

ONCOGENE HER2



erbB and HER2

- ❖ HER2 activating mutations act as oncogenic drivers in various cancer types in humans
- ❖ causative for cancer initiation and progression in multiple tumor types

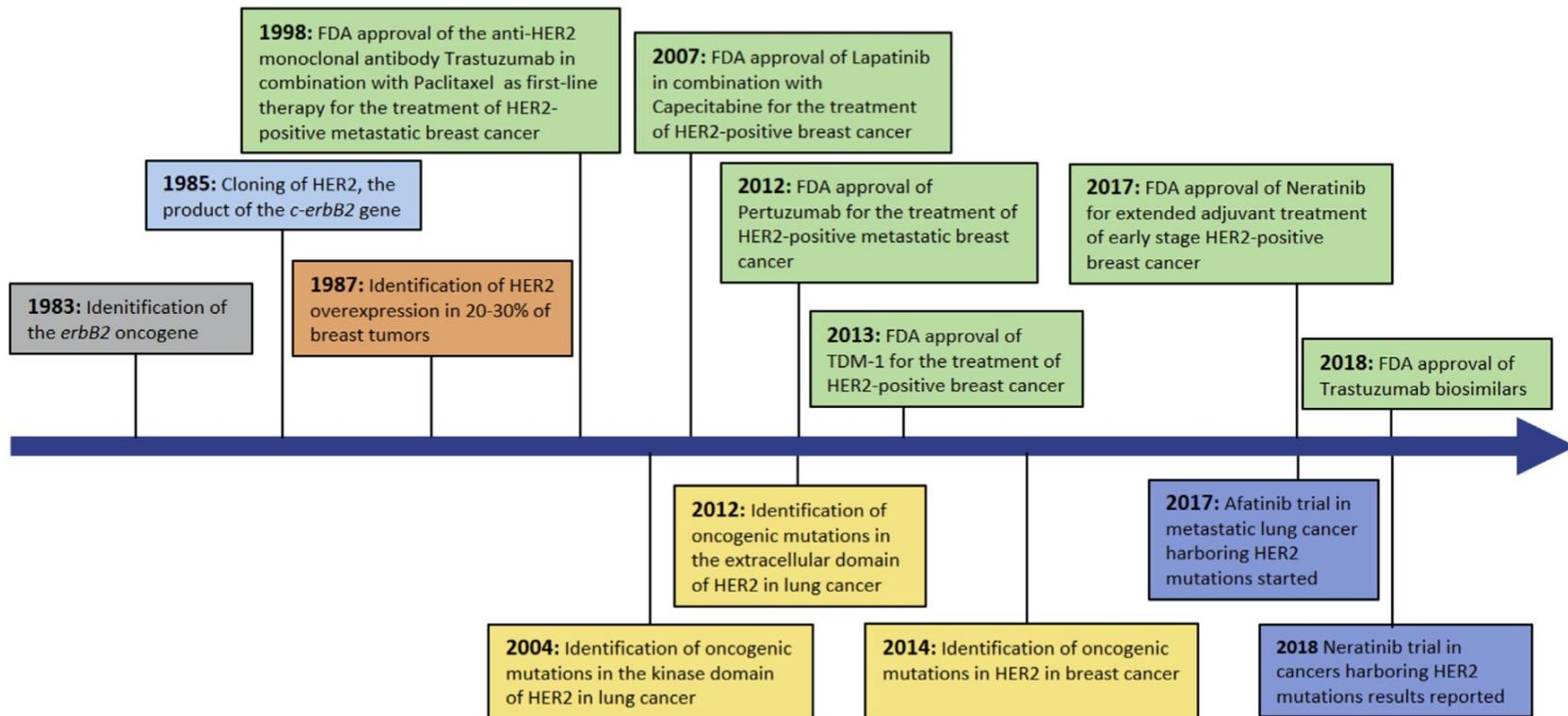


Fig. 1. Timeline of key advances relating to the biology and therapeutic targeting of HER2 signaling. Milestone discoveries that are relevant to HER2 amplified/overexpressing (boxes above the timeline arrow) and HER2 mutant tumors (boxes below the timeline arrow). Key events relating to the following fields of study are color coded as follows: FDA approval of anti-HER2 drugs (green); identification of HER2 mutation in cancers (yellow); clinical trial enrolling HER2 mutated cancers (blue).

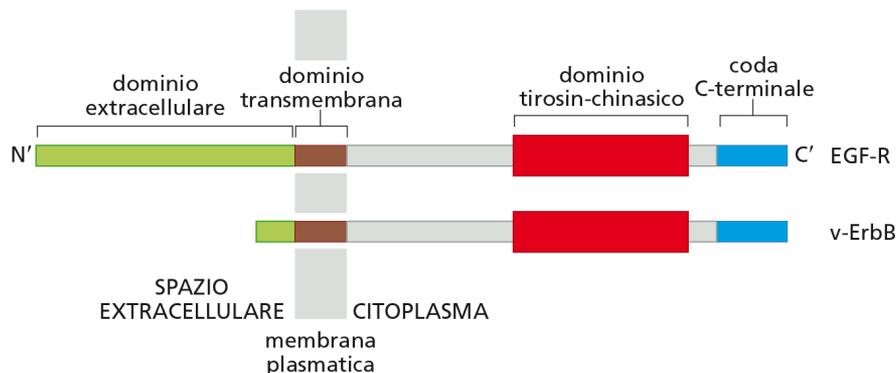
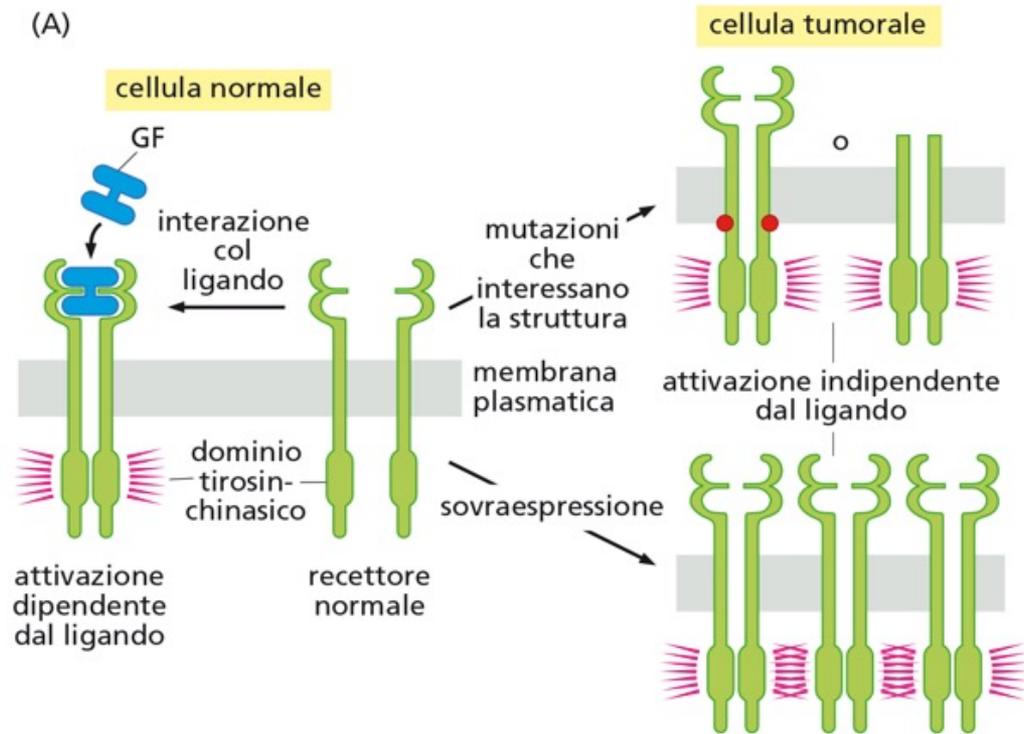
Come HER2 diventa oncogene?

❖ Ipotesi: Oncogene causa la stimolazione costitutiva della via di trasduzione del segnale indipendente da EGF

❖ Quali tipi di mutazioni?

