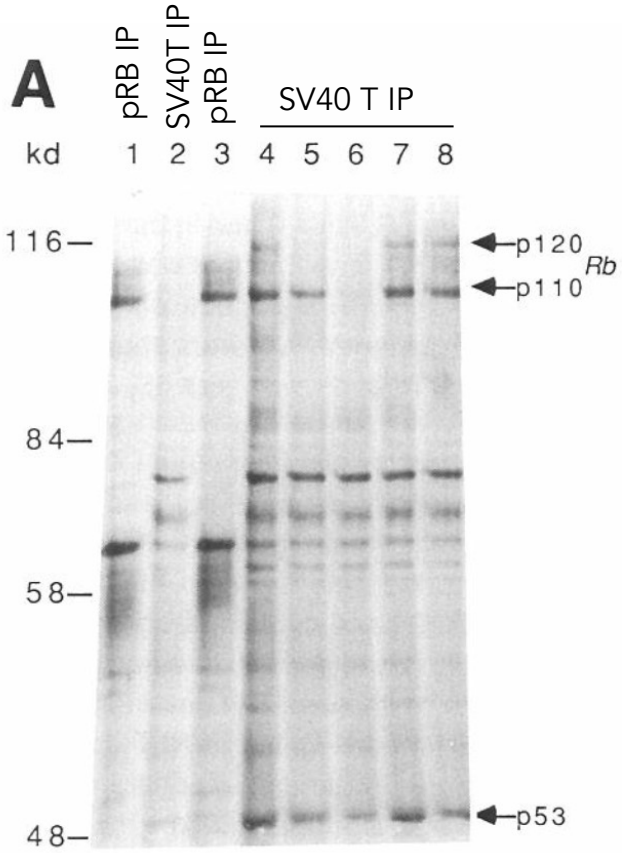
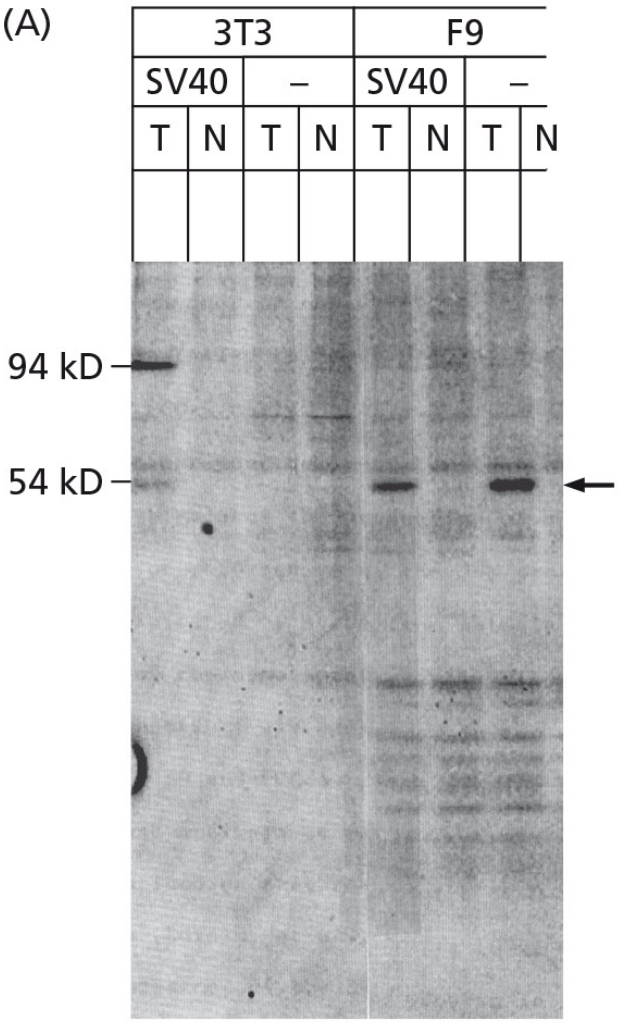
P53 E L'APOPTOSI

Scoperta di p53

1979: Large T antigen di SV40 COIP con una proteina di 53 KDa in cellule infettate

Antiseri per p53 danno segnale anche il cellule non infettate → *p53* è un gene cellulare

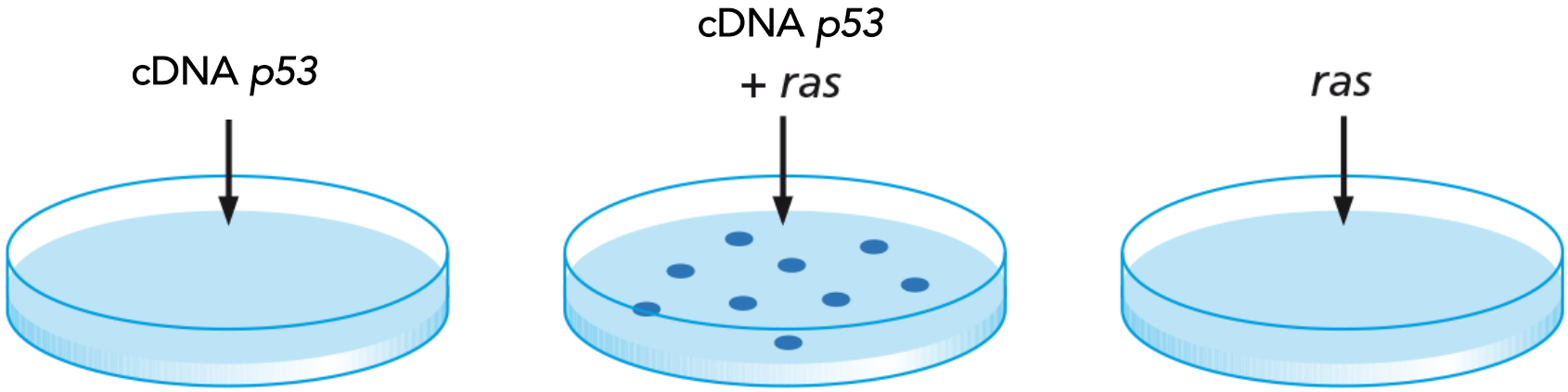




Weinberg, la Biologia del Cancro

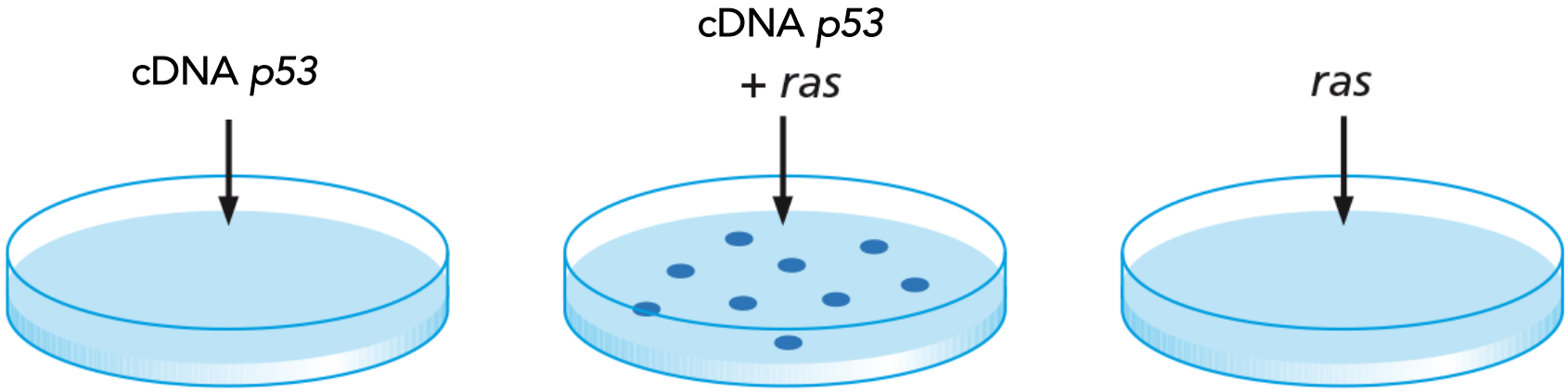
Attività trasformante di p53

Cotrasfezione cDNA *p53* con oncogene *ras* in fibroblasti embrionali di ratto



Attività trasformante di p53

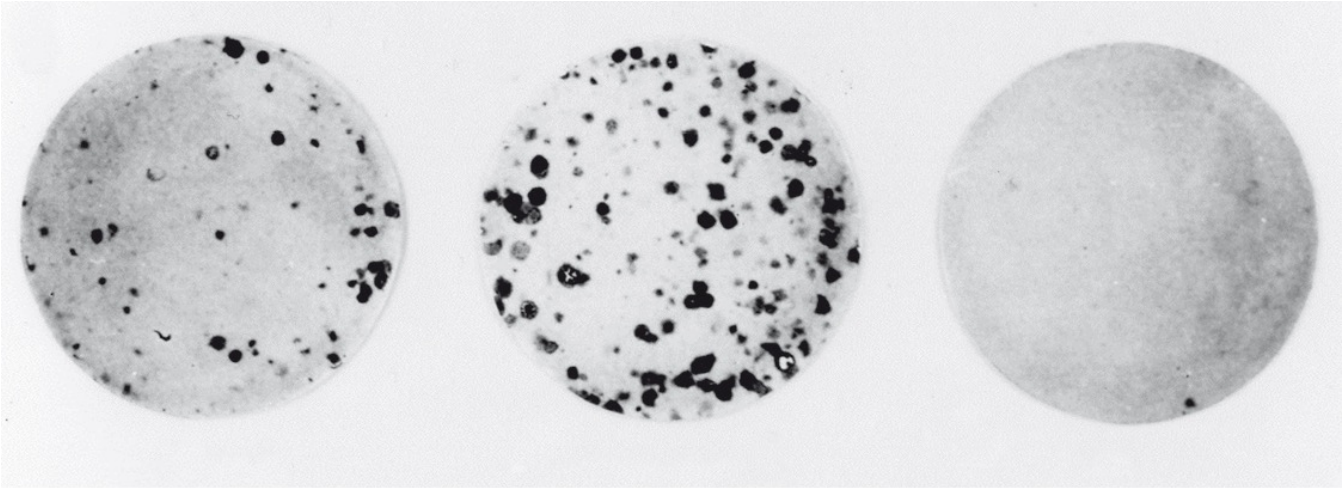
Cotrasfezione cDNA *p53* con oncogene *ras* in fibroblasti embrionali di ratto



p53 è un oncogene?

