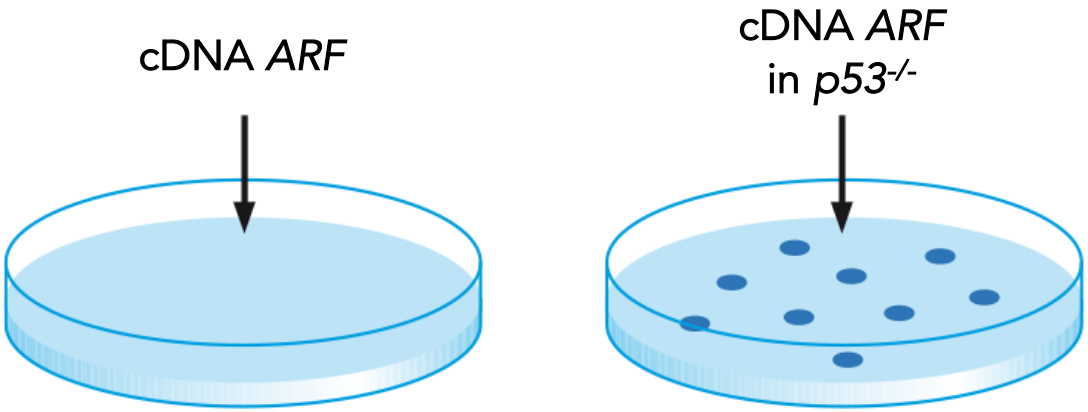
P53, APOPTOSI E TUMORI

MDM2, ARF e p53

Come sono regolati i livelli di Mdm2?

cDNA ARF in cellule normali di roditore → NO proliferazione

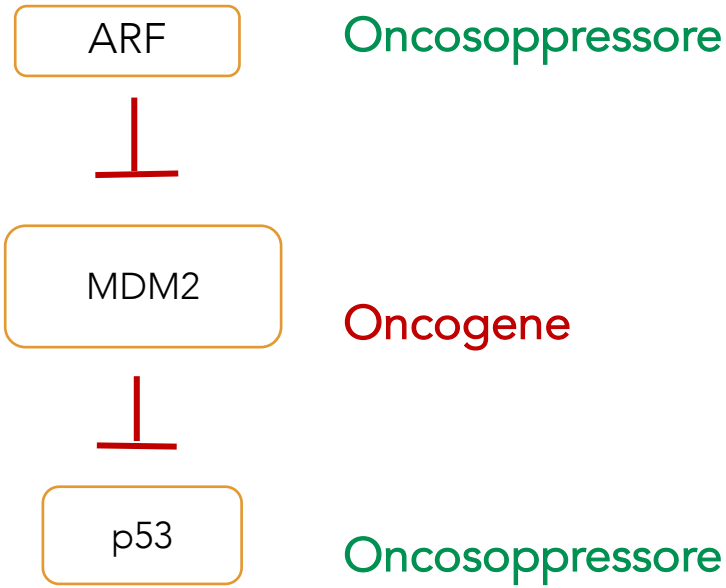
cDNA ARF in cellule $p53^{-/-}$ → proliferazione