Sovraespressione

Mutazioni strutturali



HER2 mutations in cancer

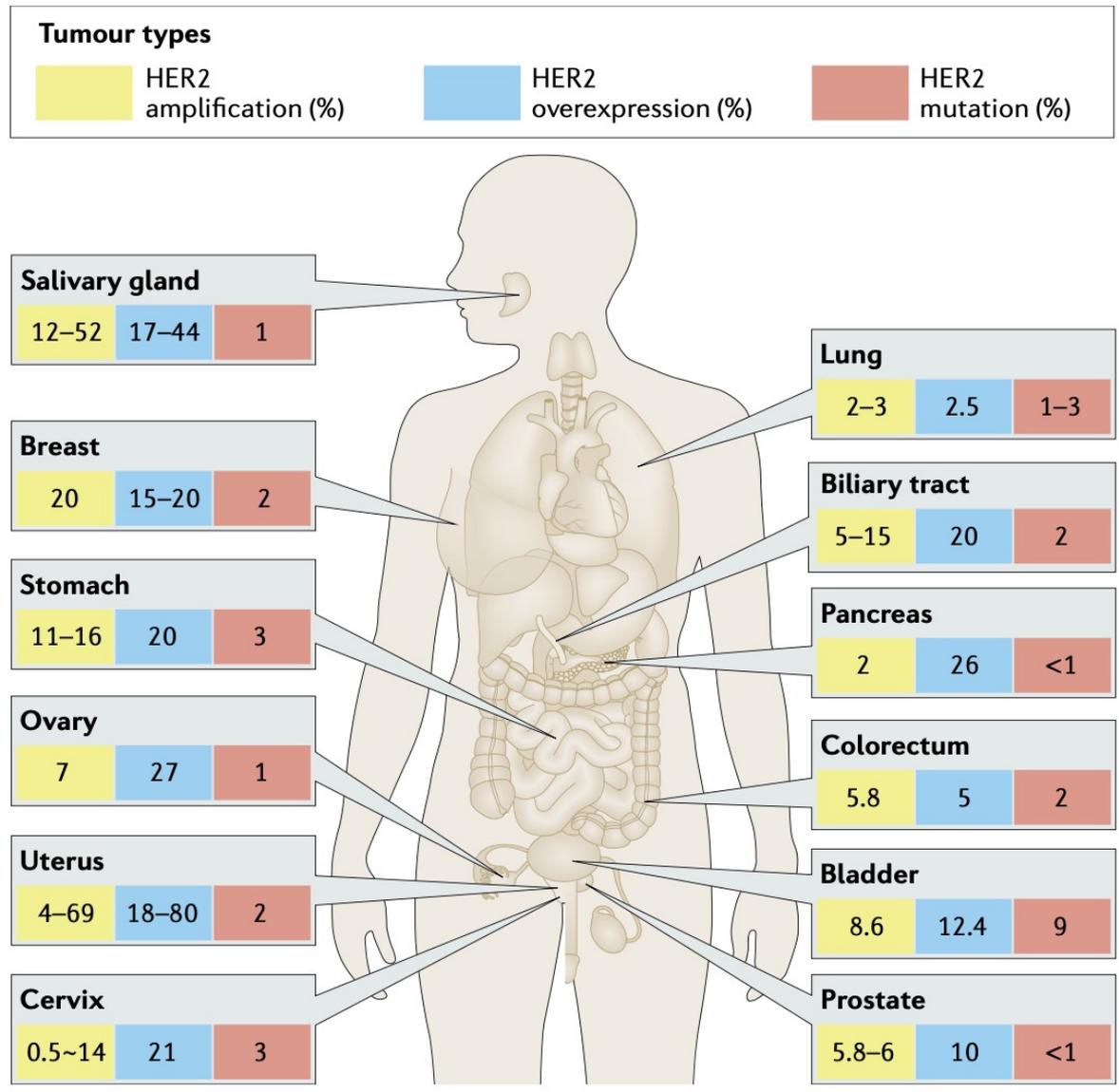
TABELLA 5.2

Recettori di fattori di crescita tirosin-chinasici alterati nei tumori umani^a

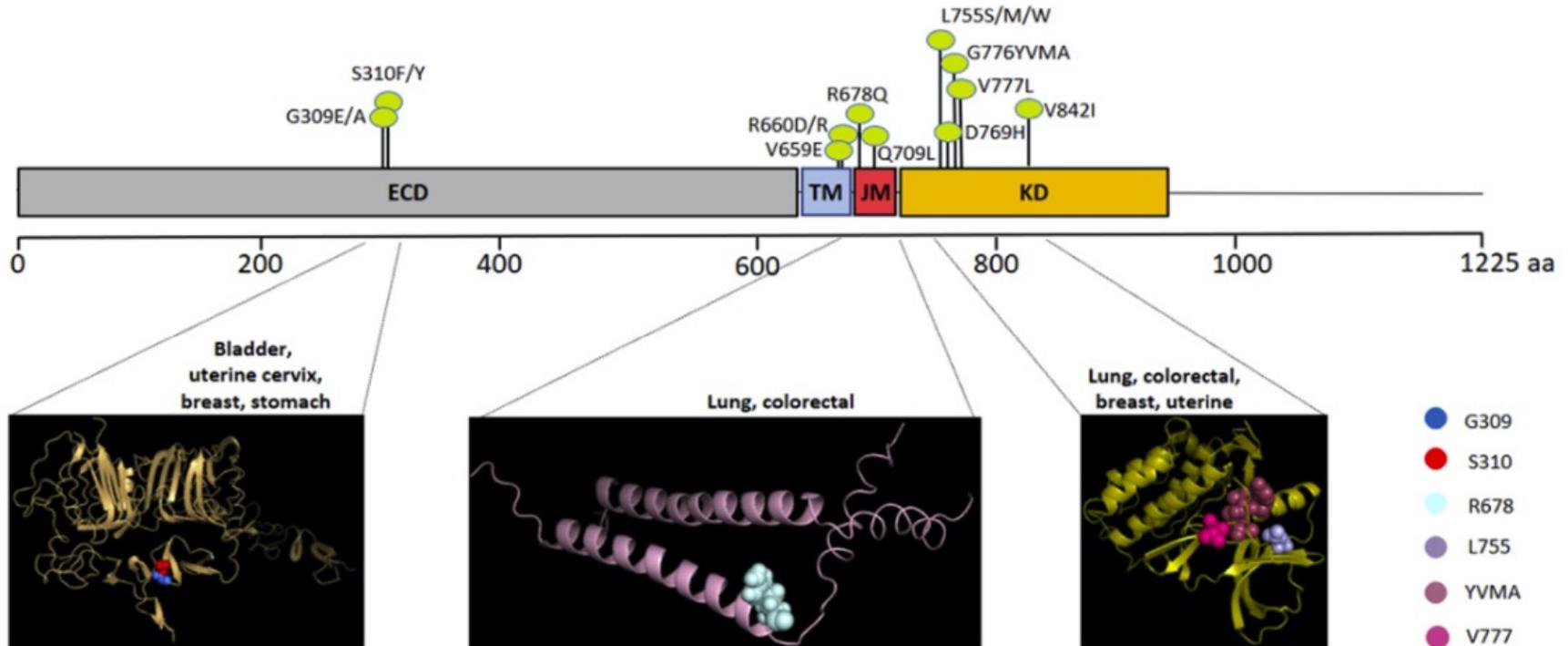
Nome del recettore	Ligando principale	Tipo di alterazione	Tipo di tumore
EGF-R/ErbB1	EGF, TGF- α	sovraespressione	tumore polmonare non a piccole cellule; carcinomi di mammella, testa-collo, stomaco, colon-retto, esofago, prostata, vescica, rene, pancreas e ovaio; glioblastoma
EGF-R/ErbB1		troncamento del dominio extracellulare	glioblastoma; carcinomi di polmone e mammella
ErbB2/HER2/Neu	NRG, EGF	sovraespressione	30% degli adenocarcinomi della mammella
ErbB3, 4	vari	sovraespressione	carcinoma a cellule squamose del cavo orale
Flt-3	FL	uplicazioni in tandem	leucemia mieloide acuta
Kit	SCF	sostituzioni amminoacidiche	tumori gastrointestinali stromali
Ret	GFL	fusione con altre proteine; mutazioni puntiformi	carcinomi papillari della tiroide; neoplasie endocrine multiple 2A e 2B
FGF-R2	FGF	sostituzioni amminoacidiche	carcinomi di mammella, stomaco, endometrio
FGF-R3	FGF	sovraespressione; sostituzioni amminoacidiche; traslocazioni	mieloma multiplo; carcinomi di vescica e cervice uterina; leucemia mieloide acuta
PDGF-R β	PDGF	traslocazioni	leucemia mielomonocitica cronica

^a Vedi anche Figura 5.16.

HER2 mutations in cancer



HER2 mutations in cancer



All these mutations converged to tyrosine phosphorylation of cellular effectors

Effects of mutations in receptor protein?

Mutazioni nella struttura

- ❖ Mutazioni che causano la dimerizzazione del recettore indipendente dal ligando e senza sovraespressione: mutazioni puntiformi o piccole delezioni
- ❖ Mutazioni che modificano l'attività del recettore indipendentemente dalla dimerizzazione: es. modifiche torsionali che inducono l'attivazione del dominio chinasi



Mutazioni nella struttura

- ❖ Es. mutazione puntiforme che causa cambiamento aminoacidico Valina → Ac. Glutamico in porzione transmembrana di **oncogene neu** (HER2) di ratto
- ❖ Esposizione femmine gravide a mutageno → progenie con tumori → stessa mutazione puntiforme Val664→Glu

❖ **Perché è così specifica?**

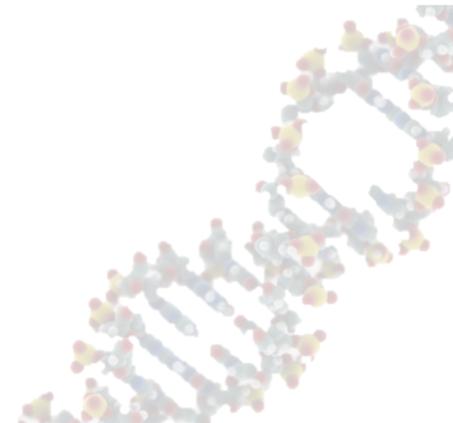


Mutazioni puntiformi oncogene *neu*

The EMBO Journal vol.7 no.7 pp.2043 – 2052, 1988

Oncogenic activation of the *neu*-encoded receptor protein by point mutation and deletion

- ❖ Proteina codificata da gene *neu* è p185
- ❖ A differenza di *v-erbB* che porta delezione, oncogene *neu* trasformante ha mutazione puntiforme Val664→Glu
- ❖ Mutazione è specifica?
- ❖ Qual è il meccanismo d'azione di questa mutazione?