Attività trasformante di p53 selvatica e mutante

Cotrasfezione cDNA *p53* mutato e wt con oncogene *ras* in fibroblasti embrionali di ratto



ras + *p53*
mutante
con delezione

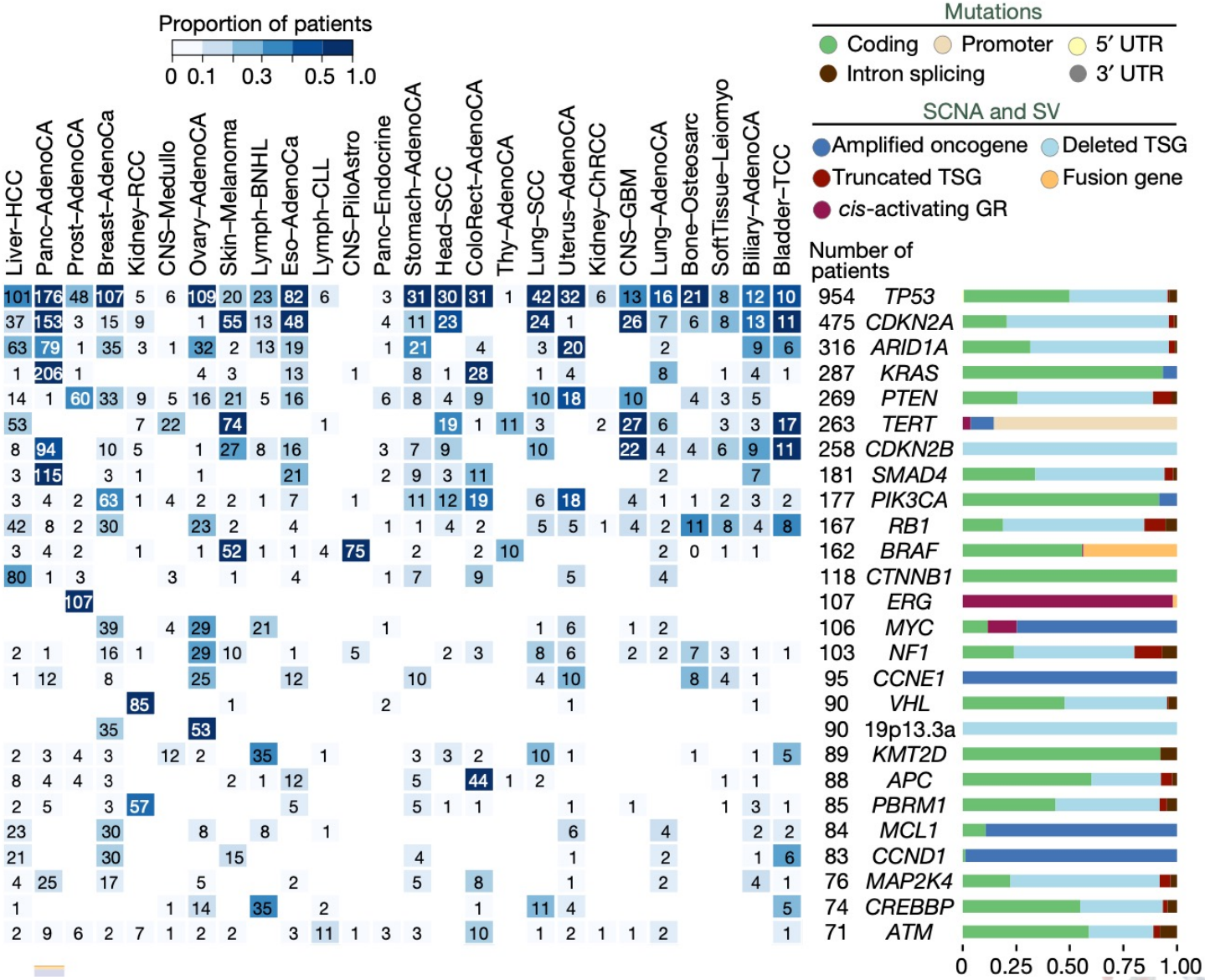
ras + *p53* val-135
con mutazione
puntiforme

ras + *p53*
normale

Weinberg, la Biologia del Cancro

Mutazioni p53 e cancro

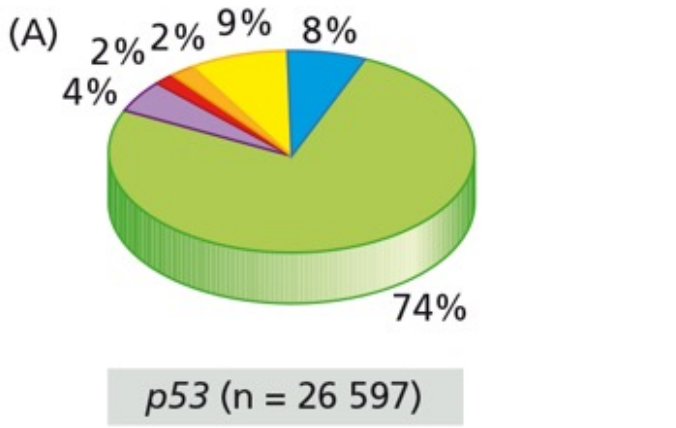
Dal 1987 p53 trovato mutato in 30-50% tumori umani

