murini indotti dal virus del poliovirus
<i>mil (= raf)</i>	<i>myc</i>	tumori del pollo indotti dal virus della leucemia aviaria MH2
<i>erbB</i>	<i>erbA</i>	tumori del pollo indotti dal virus dell'eritroblastosi aviaria
<i>pim1</i>	<i>myc</i>	tumori indotti dal virus della leucemia murina
<i>abl</i>	<i>myc</i>	tumori indotti dal virus della leucemia murina
Notch-1/2	<i>myc</i>	timomi in topi transgenici
<i>bcl-2</i>	<i>myc</i>	linfomi follicolari in topi transgenici

^a I termini "di tipo *ras*" e "di tipo *myc*" si riferiscono a classi funzionali anziché a geni che codificano elementi della stessa via di trasduzione del segnale. Gli oncogeni "di tipo *ras*" tendono a codificare componenti di cascate del segnale citoplasmatico, mentre gli oncogeni "di tipo *myc*" tendono a codificare proteine nucleari.

Ricostruire la tumorigenesi in laboratorio

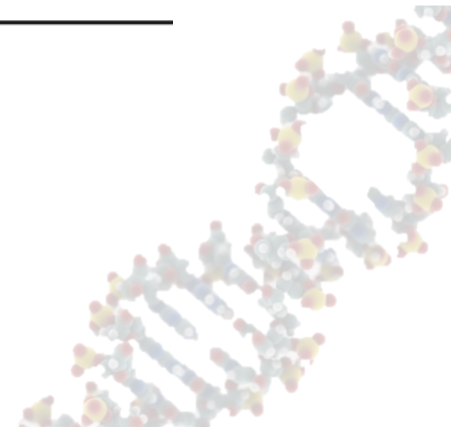


La protein-fosfatasi PP2A



La protein-fosfatasi PP2A

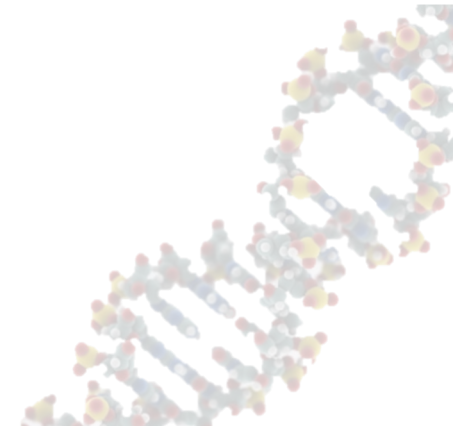
Targets of PP2A	Cellular role
p53, Cdc25	Cell cycle checkpoints and regulation
PI3K/Akt	Cell proliferation and survival
RAF, MAPK, ERK	Cell proliferation and survival
p70 S6K, mTOR	Cell proliferation, survival and translation initiation
c-Myc	Tumourigenesis
Wnt/ β -catenin	Tumourigenesis
FoxM1	Transcription factor that control expression of genes involved in the cell cycle
Bcl-2, caspase-3	Apoptosis



Le tappe verso il cancro

via	Ras	pRb	p53	telomeri	PP2A
geni/agenti usati per perturbare la via	<i>ras</i> , <i>MEK+</i> <i>Akt/PKB</i> , <i>MEK+IKBKϵ</i> , <i>PAK1+</i> <i>Akt/PKB</i>	SV40 <i>LT</i> , <i>CDK4 + D1</i> , HPV <i>E7</i> , <i>Rb shRNA</i>	SV40 <i>LT</i> , DN <i>p53</i> , HPV <i>E6</i> , <i>p53 shRNA</i>	<i>hTERT</i> , <i>myc + SV40 LT</i>	SV40 <i>sT</i> in alcune cellule: <i>myc</i> <i>Akt/PKB+Rac1</i> , <i>PI3K</i> , <i>B56 shRNA</i>

❖ Sequenziamento genoma di molti tumori umani

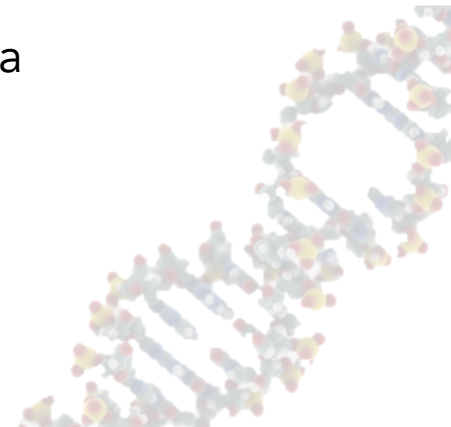


Evoluzione darwiniana del tumore: non solo frequenza di mutazione

Altri meccanismi contribuiscono alla trasformazione multi-step e complicano il modello e le tempistiche attese:

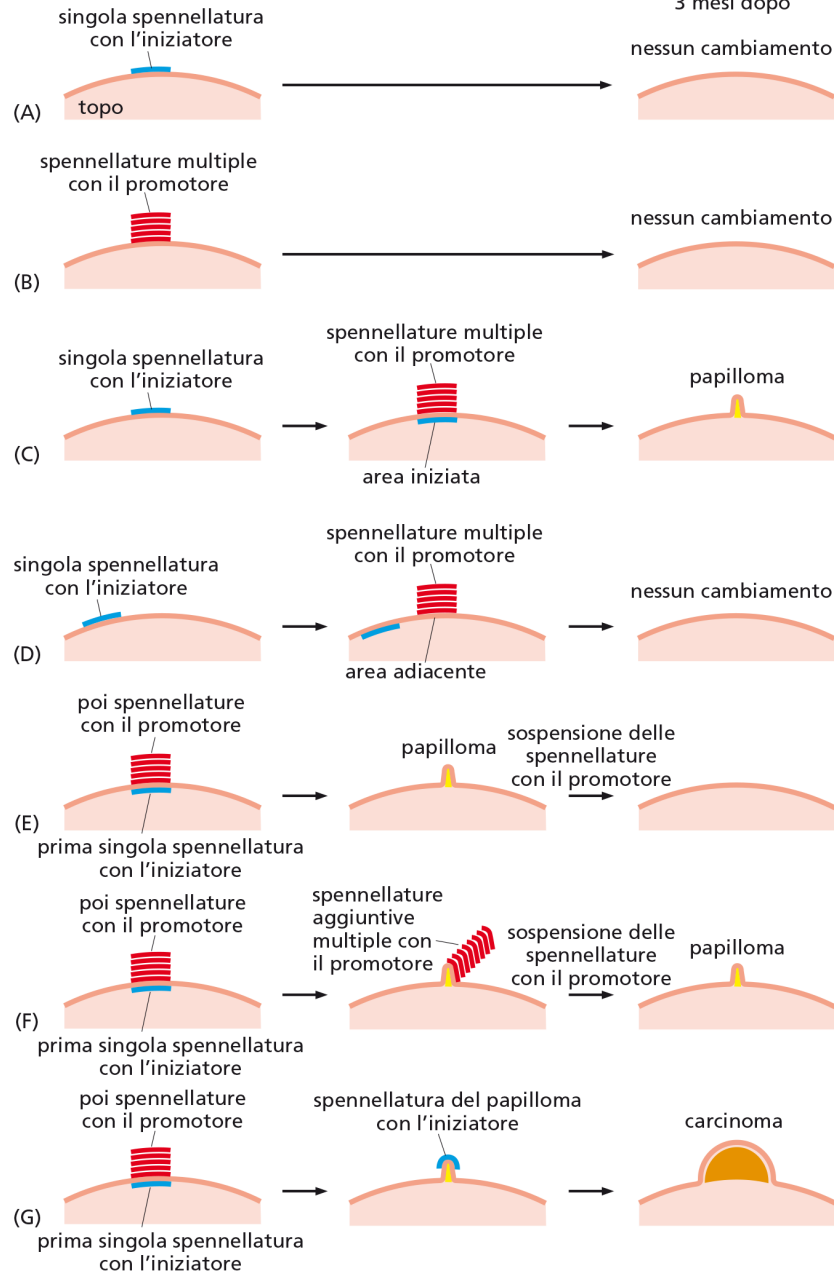
- ❖ Cambiamenti epigenetici
- ❖ Perdita oncosoppressore richiede 2 eventi mutazionali
- ❖ Mutazioni driver e mutazioni passenger

- ❖ Ambiente
- ❖ Variazione frequenza di mutazione in mutanti instabilità genetica