Mutazioni puntiformi oncogene *neu*

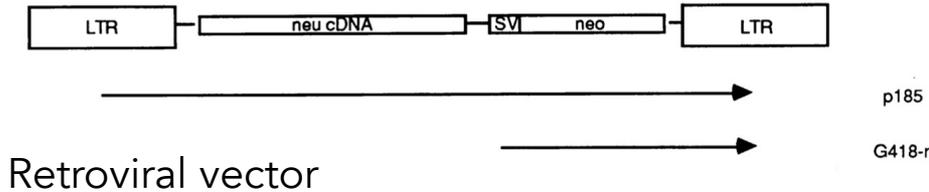
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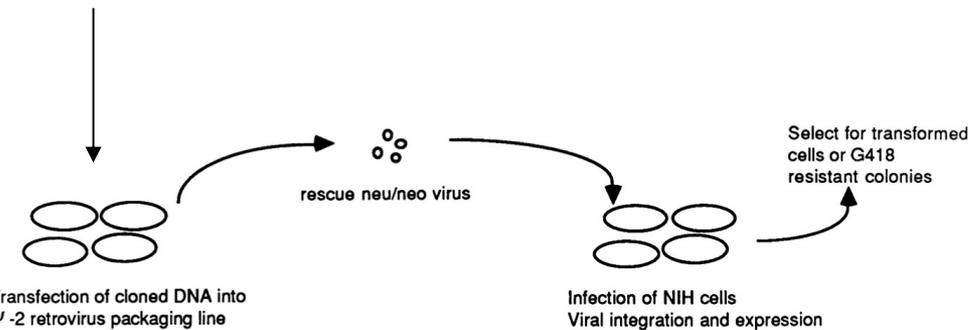
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Costruzione mutazioni puntiformi intorno a residuo 664: mutagenesi sito-diretta di Val664 in diversi altri residui o di residui adiacenti a Val664

Mutazioni puntiformi oncogene *neu*



Retroviral vector



Ratio of transformed foci to G418-resistant colonies = measurement of the specific transforming activity of each *neu* allele.

Tested 1 or 2 independent isolates for each mutation

Only two *neu* alleles have transforming activity

Table I. Biological activity of *neu* clones bearing point mutations at amino acids 663–665

Virus	Foci per 1000 <i>neo</i> colonies
Experiment 1	
Nml	10
Asp-664 (2)	10, 10
Lys-664 (2)	<5, <5
Gly-664	1
Gln-664 (2)	1000, 1000
Glu-664	1000
Experiment 2	
Nml*	1
Asp-664*	20
Gly-664*	5
Lys-664*	<5
Gln-664*	600
Glu 664*	2500
Experiment 3	
Nml*	3
Asp-664* (3)	9, 9, 8
Gly-664*	3
Lys-664*	0.1
Gln-664*	700
Glu-664*	1300
His-664	2
Tyr-664	10
Glu-663	5
Glu-665 (2)	4, 4
Asp-665	8

The number of independently derived virus producer cell lines is given in parentheses following the virus. Asterisks denote clonal producer cell lines.

Mutazioni puntiformi oncogene *neu*

The results described here with the *neu* gene suggest a different mechanism of activation. Only two out of eight residues tested at amino acid position 664 lead to full activation of the oncogenic potential of the gene, and a third gives weak activation; five out of eight substituents tested do not substantially activate the gene. Some of these substituted residues have side chains quite distinct from that of the valine, so it seems very unlikely that the *neu* gene is activated by destroying some specific property of the normally present valine residue. Rather, a novel or augmented activity must be ascribed to the presence of the activating glutamic acid and glutamine residues at this position.

Gain-of-function
mutation?

Mechanism???

Mutazioni puntiformi oncogene *neu*

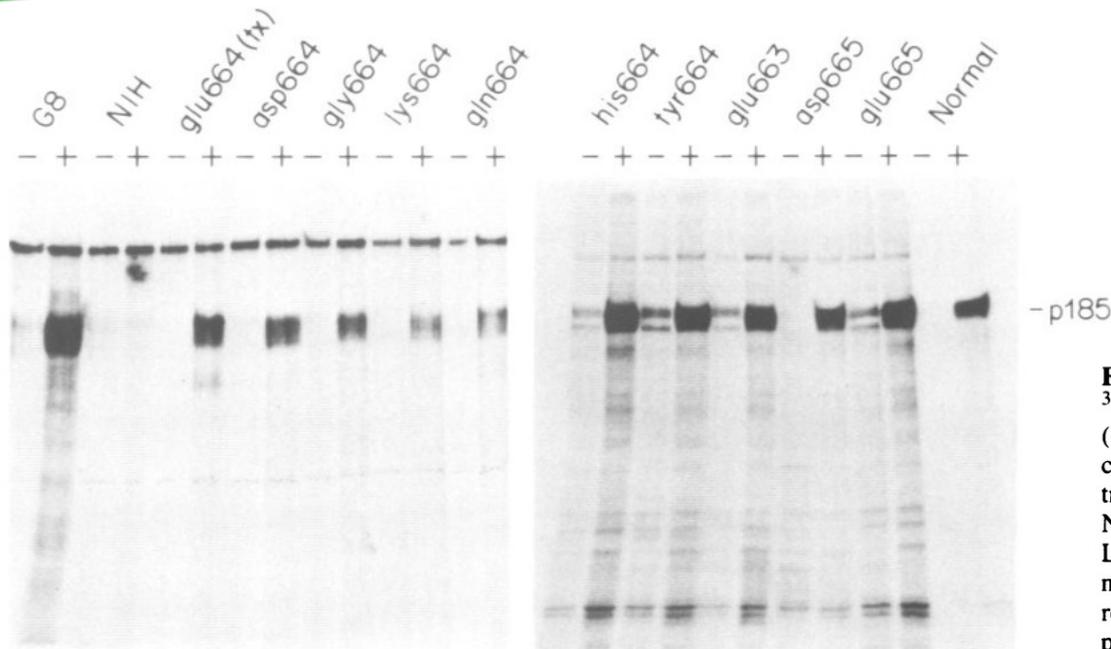


Fig. 2. Expression of p185 in cell lines bearing pDOL/*neu*. ³⁵S-labelled lysates of cells were incubated either with (+) or without (-) monoclonal anti-p185 antibody 7.16.4. (Drebin *et al.*, 1984) and collected on Protein A Sepharose. G8 (DHFR G8 cells) NIH cells transfected with a normal genomic *neu* gene (Hung *et al.*, 1986). NIH, uninfected NIH-3T3 cells. Glu-664 (tx), Asp-664, Gly-664, Lys-664, Gln-664, His-664, Tyr-664, Glu-663, Asp-665, Glu-665 and normal, clonal cell lines expanded from G418-resistant colonies resulting from infection with pDOL/*neu* viruses bearing each of the point-mutated *neu* genes.