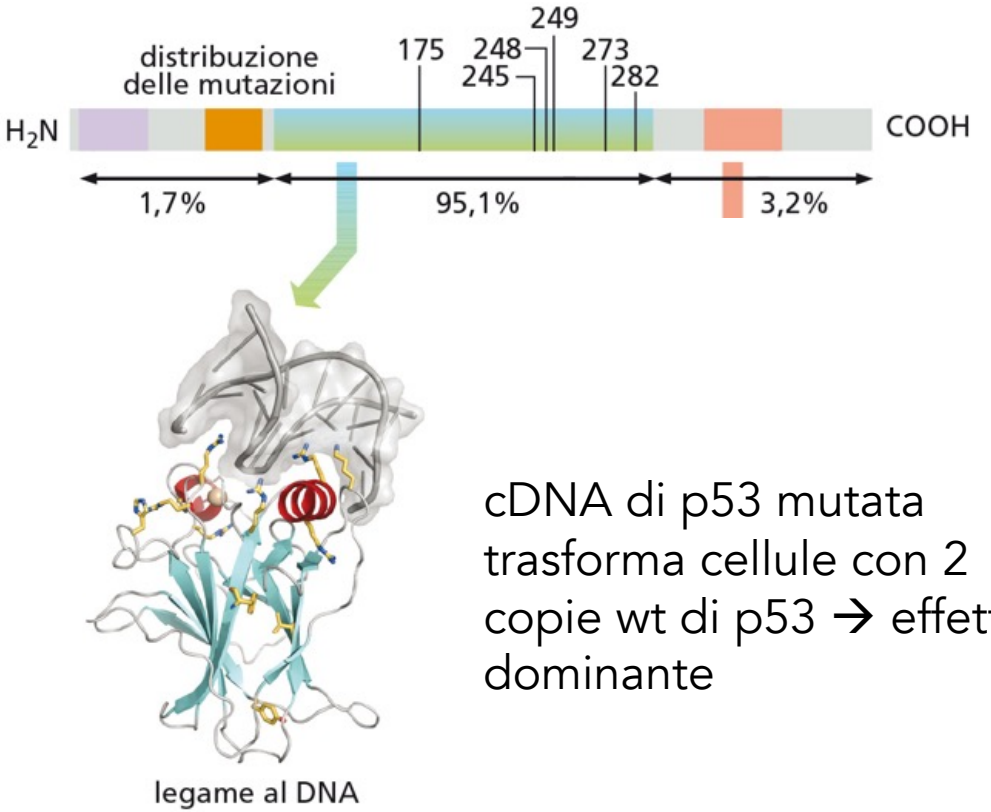


The ICGC/TCGA Pan-Cancer Analysis of Whole Genomes Consortium , 2018

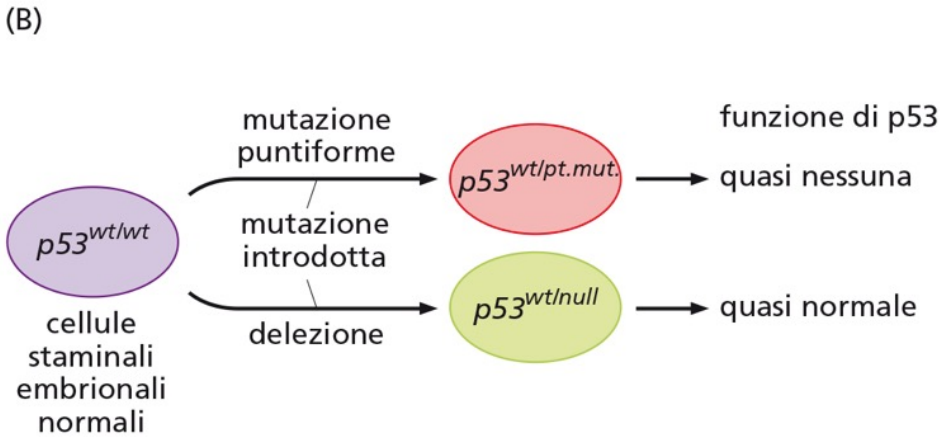
Mutazioni p53 e cancro

Mutazioni p53 in tumori sono prevalentemente missenso





cDNA di p53 mutata trasforma cellule con 2 copie wt di p53 → effetto dominante



Weinberg, la Biologia del Cancro

Mutazioni p53 e cancro

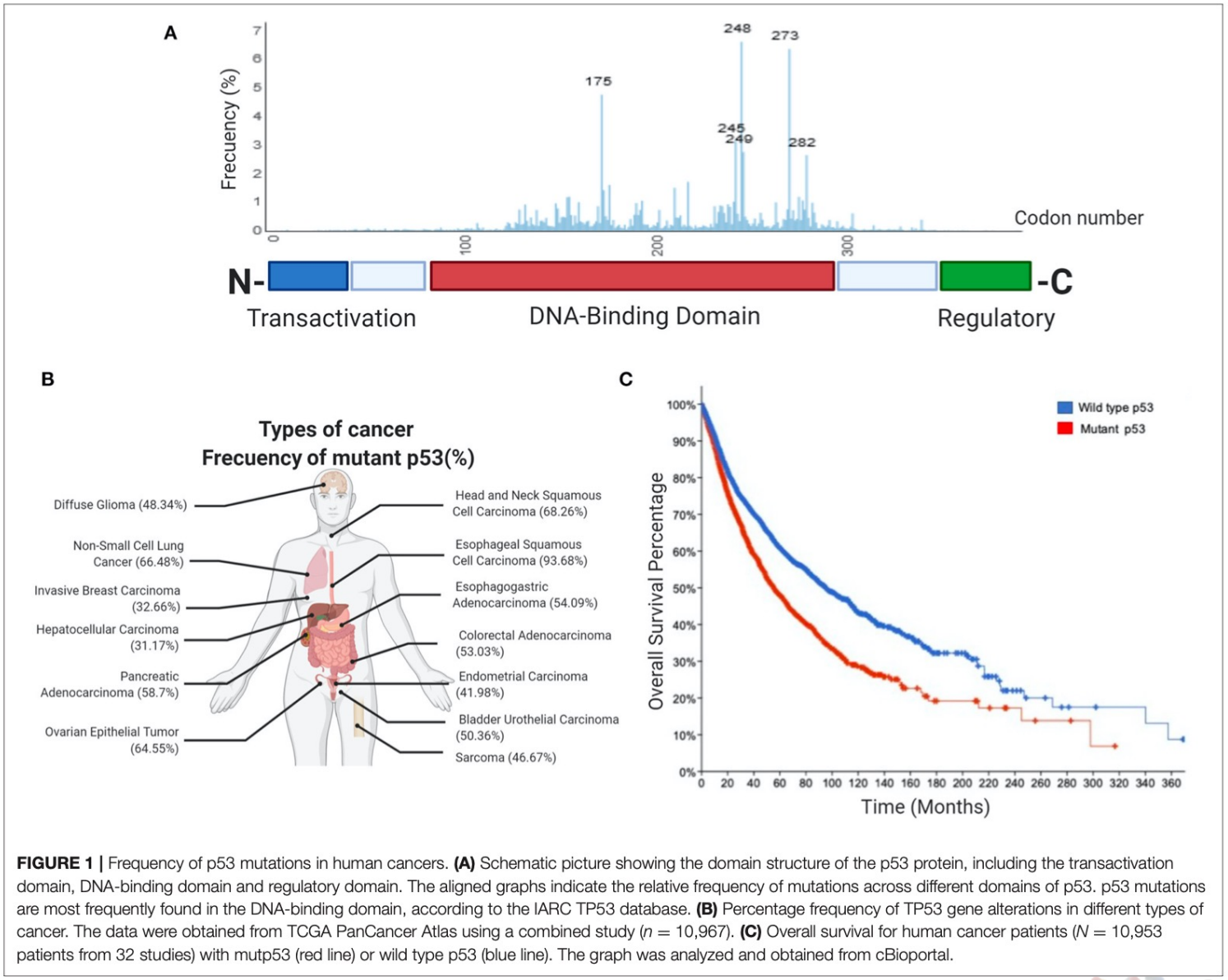
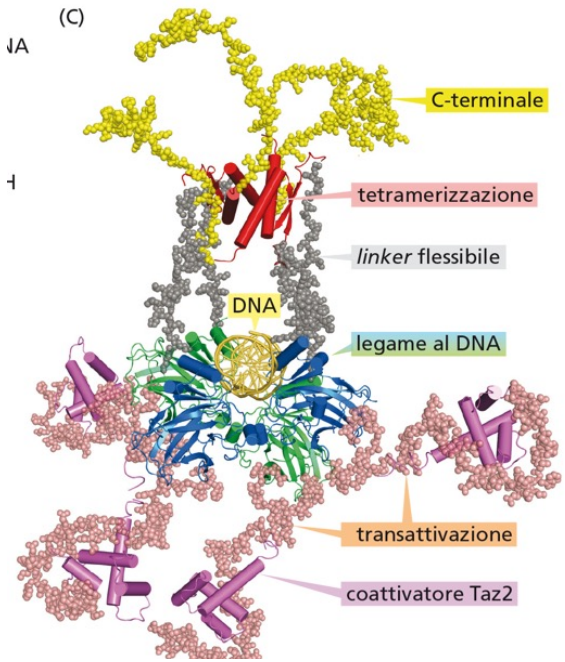


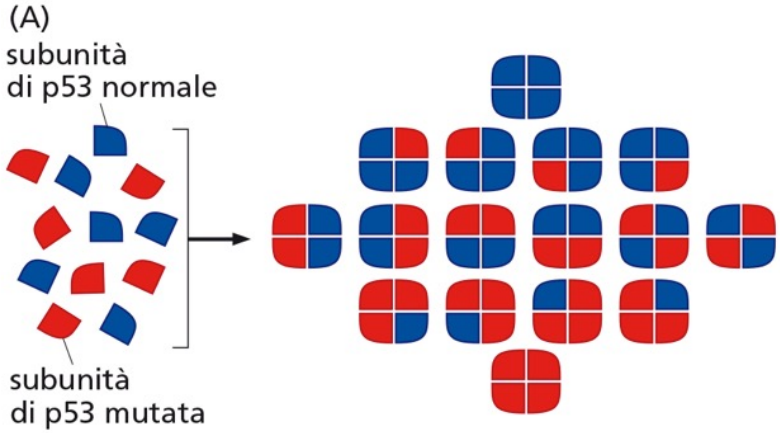
FIGURE 1 | Frequency of p53 mutations in human cancers. **(A)** Schematic picture showing the domain structure of the p53 protein, including the transactivation domain, DNA-binding domain and regulatory domain. The aligned graphs indicate the relative frequency of mutations across different domains of p53. p53 mutations are most frequently found in the DNA-binding domain, according to the IARC TP53 database. **(B)** Percentage frequency of TP53 gene alterations in different types of cancer. The data were obtained from TCGA PanCancer Atlas using a combined study (n = 10,967). **(C)** Overall survival for human cancer patients (N = 10,953 patients from 32 studies) with mutp53 (red line) or wild type p53 (blue line). The graph was analyzed and obtained from cBioportal.

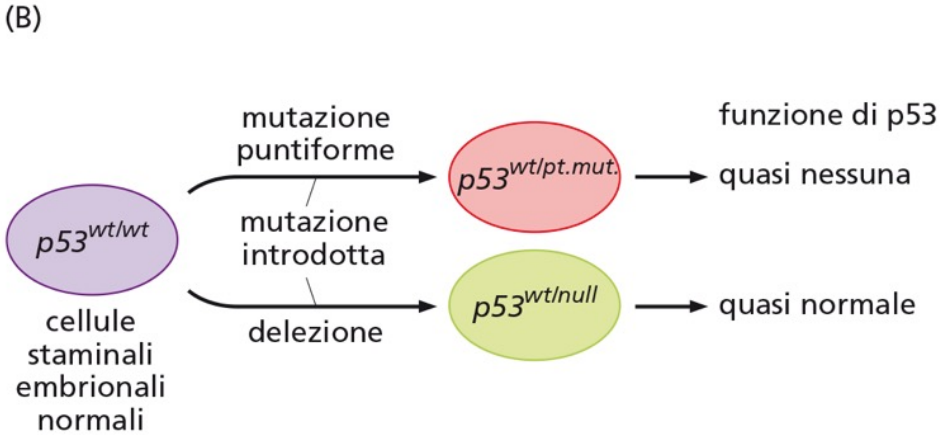
Alvarado-Ortiz et al., 2021

Mutazioni p53 e cancro

- Descrizione effetto dominante negativo nei lieviti
- Analisi biochimica di p53: p53 forma omotetrameri
- Basta una copia non funzionante per inattivare il complesso: 1/16 complessi mantengono la funzione







Weinberg, la Biologia del Cancro

Mutazioni p53 e cancro

Supplementary Sidebar 9.3 Dominant-negative functions of mutant *p53* alleles: functional interactions between p53 and its p63 and p73 cousins Evidence is rapidly accumulating that p53 strongly influences the functioning of p63 and p73, almost certainly by forming mixed tetramers or mixed oligomers with the two paralogous proteins. Perhaps the most graphic evidence comes from experiments with a *p53* mutant allele that carries a mutation in the sequence encoding the DNA-binding domain of p53. The resulting amino acid substitution not only causes a loss of p53's ability to bind its usual target sequences in chromosomal DNA, but also causes p53 to undergo partial denaturation, leading to the formation of aggregates that accumulate in the cytoplasm, that is, away from p53's normal site of functioning in the nucleus. The resulting complexes also involve p63 and p73 proteins, which form mixed aggregates with p53 in the cytoplasm, ostensibly compromising their ability to function as transcription factors because of this cytoplasmic sequestration (Figure S9.3). These aggregates testify to the ability of mutant alleles of the *p53* gene to operate in a dominant-negative fashion to compromise the functions not only of wild-type *p53* alleles but also of wild-type *p63* and *p73* alleles.