Effetto dell'ambiente

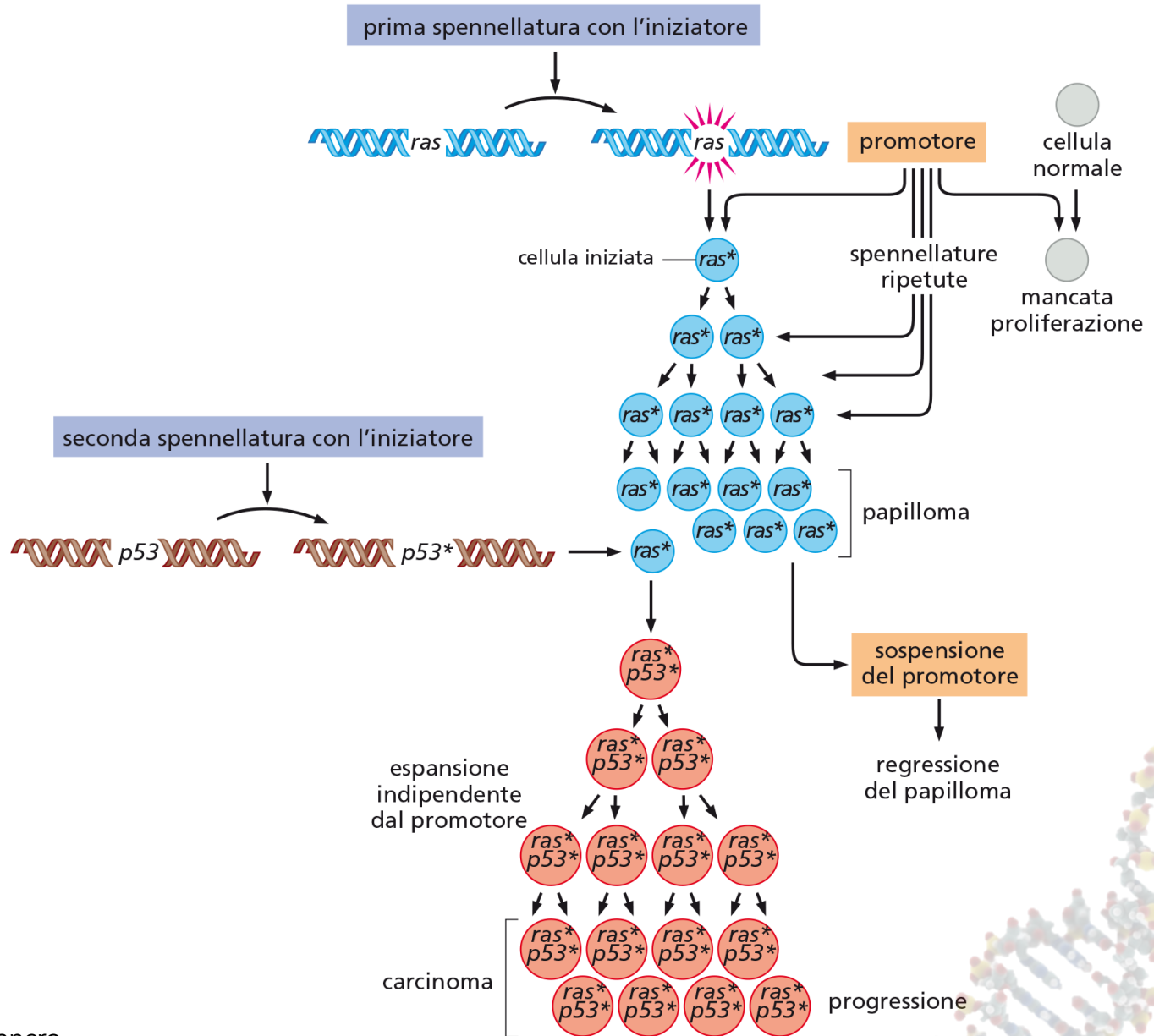
3 mesi dopo



❖ Iniziatori e promotori della tumorigenesi

Effetto dell'ambiente

❖ Iniziatori e promotori della tumorigenesi



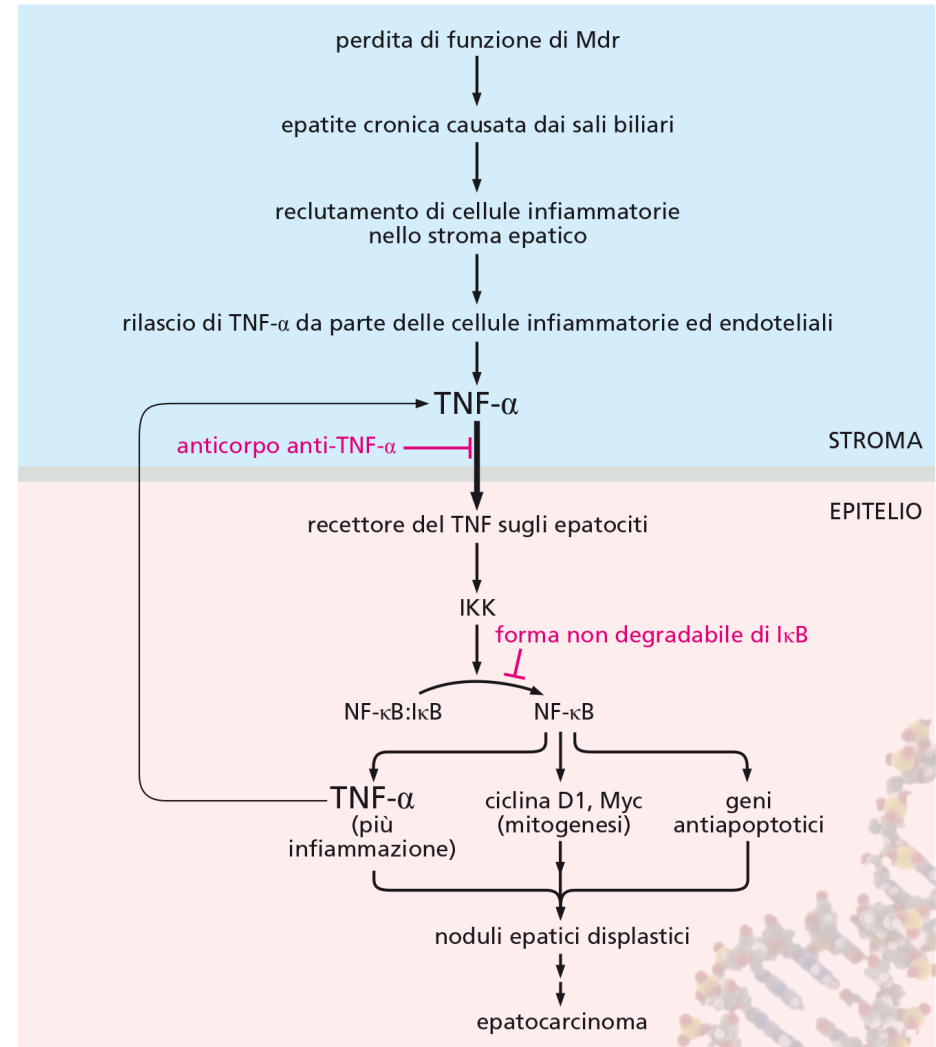
Inflammation

- ❖ Inflammation chronic and activation of specific signaling pathways

TABELLA 11.3
Condizioni infiammatorie e sviluppo tumorale

Tumore umano	Condizione infiammatoria o agente che causa infiammazione
Carcinoma della vescica	schistosomiasi, cistite cronica
Carcinoma gastrico	gastrite indotta da <i>H. pylori</i>
Epatocarcinoma	virus dell'epatite B/C
Carcinoma bronchiale	silicio
Mesotelioma	asbesto
Carcinoma ovarico	endometriosi
Carcinoma esofageo	reflusso
Carcinoma papillare della tiroide	tiroidite