- ❖ The p185 protein was present in similar amounts in all cell lines

Mutazioni puntiformi oncogene *neu*

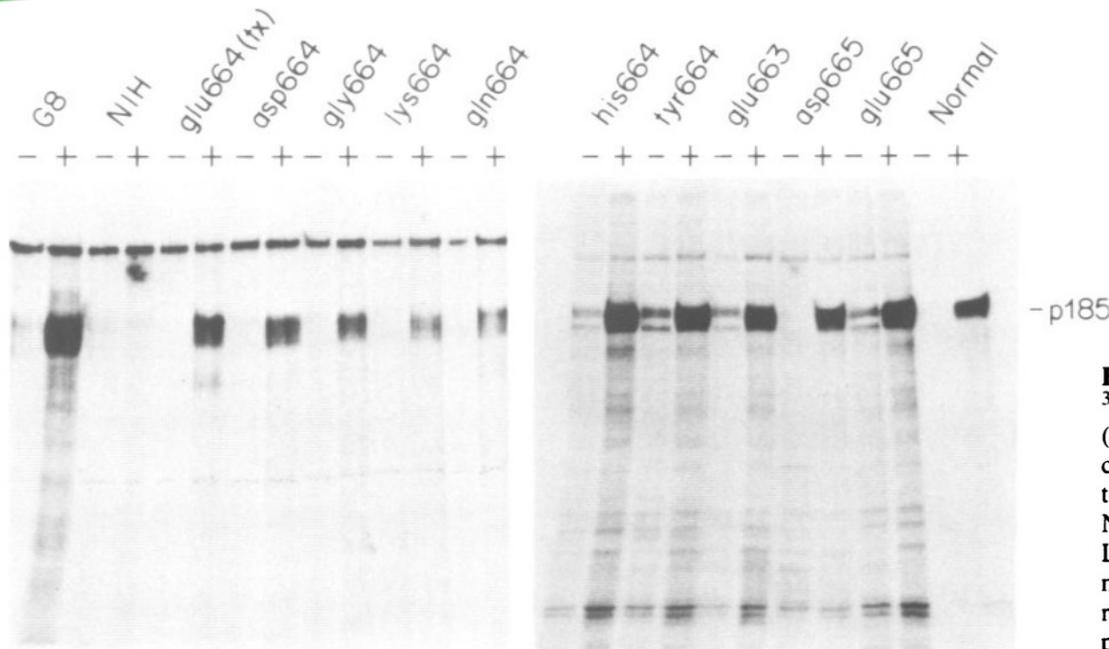


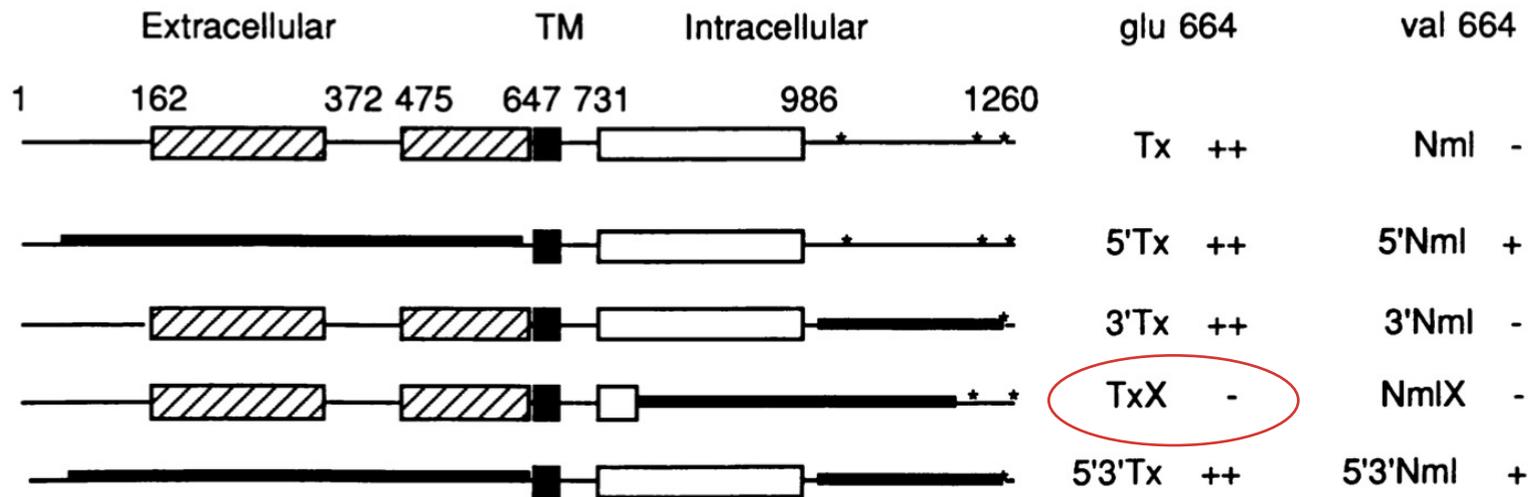
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- ❖ The p185 protein was present in similar amounts in all cell lines
 - ❖ Normal and transforming p185 molecules are both predominantly expressed at the cell surface
 - ❖ Similar tyrosine kinase activity
 - ❖ Glu is hydrophobic → conformational change
- Kinase activity is important for oncogenic activity of *neu*-V664Q/E?

Mutazioni puntiformi oncogene *neu*

La mutazione puntiforme trasformante è in dominio transmembrana. L'attività chinase di *neu* serve per trasformare?

Delezioni e inserimenti di linker in oncogene *neu* Val664 -->Glu



Mutazioni puntiformi oncogene *neu*

CONCLUSIONS:

- Only a few specific amino acids at 664 position activate the oncogenic potential of the *neu* gene
- Correlation between transforming activity and kinase activity

Point mutations induce transformation through the kinase activity

Oncogene *c-neu* e induzione tumori nei topi

Costrutto: Promotore MMTV-LTR (Mouse mammary tumor virus) + rat *c-neu* activated cDNA

Cell, Vol. 57, 931-936, June 16, 1989, Copyright © 1989 by Cell Press

Stochastic Appearance of Mammary Tumors in Transgenic Mice Carrying the MMTV/*c-neu* Oncogene

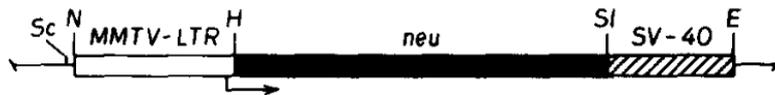


Figure 1. Schematic Representation of the Injected MMTV/*neuT* Fusion Gene

The arrow indicates the transcriptional start site located 110 bp upstream of the junction HindIII site. The restriction sites are: E, EcoRI; H, HindIII; N, NcoI; Sc, SacII; SI, SalI.

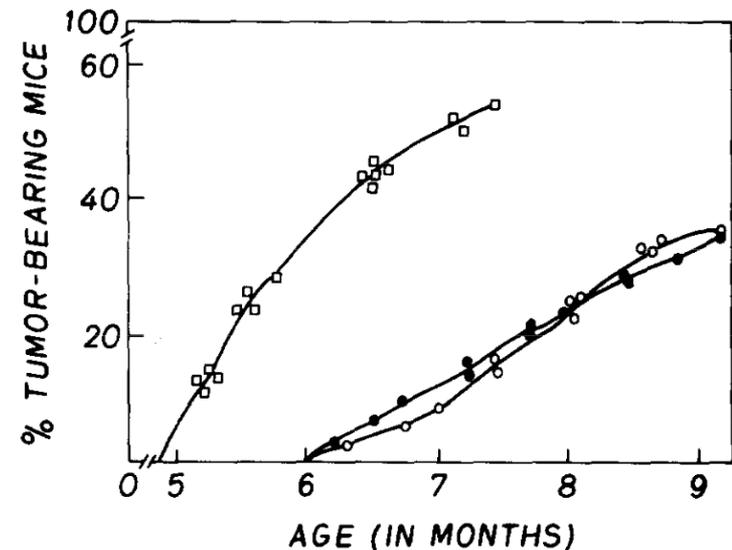
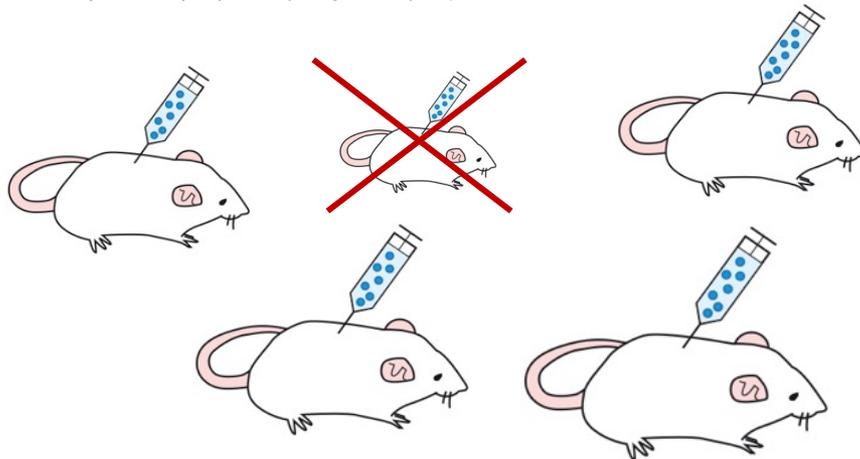
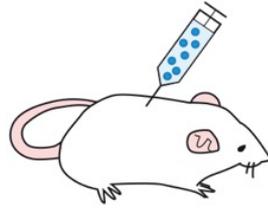


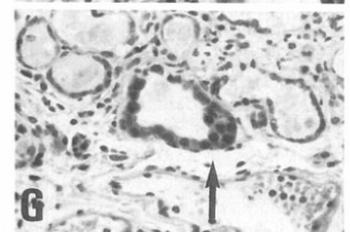
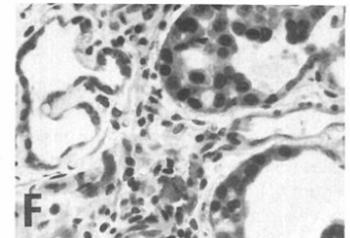
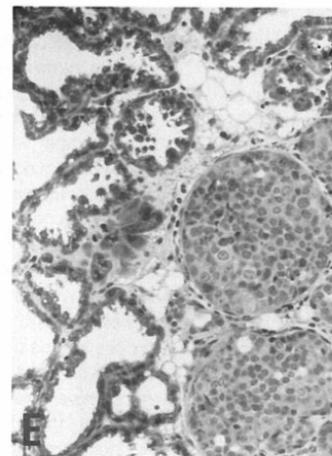
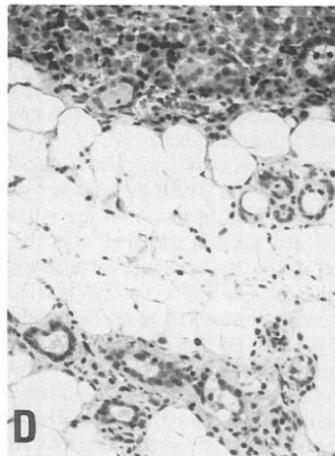
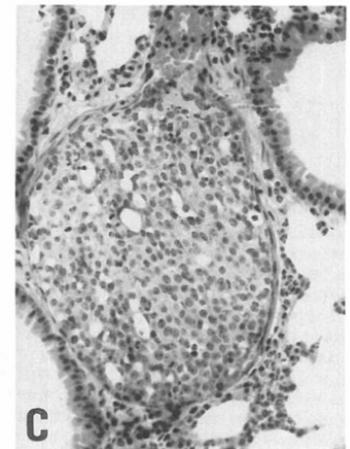
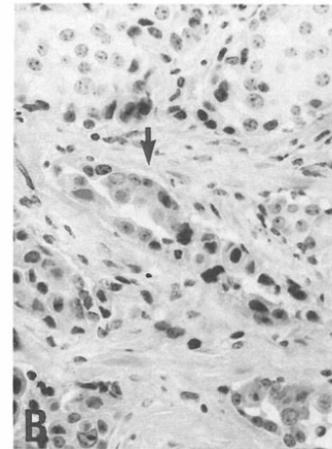
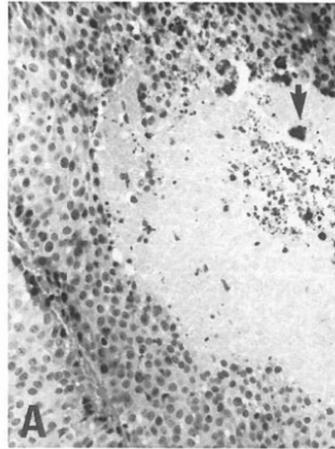
Figure 2. Cumulative Incidence of Mammary Tumors in Transgenic Females

The percentage of animals with tumors is plotted as a function of age. Data include 29, 35, and 34 females from line MN-9 (□), MN-10 (●), and MN-17 (○), respectively.