- Effetto dominante negativo anche su proteine della stessa famiglia

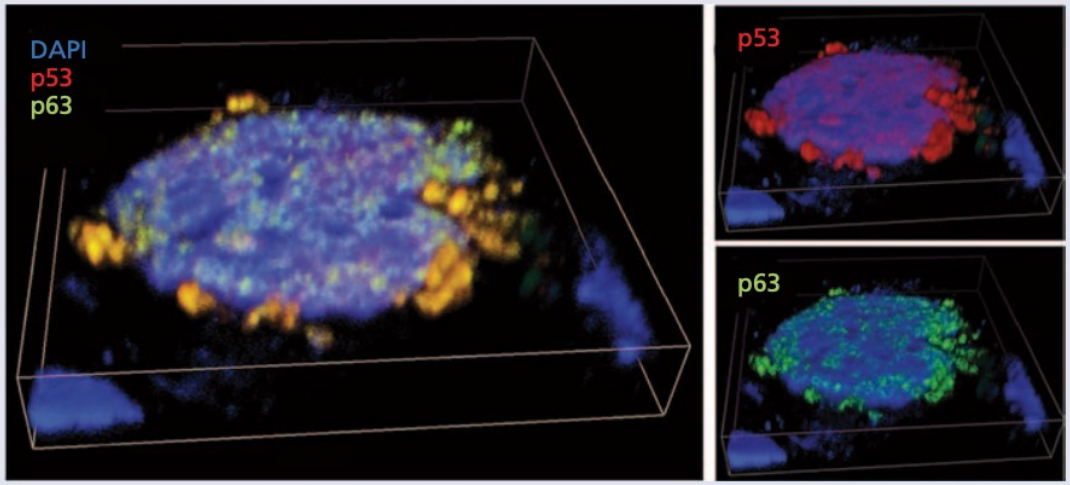
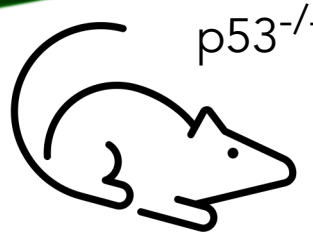


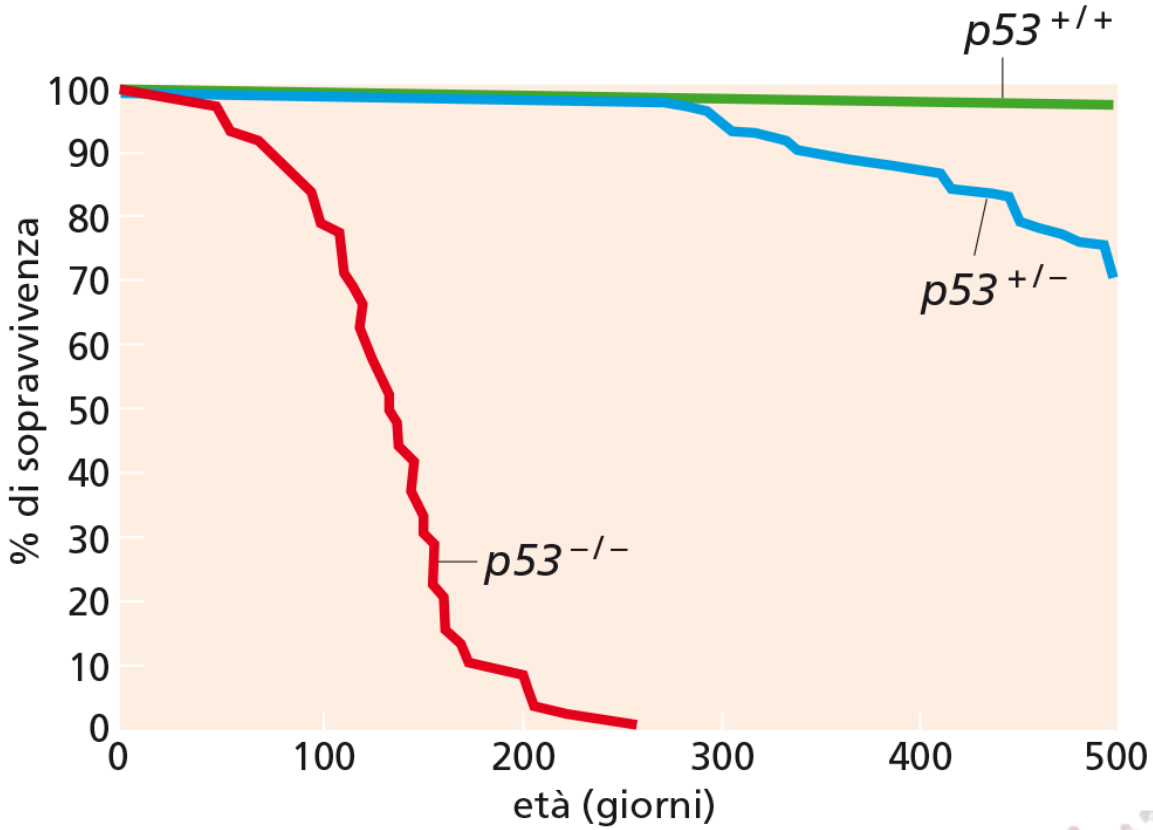
Figure S9.3 Sequestration of p63 in cytoplasmic complexes with mutant p53 The products of certain mutant alleles of *p53*, such as the R110P allele studied here, undergo partial denaturation within cells—a consequence of amino acid substitutions in their DNA-binding domains. The mutant p53 proteins then form aggregates in the cytoplasm that include both p63 and p73; only effects on p63 are shown here. These aggregates provide evidence that p53 has a natural tendency to associate with its p63 and p73 cousins, ostensibly forming mixed tetramers and possibly higher-order complexes in normal cells. These images are 3-dimensional computer-generated reconstructions of confocal micrograph serial sections that image an individual cell stained with an anti-p53 antibody (*red*), an anti-p63 antibody (*green*), and DAPI stain for DNA (*blue*). Any overlap of *red* and *green* (*right panels*) staining yields computer-generated *yellow* regions (*left panel*). (From J. Xu et al., *Nat. Chem. Biol.* 7:285–295, 2011.)

Funzioni di p53



Topi $p53^{-/-}$ nascono e non hanno difetti di sviluppo. Muoiono per linfomi e sarcomi

Diversi da topi $Rb^{-/-}$ che hanno difetti di sviluppo



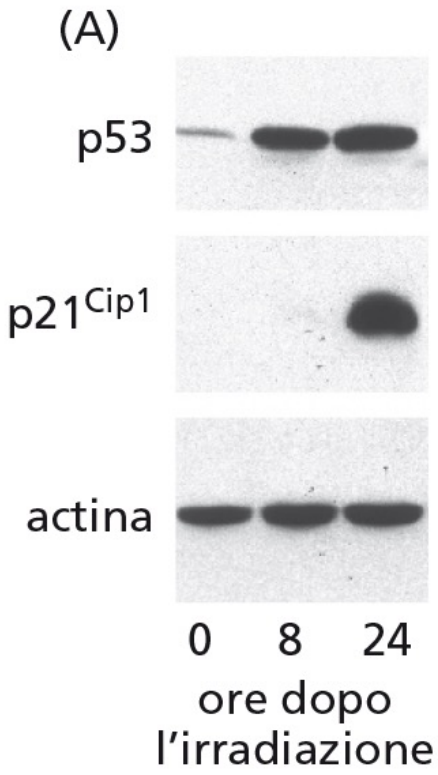
P53 non è regolatore del ciclo cellulare come pRB

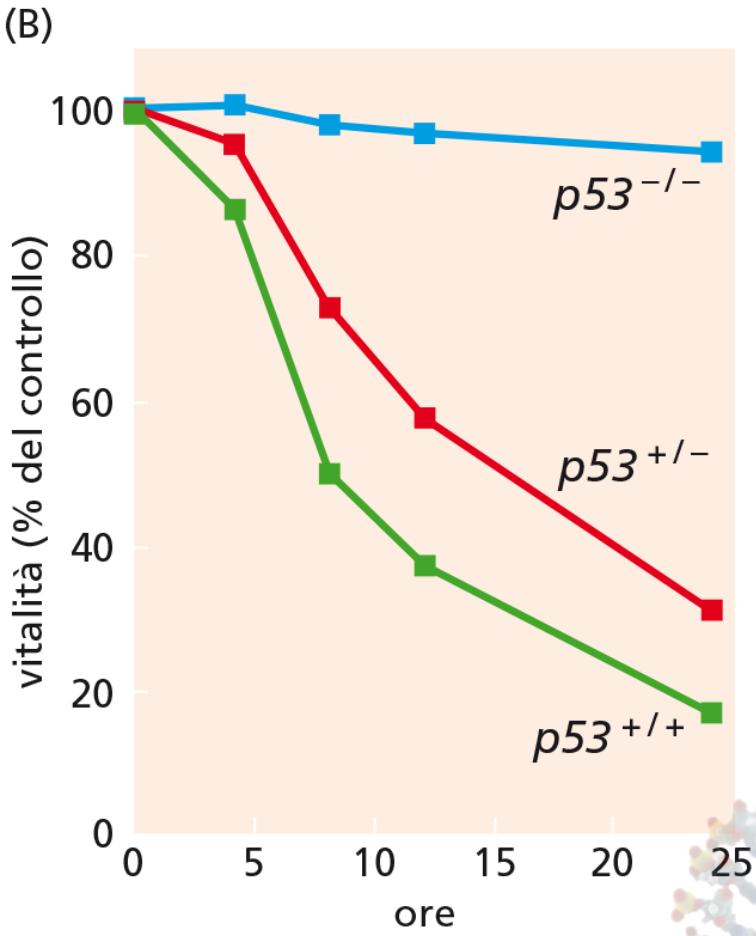
Funzioni di p53

P53 è un fattore trascrizionale?