Carcinoma prostatico	prostatite
Carcinoma polmonare	bronchite cronica
Carcinoma della colecisti	colecistite cronica
Carcinoma cutaneo a cellule squamose	osteomielite cronica

datata da F. Balkwill, K.A. Charles e A. Mantovani, *Cancer Cell* 7:211-217, 2005.

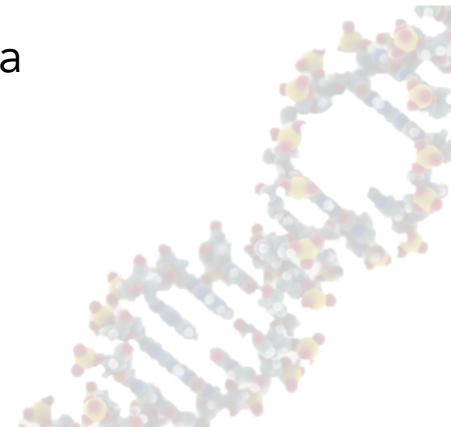


Evoluzione darwiniana del tumore: non solo frequenza di mutazione

Altri meccanismi contribuiscono alla trasformazione multi-step e complicano il modello e le tempistiche attese:

- ❖ Cambiamenti epigenetici
- ❖ Perdita oncosoppressore richiede 2 eventi mutazionali
- ❖ Mutazioni driver e mutazioni passenger

- ❖ Ambiente
- ❖ Variazione frequenza di mutazione in mutanti instabilità genetica
- ❖ Presenza di cellule diverse nel tumore



Eterogeneità intratumorale