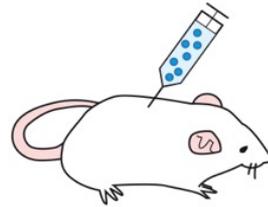
Oncogene *c-neu* e induzione tumori nei topi



Morfologia del tumore
simile a adenocarcinoma
duttale umano



Oncogene *c-neu* e induzione tumori nei topi



Altri tumori e fenotipi

Table 1. Pathologies Associated with Transgenic Mice Carrying the MMTV/*neuT* Fusion Gene

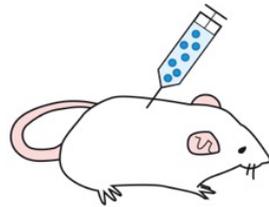
Pathology	Sex	% of Mice Affected (no./total) in Line			
		MN-9	MN-10	MN-12	MN-17
Mammary tumor ^a	Female	50 (18/36)	44 (18/41)	28 (2/7)	35 (15/43)
	Male	0 (0/16)	0 (0/40)	0 (0/9)	0 (0/34)
Salivary gland tumor ^a	Female	0 (0/36)	5 (2/41)	0 (0/7)	0 (0/43)
	Male	0 (0/16)	0 (0/40)	33 (3/9)	0 (0/34)
Splenomegaly ^b	Female	100 (10/10)	100 (7/7)	100 (1/1)	100 (2/2)
	Male	0 (0/3)	0 (0/1)	0 (0/1)	—
Seminal vesicle and epididymis enlargement ^b	Male	100 (3/3)	100 (1/1)	100 (1/1)	—
Unilateral proptosis ^a	Female	0 (0/36)	5 (2/41)	0 (0/7)	0 (0/43)
	Male	0 (0/16)	8 (3/40)	0 (0/9)	0 (0/34)

^a Only data from animals older than 5 months were included in this group.

^b These lesions were observable at autopsy. Thus, only autopsied animals are included in this group.

Oncogene *c-neu* e induzione tumori nei topi

Analisi di espressione del transgene per northern blot



Transgenic mice carrying the MMTV-*neu* oncogene have a strong heritable predisposition to the development of mammary adenocarcinomas.

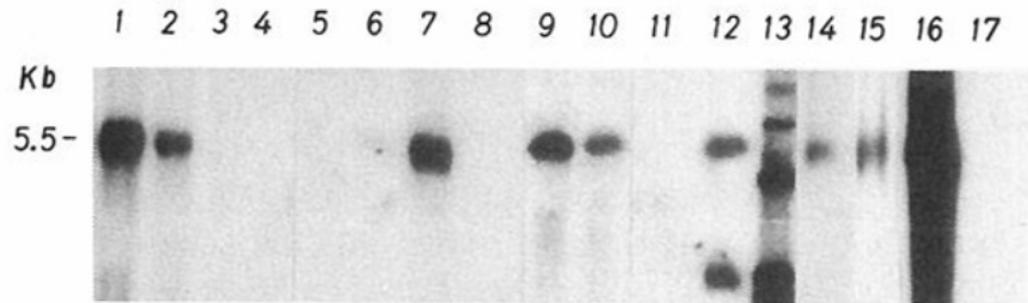


Figure 4. Northern Blot Analysis of the MMTV-*neu* Expression in Representative Normal and Tumor Tissues of Transgenic Mice

Hybridization was with a ^{32}P -labeled SV40 Sall-EcoRI fragment from MMTV*neuT* DNA. RNA (20 μg) from nontumoral tissues of mice from line MN-9: two independent lactating mammary glands at 4 months of age (lanes 1 and 2), brain (lane 3), harderian gland (lane 4), testis (lane 5), seminal vesicle (lane 6), epididymis (lane 7), and kidney (lane 8). RNA from tumors: two distinct mammary tumors from mouse MN-158-9 (lanes 9 and 10), salivary gland tumor from mouse MN-250-12 (lane 14), enlarged epididymis from mouse MN-291-9 (lane 15), enlarged seminal vesicle from mouse MN-250-12 (lane 16), and enlarged spleen from mouse MN-39-9 (lane 17). Negative control rat-1 cells (lane 11) and positive control MMTV*neuT*-transformed rat-1 cells (lane 12). HindIII-digested ^{32}P -labeled lambda DNA was used as a marker (lane 13).

Sovraespressione recettori

Deregolazione trascrizionale

Amplificazione genica

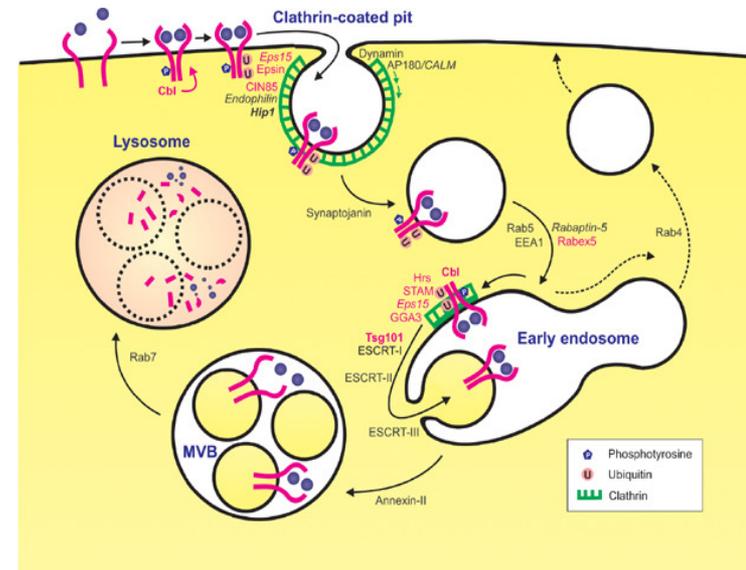
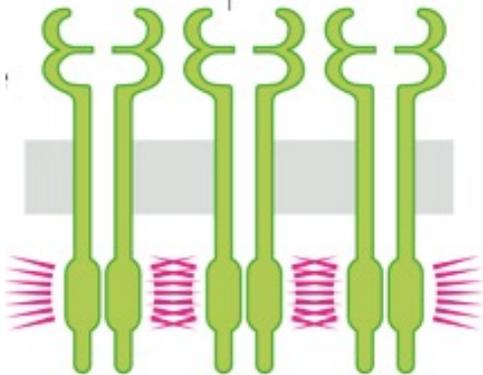
Difetti di endocitosi



Sovraespressione

Cellule iper-responsive a EGR

attivazione indipendente dal ligando

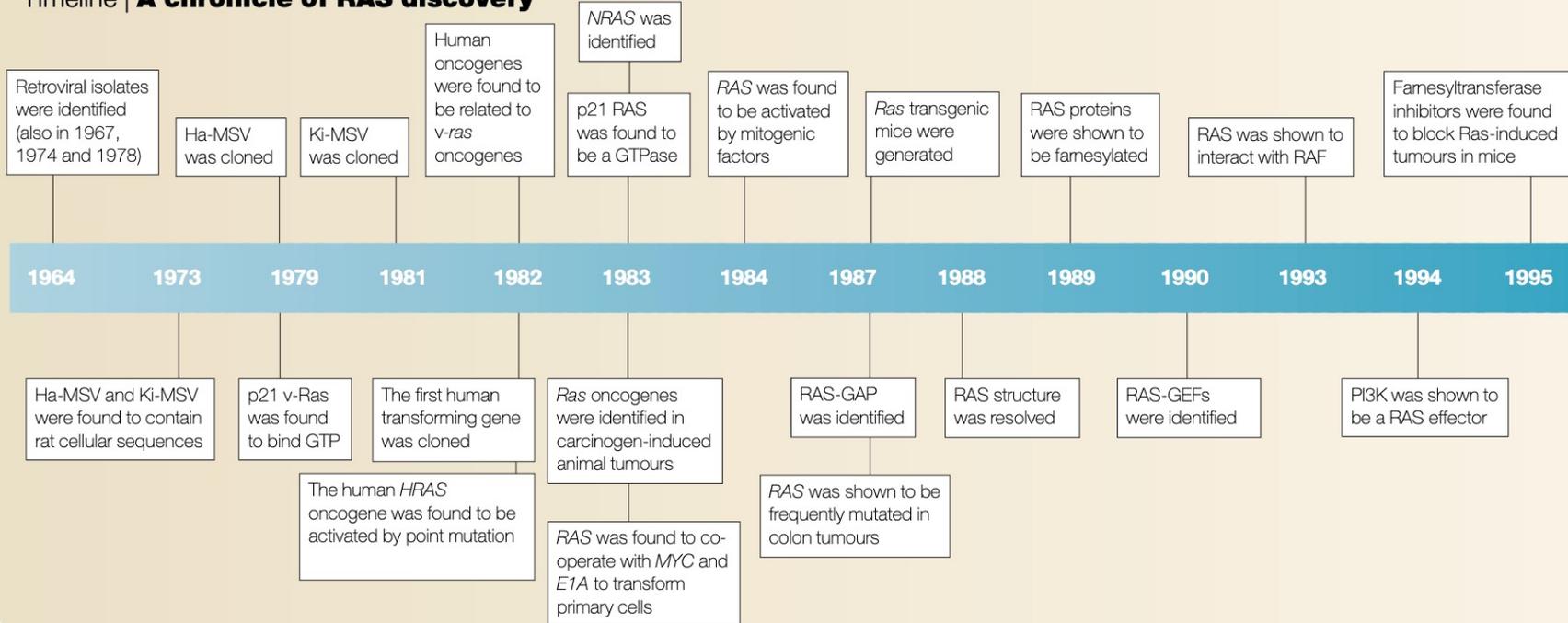


L'ONCOGENE RAS E LA TRASDUZIONE DEL SEGNALE



RAS history

Timeline | A chronicle of RAS discovery



Jennifer Harvey, 1964: preparazione di virus della leucemia di topo induce sarcomi in topi e ratti
RAS: RAt Sarcoma