- Livelli p53 molto variabili in tipi cellulari diversi.
- Localizzazione nucleare

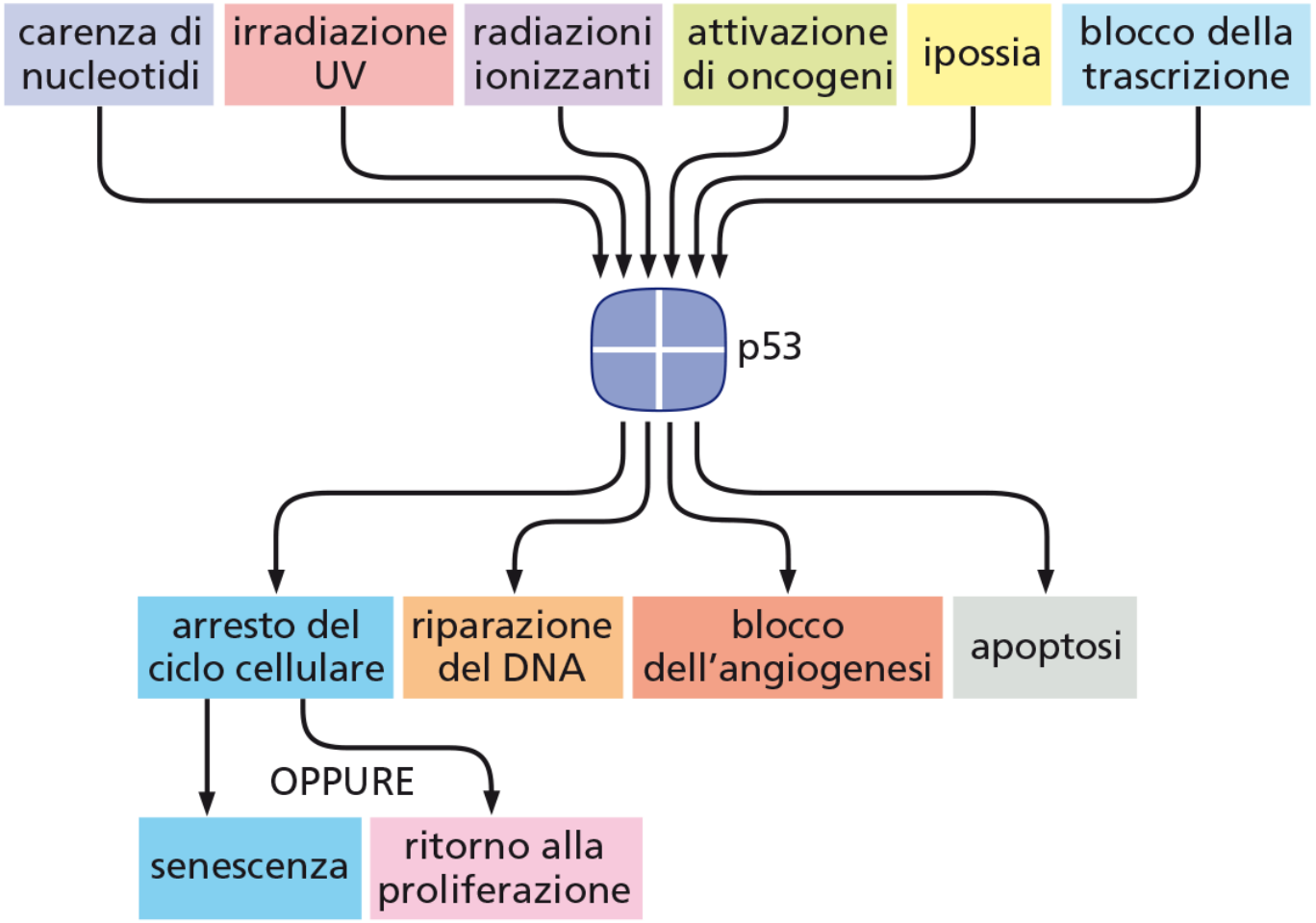
- Livelli proteici variano in risposta a qualche stimolo?





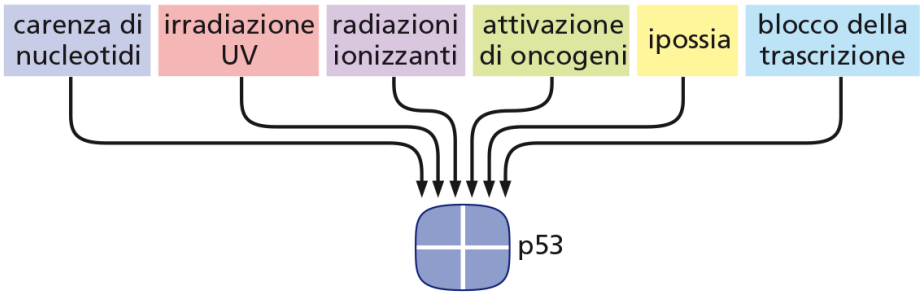
Weinberg, la Biologia del Cancro

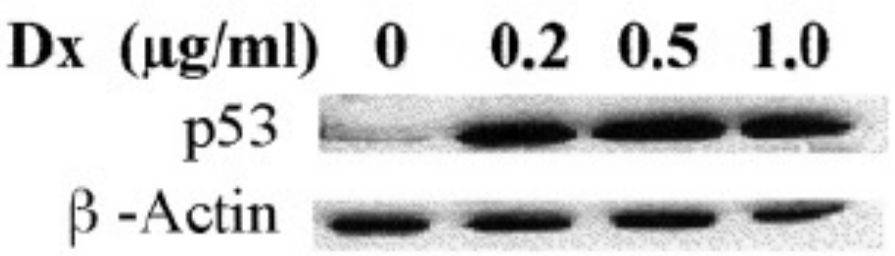
Funzioni di p53



Weinberg, la Biologia del Cancro; Martinez et al., 2002

Funzioni di p53

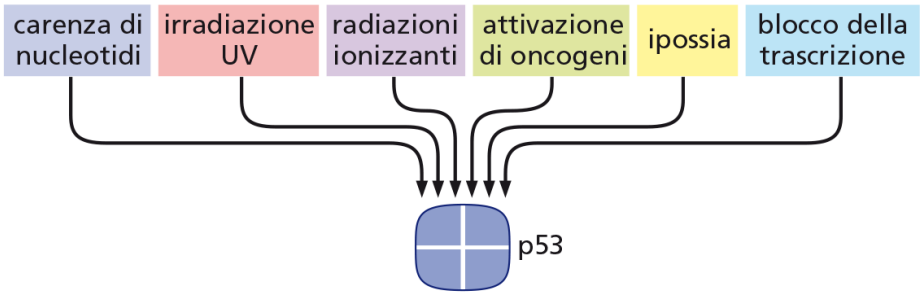


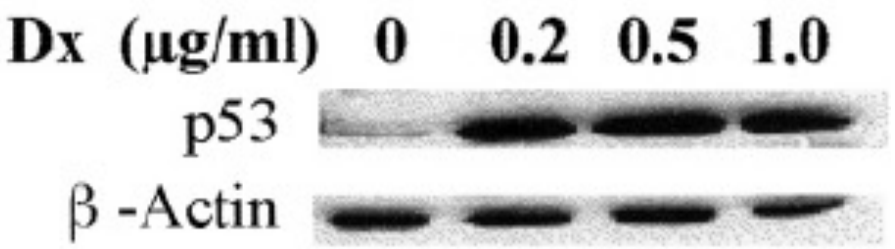


Come sono regolati i livelli di p53?

Weinberg, la Biologia del Cancro; Martinez et al., 2002

Funzioni di p53





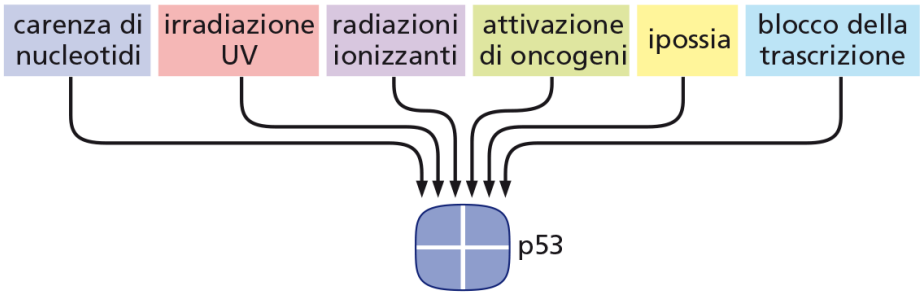
Come sono regolati i livelli di p53?

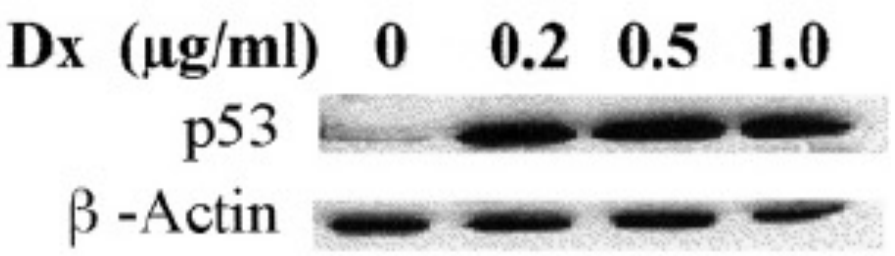
Trascrizione?

Degradazione e turnover?