Geni umani trasformanti

Cellule umane di cancro della vescica

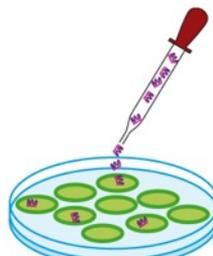


DNA

trasfezione mediante
coprecipitazione
con fosfato di calcio



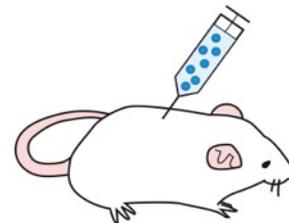
DNA



fibroblasti
murini
normali
NIH3T3

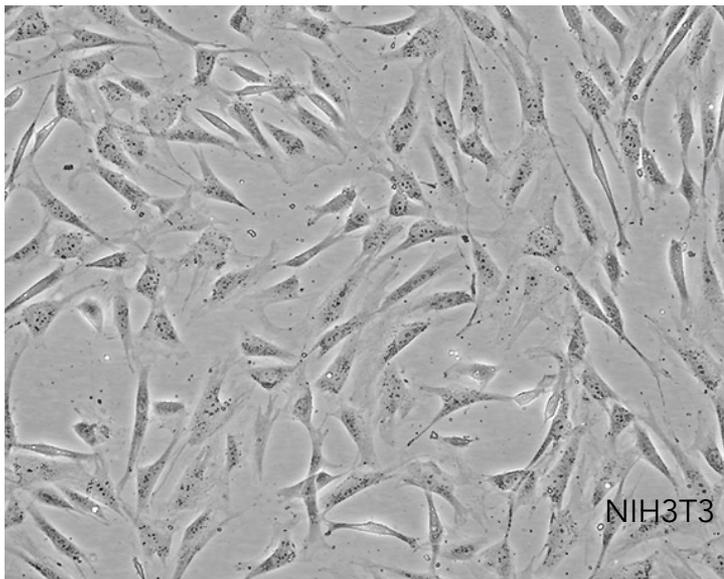


formazione
di un *focus*
di cellule
morfologicamente
trasformate



inoculo
delle cellule
morfologicamente
trasformate
in un topo

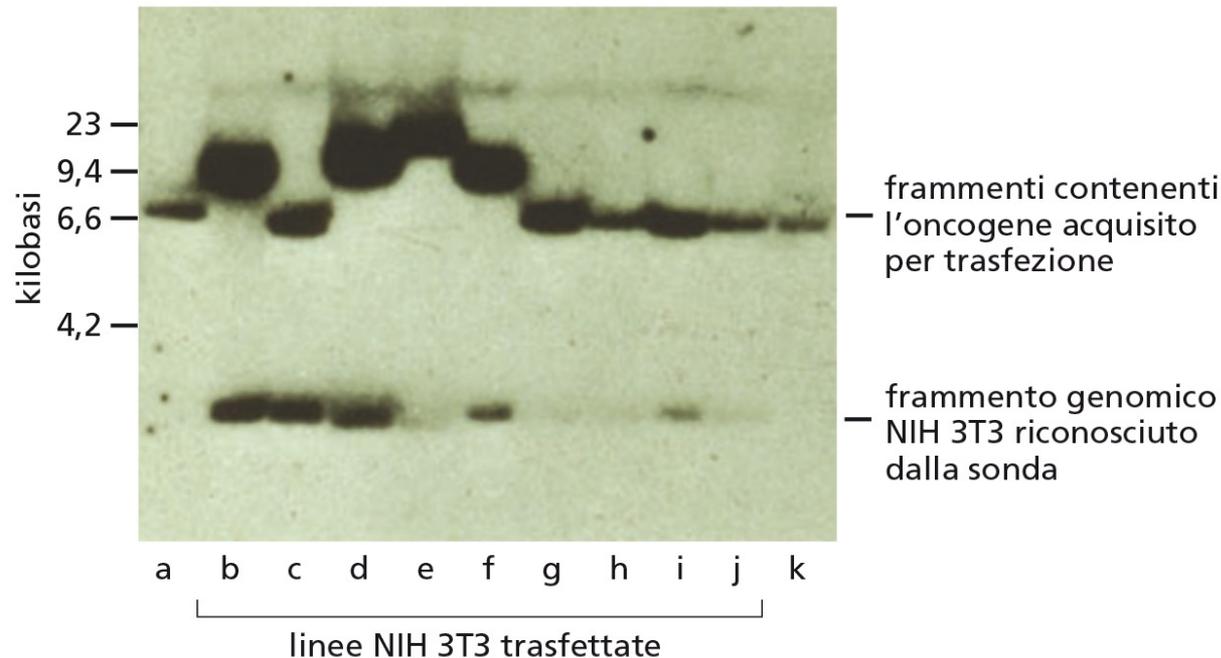
tumore



NIH3T3

Oncogeni virali e oncogeni cellulari

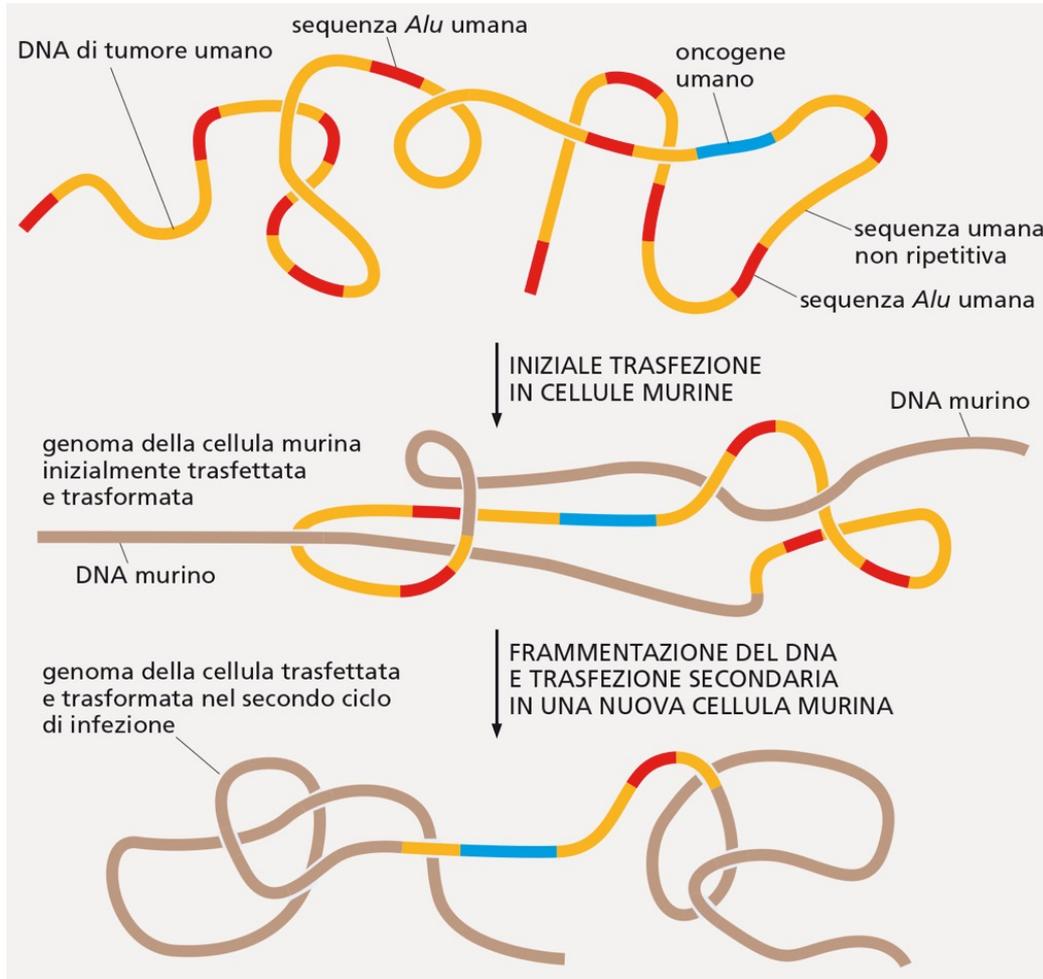
Proto-oncogeni attivati da virus o da mutageni sono diversi?



Sonda H-ras da sarcoma murino di Harvey (virale) riconosce DNA di cellule NIH-3T3 trasfettate con DNA da cellule umane di carcinoma della vescica

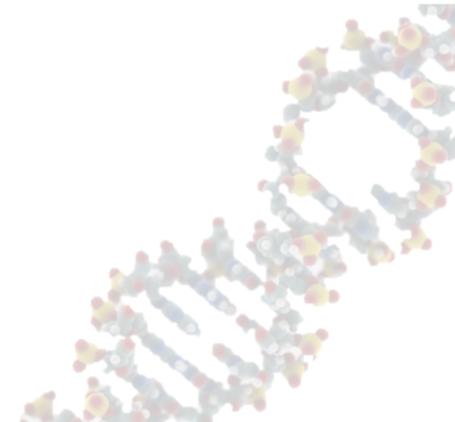
Ricerca dei «geni del cancro»

Clonaggio molecolare degli oncogeni



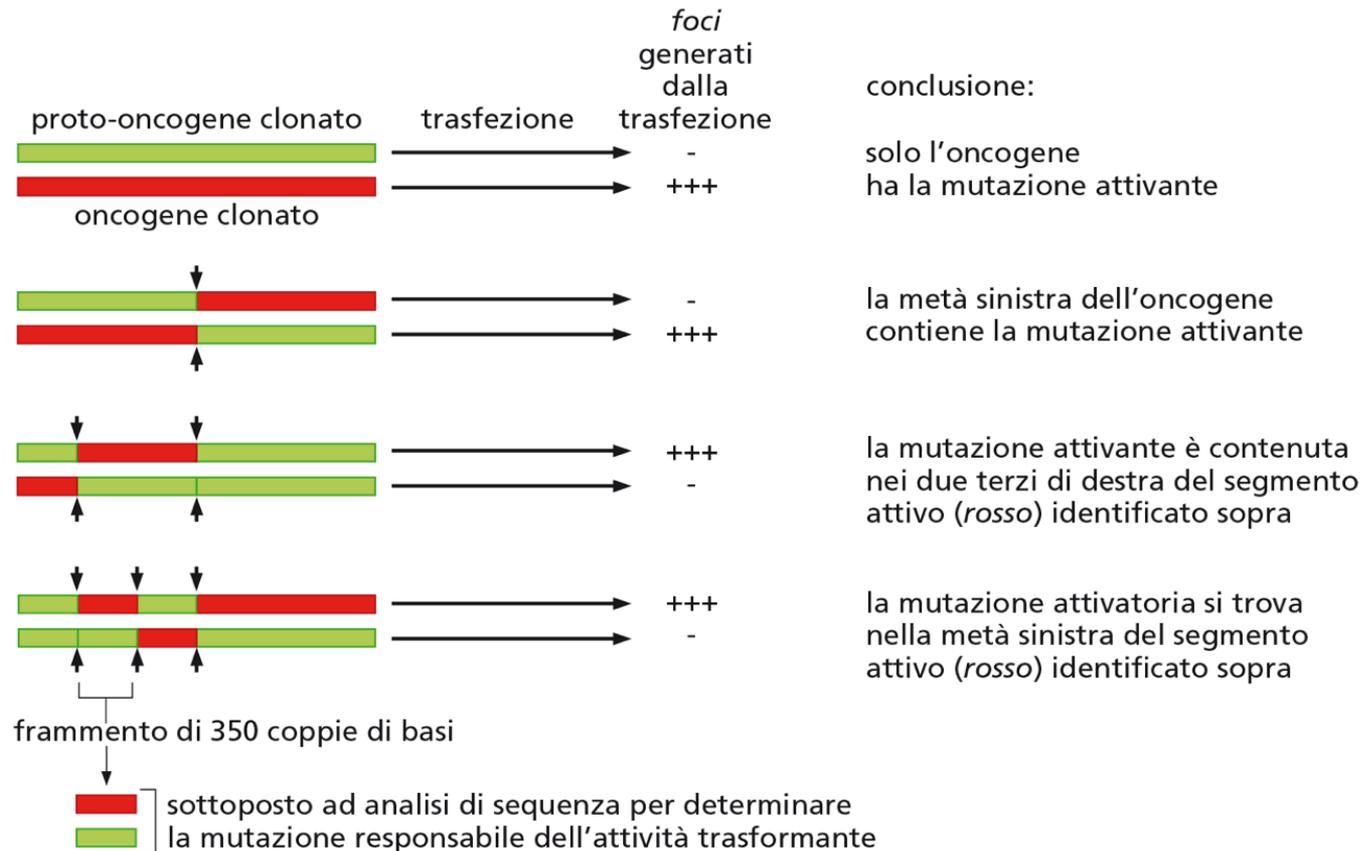
Sequenze *Alu* disseminate nel genoma umano ogni circa 5 kb e assenti in topo.

Dai secondi trasfettanti crea una libreria genomica



Mutazioni puntiformi nell'oncogene *H-ras*

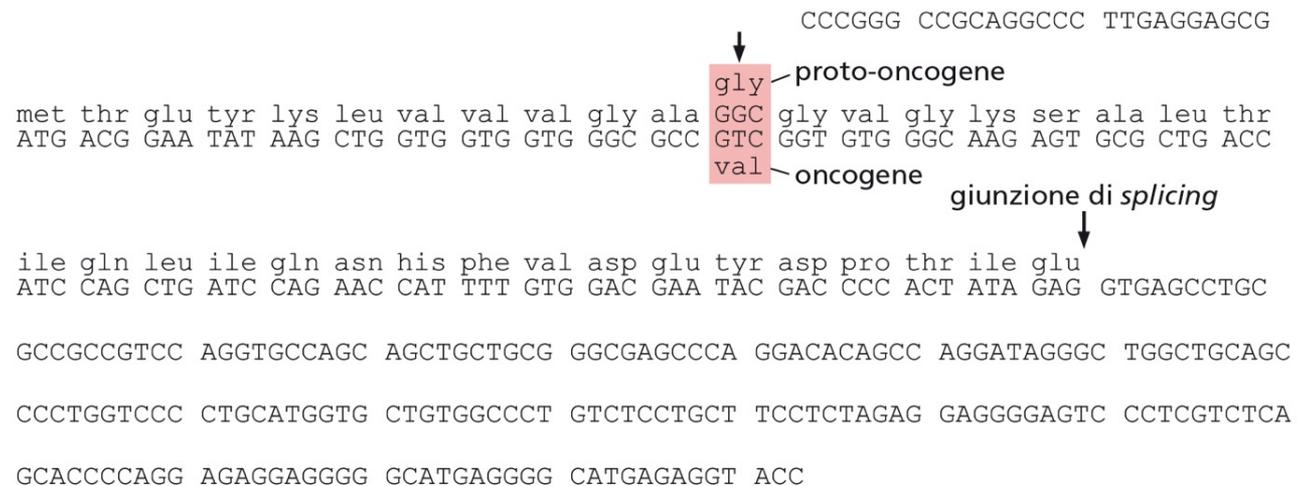
- ❖ Singola copia in carcinoma della vescica → No amplificazione
- ❖ Mappato sullo stesso frammento in cellule normali e tumorali → stessa struttura del gene
- ❖ Ricombinazione segmenti di proto-oncogene e oncogene



Mutazioni *H-ras*

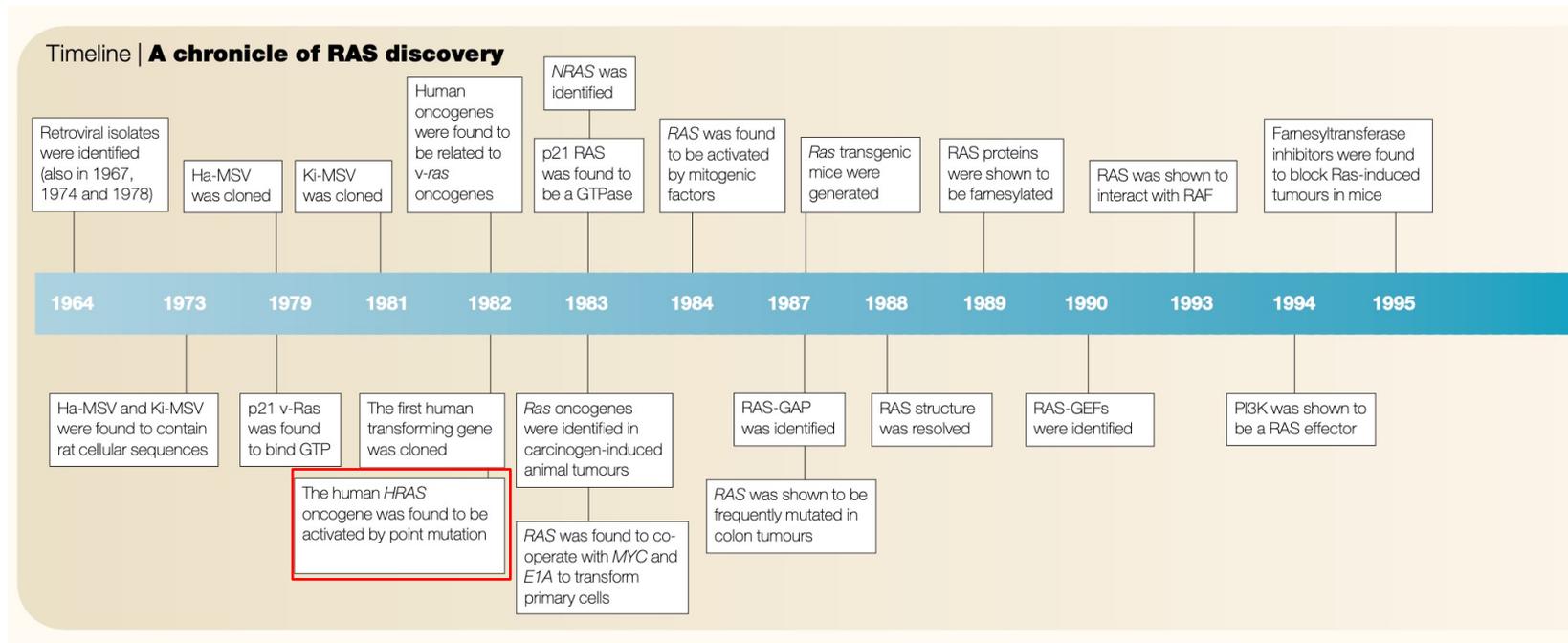
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- ❖ Mappato sullo stesso frammento in cellule normali e tumorali → stessa struttura del gene
- ❖ Ricombinazione segmenti di proto-oncogene e oncogene → regione critica
- ❖ Sequenza regione critica