Weinberg, la Biologia del Cancro; Martinez et al., 2002

Funzioni di p53



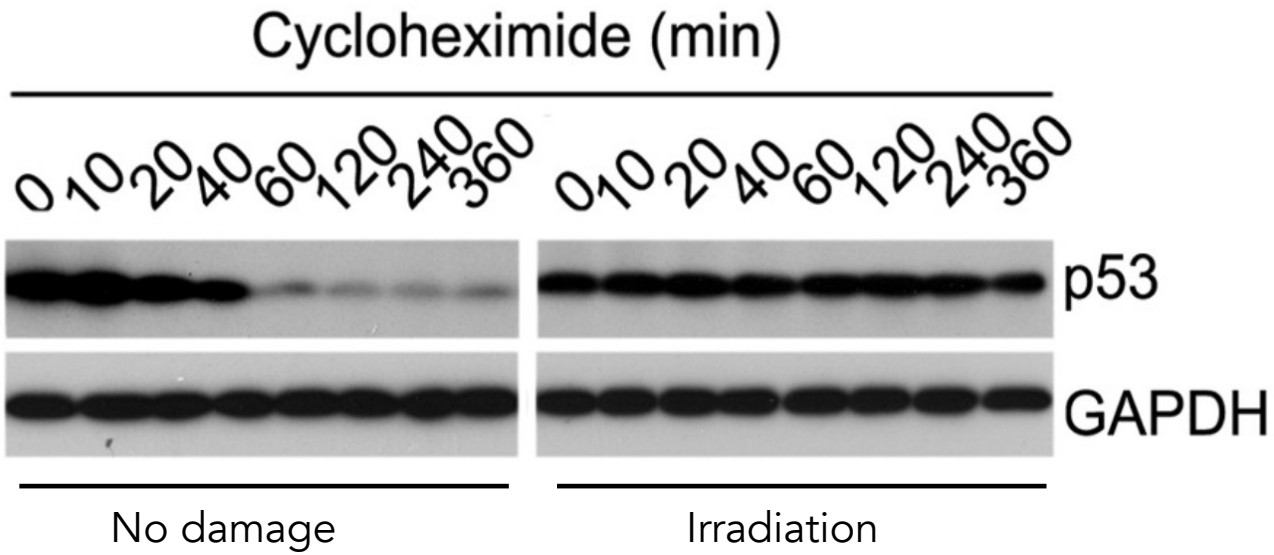


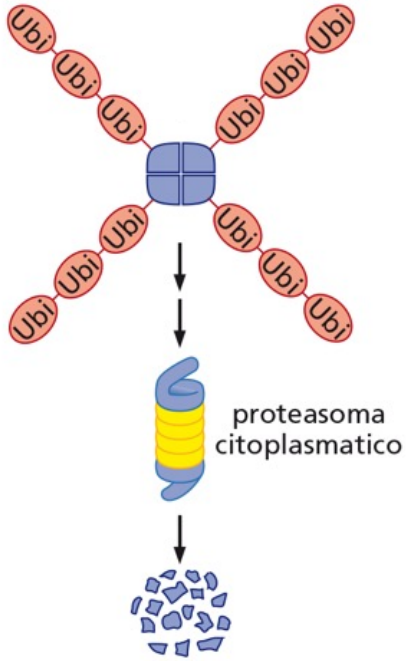
Come sono regolati i livelli di p53?

- Livello mRNA TP53 non varia
- Trascrizione TP53 elevata anche in cellule non trattate

Weinberg, la Biologia del Cancro; Martinez et al., 2002

Regolazione di p53





Emivita p53 è di circa 20 minuti

Chi regola i livelli di p53 e
la sua degradazione?

Ubiquitilazione e degradazione

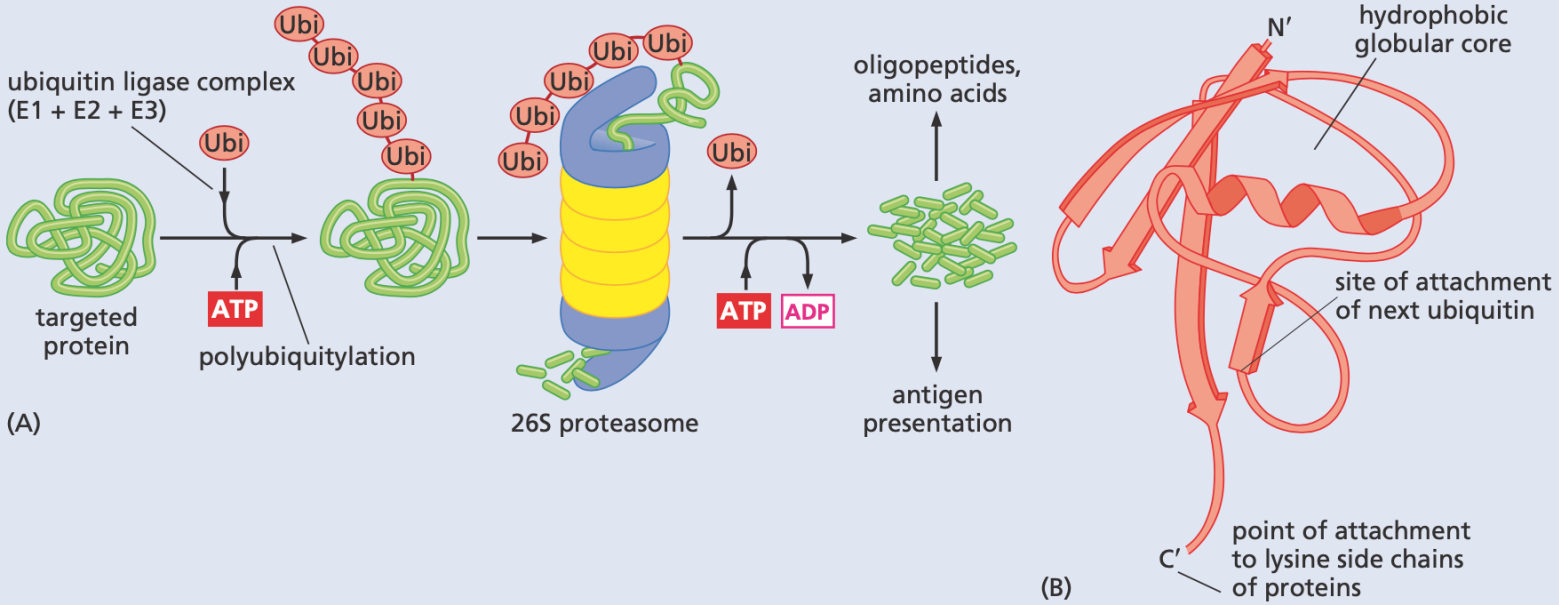
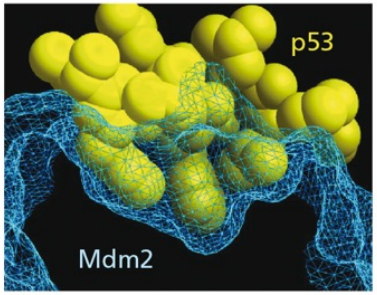
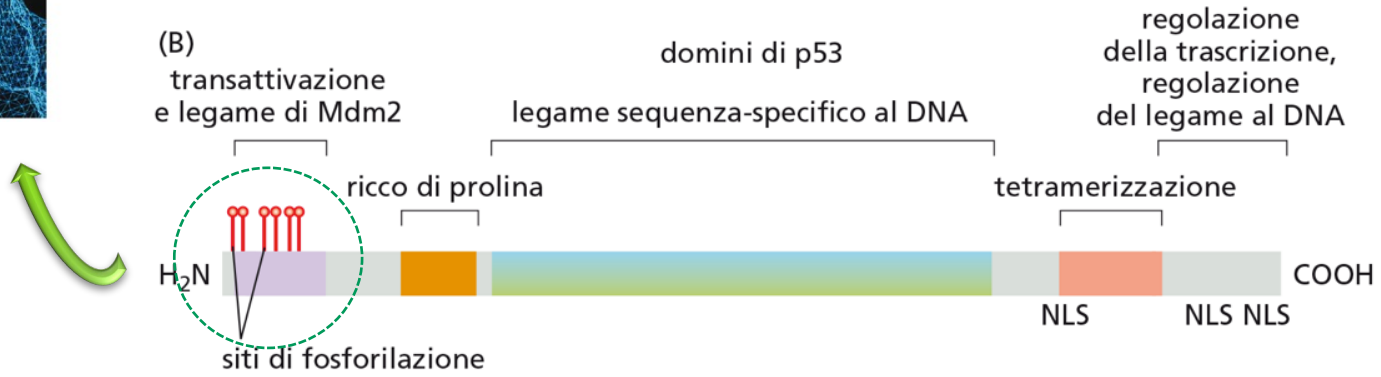


Figure S7.3 Ubiquitylation and proteasomes Much of protein degradation in the mammalian cell is carried out by the ubiquitin–proteasome pathway. (A) A complex of three proteins (E1, E2, and E3), which together constitute a ubiquitin ligase, recognizes a protein destined for degradation and tags this protein with a chain of ubiquitins (Ubi). Following polyubiquitylation, the protein is conveyed to a proteasome (see Figure S7.4), where it is de-ubiquitylated and degraded into oligopeptides that are either degraded further into amino acids or used for antigen presentation by the immune system; see Section 15.3. (In fact, a specialized proteasome is used to process proteins for antigen presentation.) (B) Ubiquitin itself is a relatively short protein of 76 amino acid residues whose amino acid sequence and structure are almost totally conserved among all eukaryotic cells. Its structure is indicated here in this ribbon diagram determined by X-ray crystallography.

Regolazione di p53

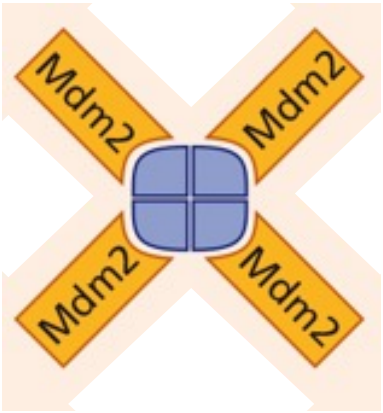
MDM2 sovraespresso in diversi tumori





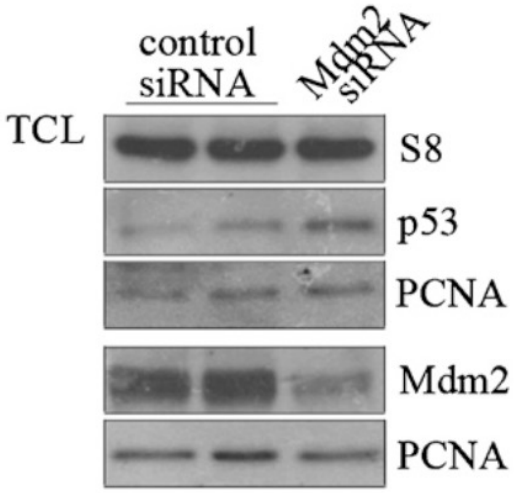
Topi KO *Mdm2*$^{-/-}$ → Letalità embrionale

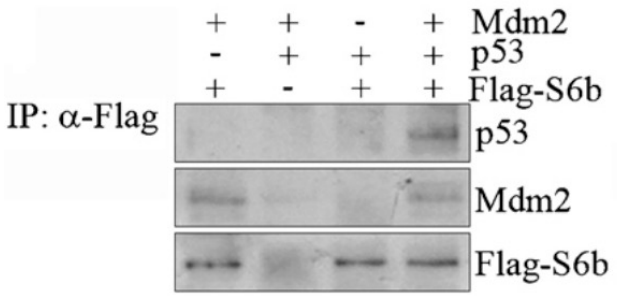
Topi KO *Mdm2*$^{-/-}$ *p53*$^{-/-}$ → Embrioni normali