Una mutazione puntiforme attiva l'oncogene *H-ras*



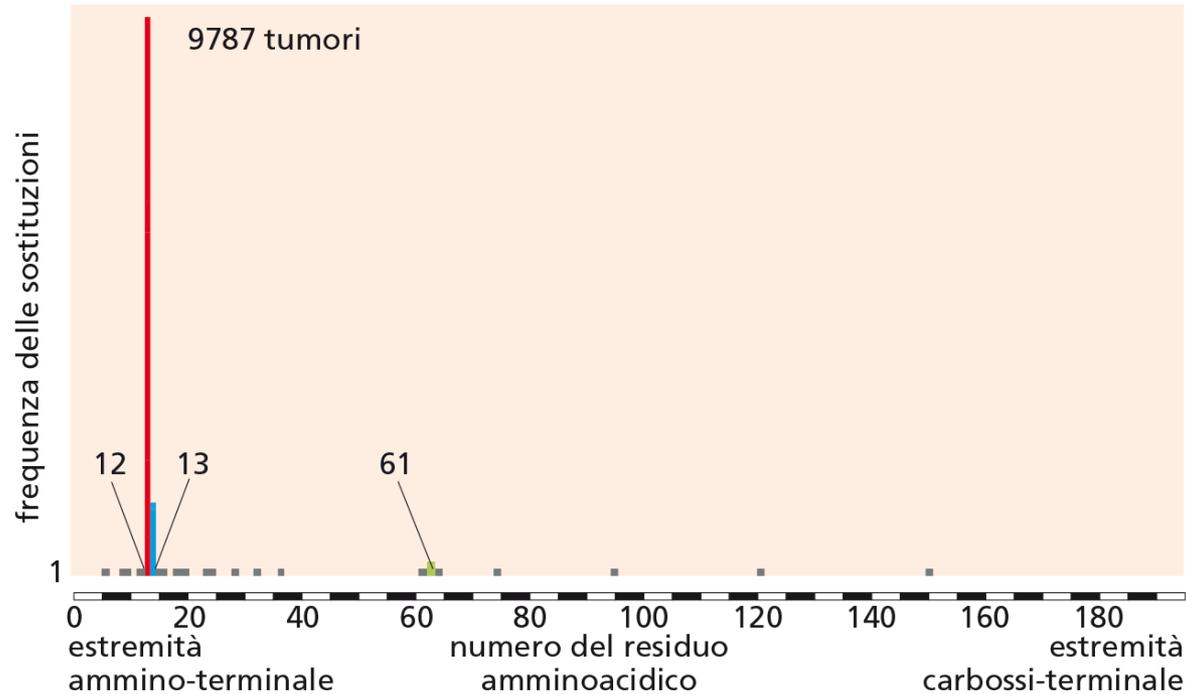
1. Reddy, E. P., Reynolds, R. K., Santos, E. & Barbacid, M. A point mutation is responsible for the acquisition of transforming properties by the T24 human bladder carcinoma oncogene. *Nature* 300, 149–152 (1982).
2. Tabin, C. J. et al. Mechanism of activation of a human oncogene. *Nature* 300, 143–147 (1982).
3. Taparowsky, E. et al. Activation of the T24 bladder carcinoma transforming gene is linked to a single amino acid change. *Nature* 300, 762–765 (1982).

Mutazioni *H-ras*



- ❖ 3 geni ras nei mammiferi: *H-ras*, *K-ras* e *N-ras*.
- ❖ Codificano per 4 proteine perché *K-ras* fa splicing alternativo
- ❖ Mutazione puntiforme trasforma cellule NIH-3T3 in vitro ma non riesce a trasformare cellule primarie

Mutazioni *ras*



- ❖ 3 geni *ras* nei mammiferi: *H-ras*, *K-ras* e *N-ras*.
- ❖ Mutazioni puntiformi in residui 12, 13, 61 di oncoproteine Ras trovate in diversi tipi di tumori
- ❖ Mutazioni molto specifiche perché attivanti



RAS

3 geni *ras* in cellule di mammifero codificano per 4 proteine Ras diverse perché *K-ras* fa splicing alternativo

Proteine di circa 21 kDa con struttura quasi identica

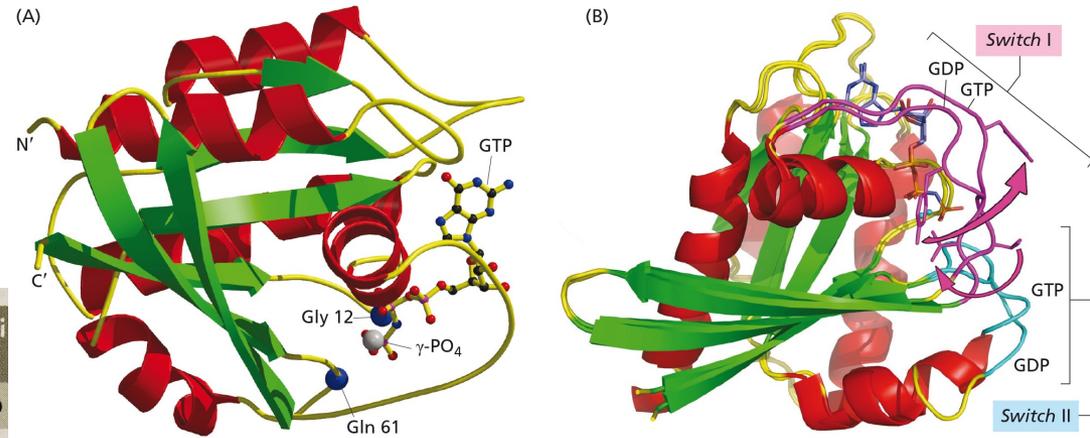


TABELLA 4.2

Lista degli oncogeni *ras* alterati per mutazione puntiforme in una serie di tumori umani

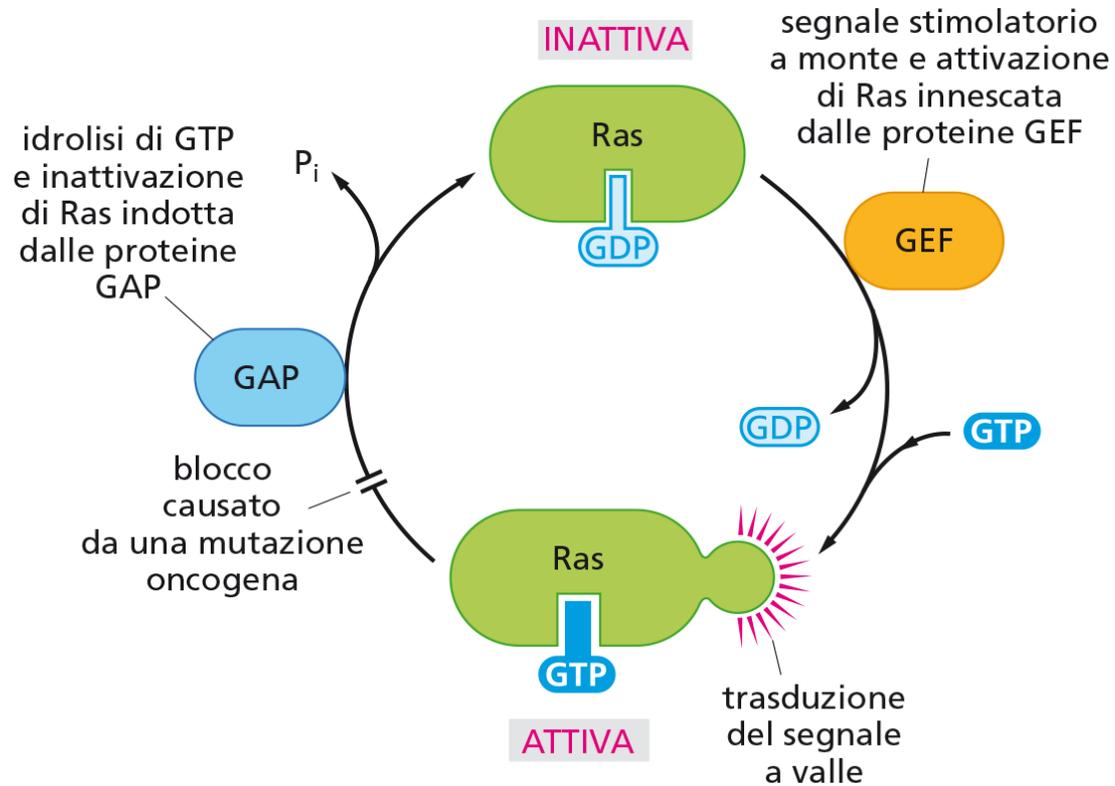
Tipo di tumore	Percentuale di tumori che hanno
Pancreas	90 (K)
Tiroide (papillare)	60 (H, K, N)
Tiroide (follicolare)	55 (H, K, N)
Colo-rettale	45 (K)
Seminoma	45 (K, N)
Mielodisplasia	40 (N, K)
Polmone (non a piccole cellule)	35 (K)
Leucemia mieloide acuta	30 (N)
Fegato	30 (N)
Melanoma	15 (N)
Vescica	10 (H, K)
Rene	10 (H)

^aH, K ed N si riferiscono, rispettivamente, ai geni umani *H-RAS*, *K-RAS*, *N-RAS*.
Adattata da J. Downward, *Nature Rev. Cancer* 3:11-22, 2003.

Poche mutazioni puntiformi trovate in vari tumori → perchè?

Mutazioni specifiche che mantengono attiva Ras. Altre mutazioni o delezioni che inattivano Ras hanno effetto opposto su crescita cellulare

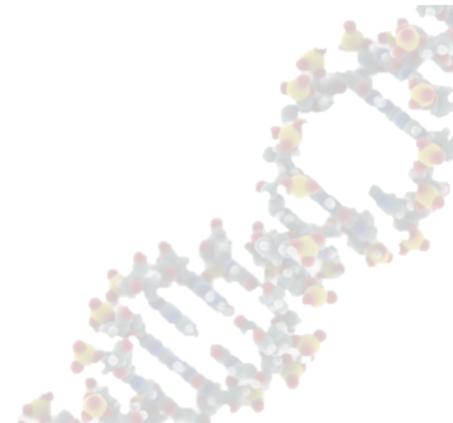
RAS



Attivazione Ras quando legata a GTP

Inattivazione richiede GAP che induce idrolisi GTP

Nuovo ciclo di attivazione di Ras richiede GEF che scambia
GDP con GTP



RAS