Weinberg, la Biologia del Cancro; Martinez et al., 2002

Regolazione di p53





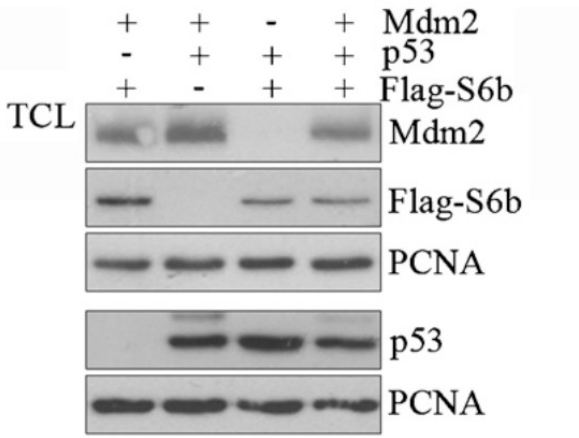


Fig. 2. p53, Mdm2 and the proteasome form a ternary complex. (*A*) H1299 cells were transfected with 5 μg of a plasmid encoding p53 together with 5 μg of a plasmid encoding Mdm2 and with a plasmid encoding Flag-tagged S6b, where the amount of transfected plasmid was adjusted to receive equal levels. 4 h prior to harvest, 10 μM MG132 were added. **IP:α-Flag**: Flag-tagged S6b was precipitated and associated p53 and Mdm2 were determined by Western blotting. **TCL**: 50 μg of total cell lysate were separated on a SDS-PAGE gel. Mdm2, p53 and S5a were determined by Western blotting.

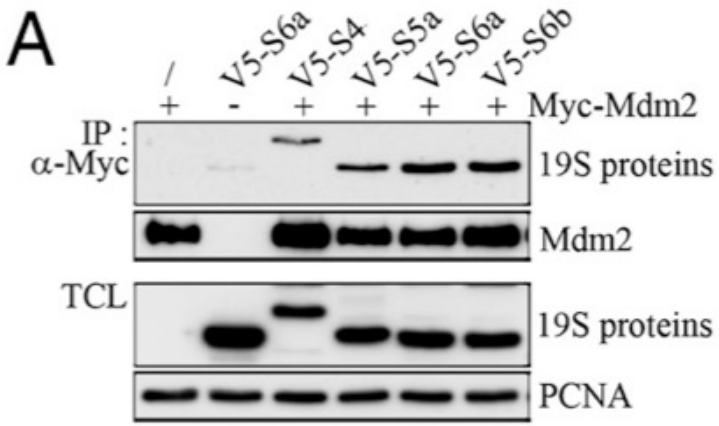


Fig. 1. Mdm2 associates with proteasomal proteins. (*A*) 293T cells were transfected with 7.5 μg of a plasmid encoding Myc-tagged Mdm2 and with 7.5 μg of a plasmid encoding the indicated V5-tagged proteins of the 19S complex. **IP**: Mdm2 was precipitated and associated proteasomal proteins were detected by Western blotting. **TCL**: Aliquots of cellular lysates were assayed by Western blotting for protein expression. (*B*) Section I: Mdm2

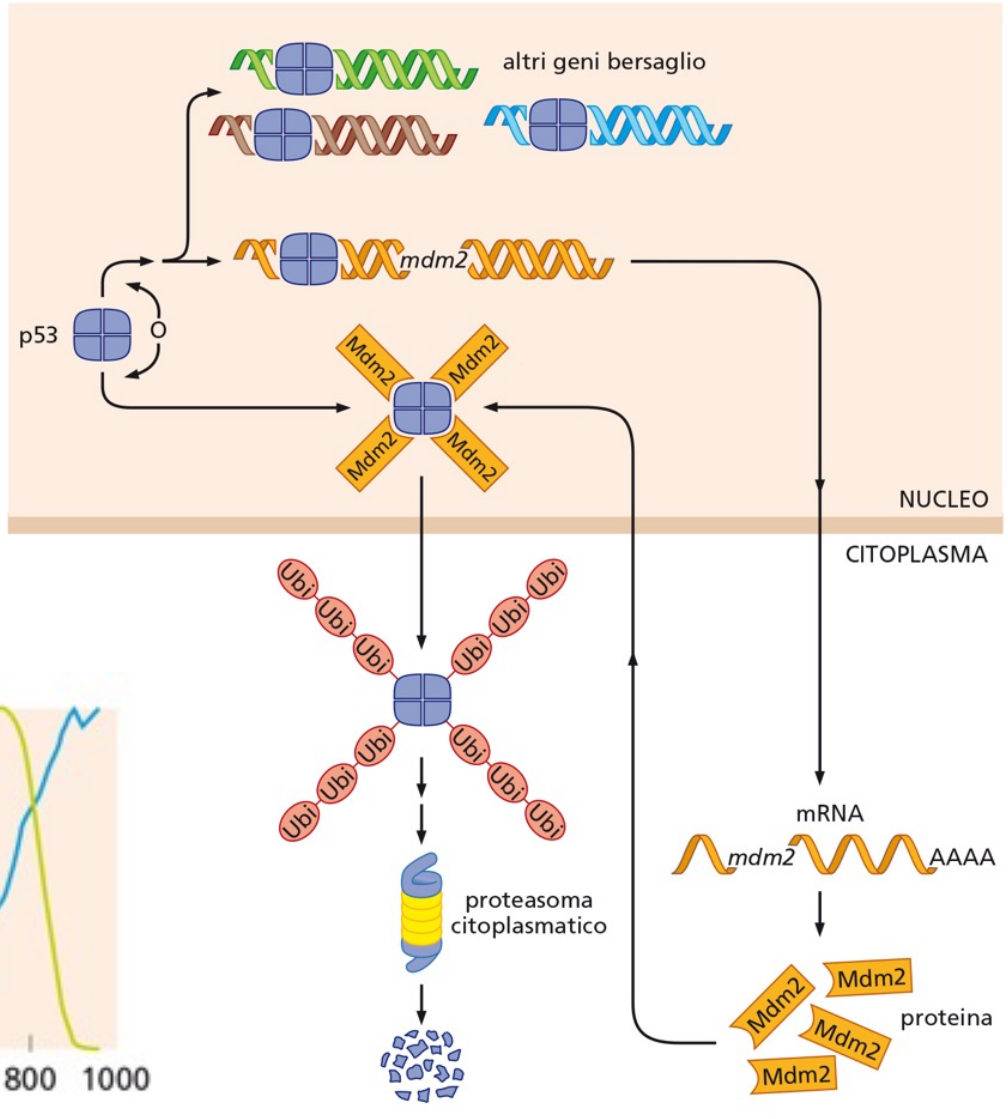
Kulikov et al., 2010 doi:10.1073/pnas.0911716107/-/DC

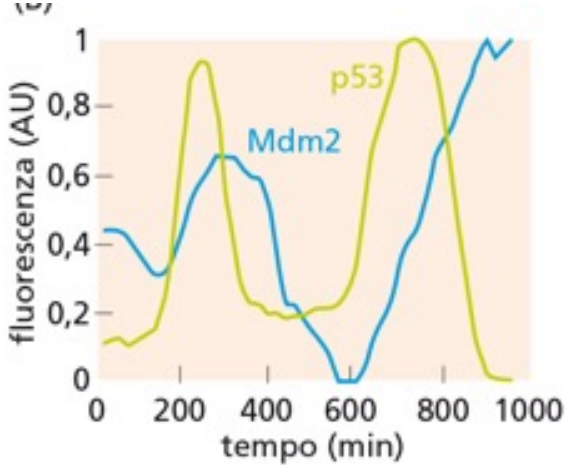
Regolazione di p53

MDM2 inibisce p53:

- Impedisce legame p53-p300/CBP (HAT)
- Recluta HMT che inibiscono trascrizione
- Media mono-ubiquitinazione p53 che lo indirizza nel citoplasma

P53 ha tra i bersagli *Mdm2*





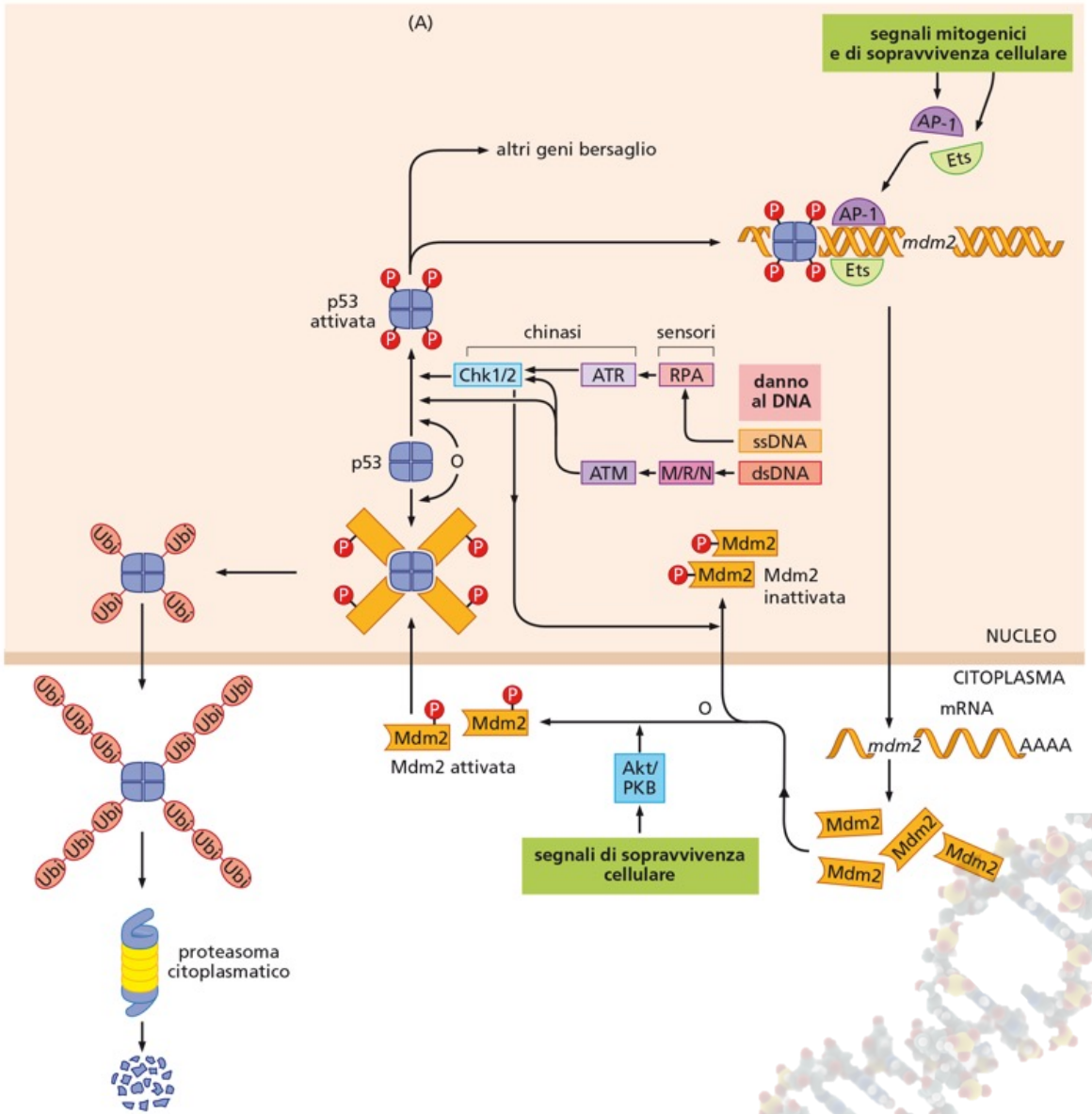
Weinberg, la Biologia del Cancro; Martinez et al., 2002

MDM2 e p53

Danni al DNA attivano p53:
-Fosforilazione p53 impedisce legame Mdm2
-Le stesse chinasi fosforilano Mdm2 e la inibiscono

Regolazioni positiva e negativa Mdm2:
-positiva da segnali mitogenici: fosforilazione attivatoria da Akt/PKB
-positiva da Ras-MAPK-AP1 su trascrizione

-negativa da $p14^{ARF}/p19^{ARF}$



Weinberg, la Biologia del Cancro

Iperattivazione p53

p53 mutant mice that display early ageing-associated phenotypes

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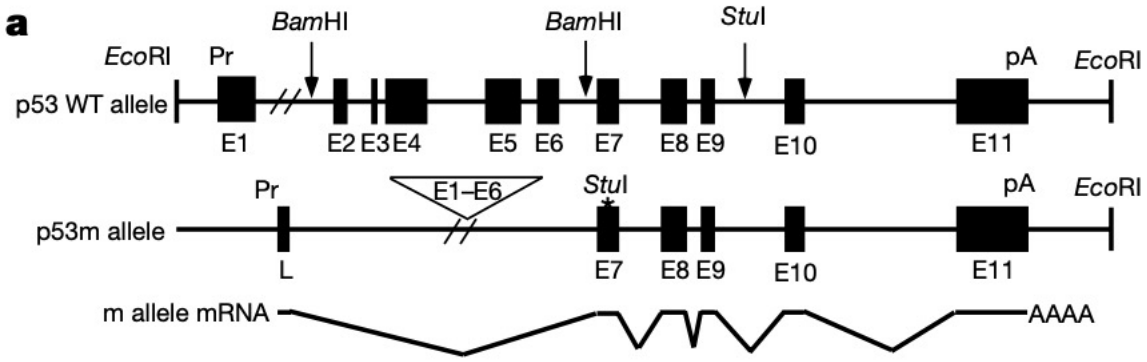


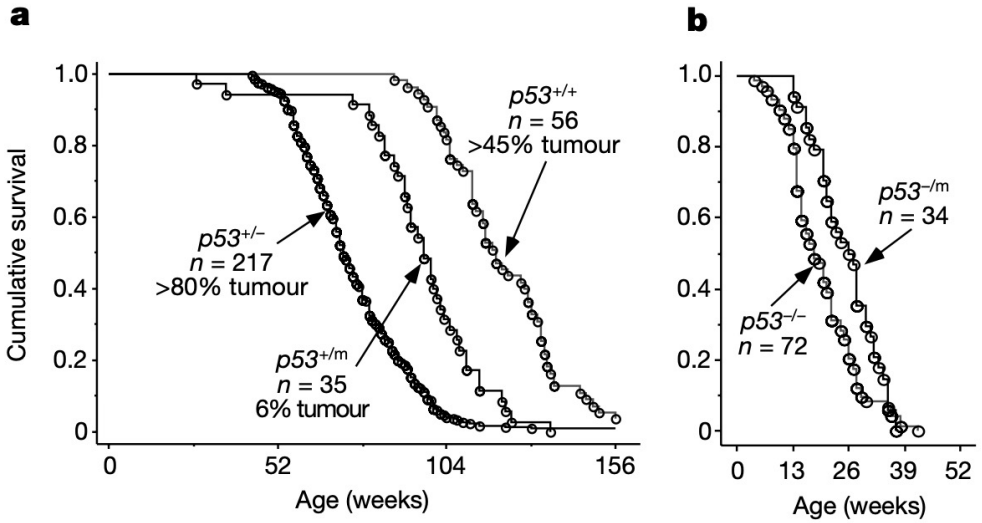
Table 1 Tumour types arising in $p53^{+/-}$, $p53^{+/+}$, $p53^{+/m}$ and pL53 mice

Tumour type	$p53^{+/-}$ (n = 217)	$p53^{+/+}$ (n = 56)	$p53^{+/m}$ (n = 35)	pL53 (n = 66)
Lymphoma	49 (28%)	18 (67%)	0	9 (56%)
Osteosarcoma	61 (34%)	2 (7%)	0	0
Soft tissue sarcoma	47 (27%)	3 (11%)	0	1 (6%)
Carcinoma	20 (11%)	4 (15%)	1 (50%)*	5 (31%)*
Other	0	0	1 (50%)†	1 (6%)
Total	177	27	2	16

Values in parentheses indicate the percentage of total tumours.
* Lung adenocarcinoma.
† Small focal lung adenoma.

Tyner et al., 2002

Iperattivazione p53



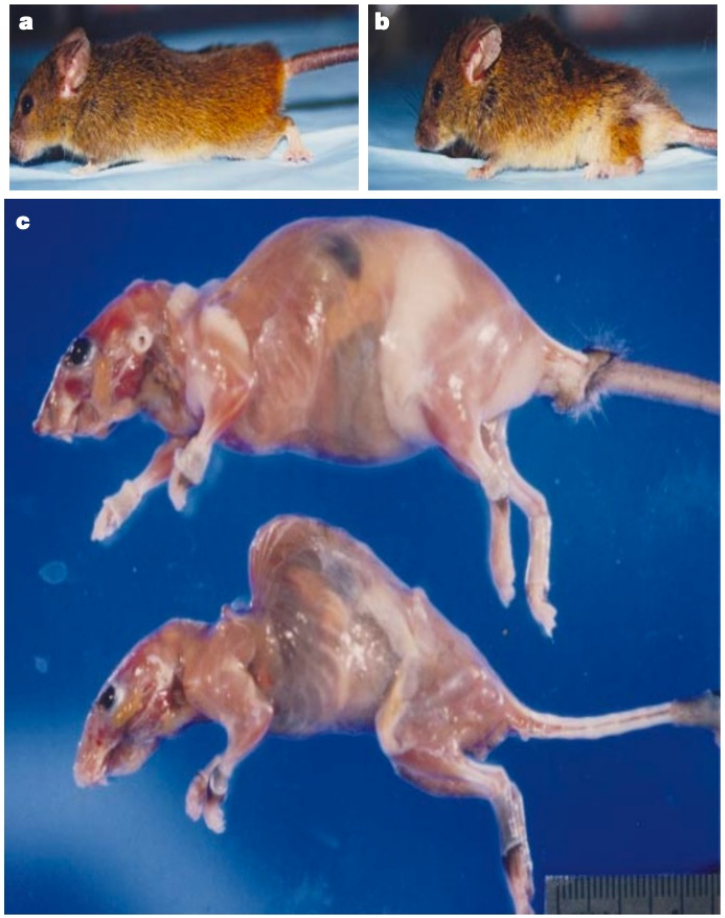
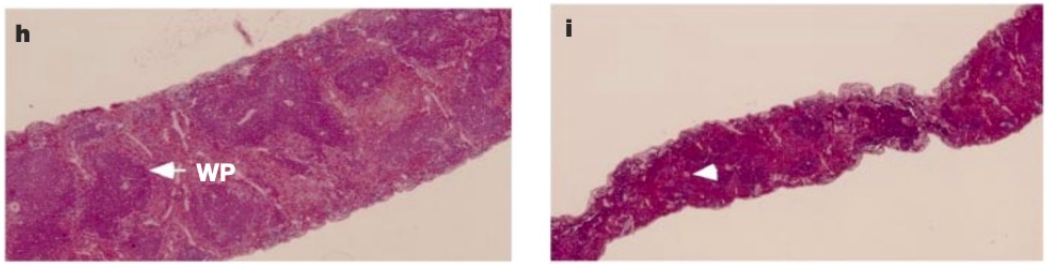


Figure 2 Longevity in $p53^{+/+}$, $p53^{+/-}$, $p53^{+/m}$, $p53^{-/-}$ and $p53^{-/m}$ mice. **a**, Survival of $p53^{+/-}$, $p53^{+/+}$ and $p53^{+/m}$ mice. More than half of the tumours in wild-type mice appeared while many of the $p53^{+/m}$ mice were still alive. **b**, Survival of $p53^{-/-}$ and $p53^{-/m}$ mice. Virtually all of the $p53^{-/-}$ and $p53^{-/m}$ mice developed cancer.



Tyner et al., 2002