MDM2, ARF e p53

ARF regola positivamente p53, sequestrando e inattivando Mdm2

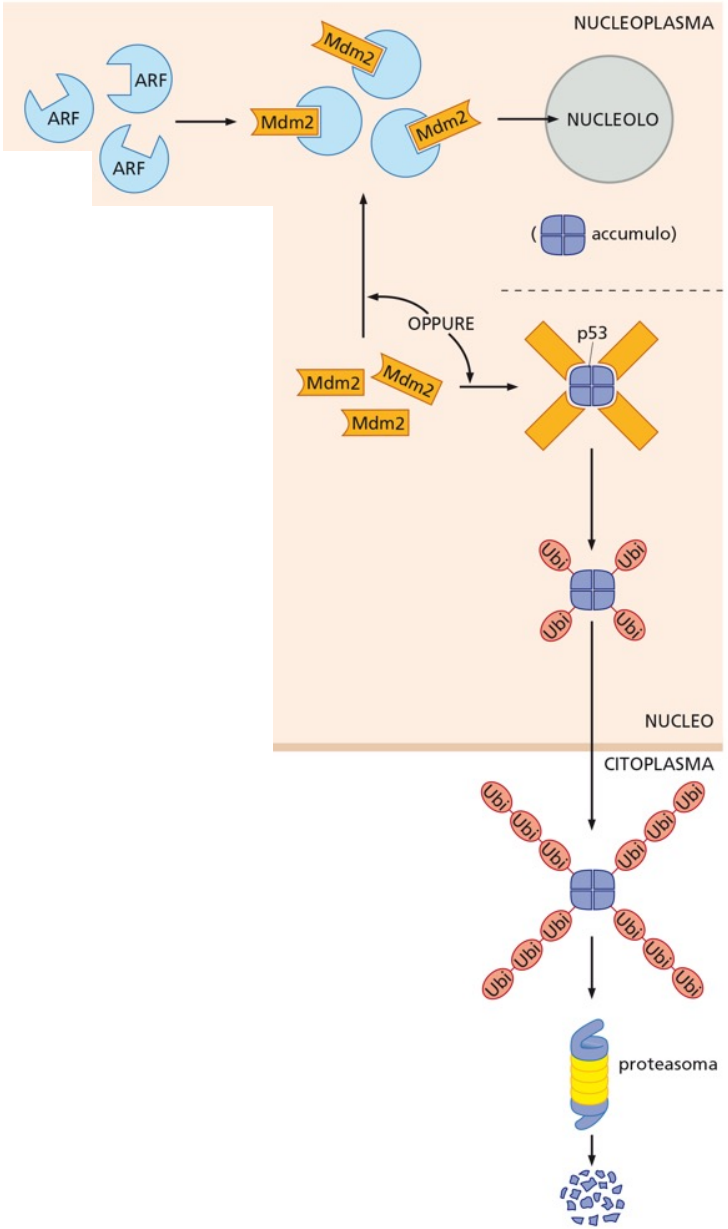
ARF è oncosoppressore



Oncosoppressore

Oncogene

Oncosoppressore



Hershko and Ginsberg, 2004

MDM2, ARF e p53 nei tumori

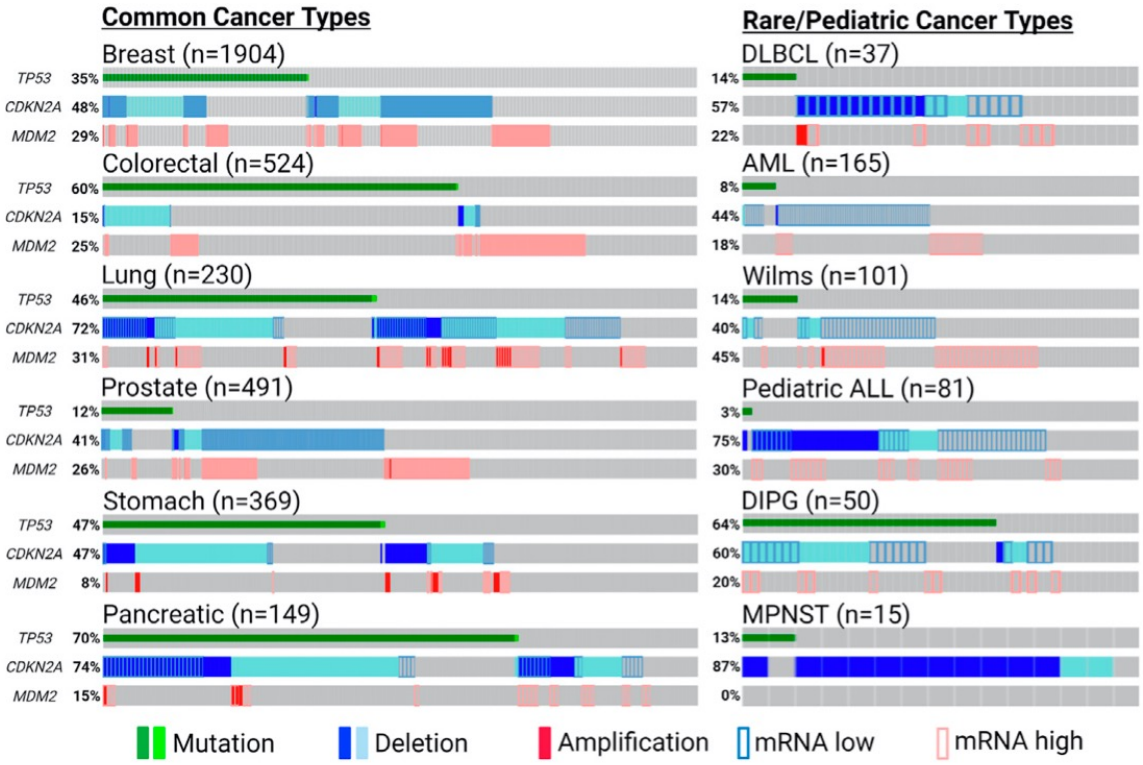
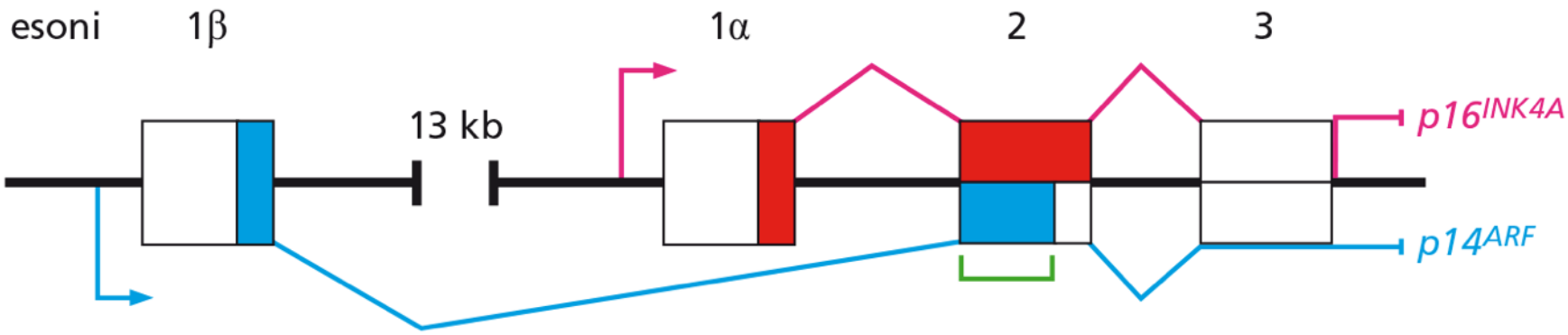


FIGURE 5 | Significant genetic alterations of p53, MDM2 and ARF in cancers. Tumor-promoting genetic events of *TP53* (non-synonymous mutation), *MDM2* (amplification or induced mRNA expression) and *CDKN2A* (deletion or reduced mRNA expression) are summarized using publicly available patient data from cBioPortal (cbioportal.org) and pediatric cBioPortal (pedcbioportal.org). Percentages shown indicate accumulated fraction of patient samples with highlighted genetic alterations. The sources of the data presented are the following: Breast—METABRIC; Colorectal/DLBCL (diffuse large b cell lymphoma)/AML (acute myeloid leukemia)—TCGA PanCancer; Lung/Prostate/Stomach/Pancreatic—TCGA Firehose; DIPG (diffuse intrinsic pontine glioma)—PNOC; Wilms/pediatric ALL (acute lymphoblastic leukemia)—TARGET; MPNST (malignant peripheral nerve sheath tumor)—MSKCC. Expression of mRNA levels (except for MPNST) are shown based on expression z-scores relative to all available diploid samples (<–0.5: mRNA low; >0.5: mRNA high). Created with BioRender.com.

Kung and Weber, 2022

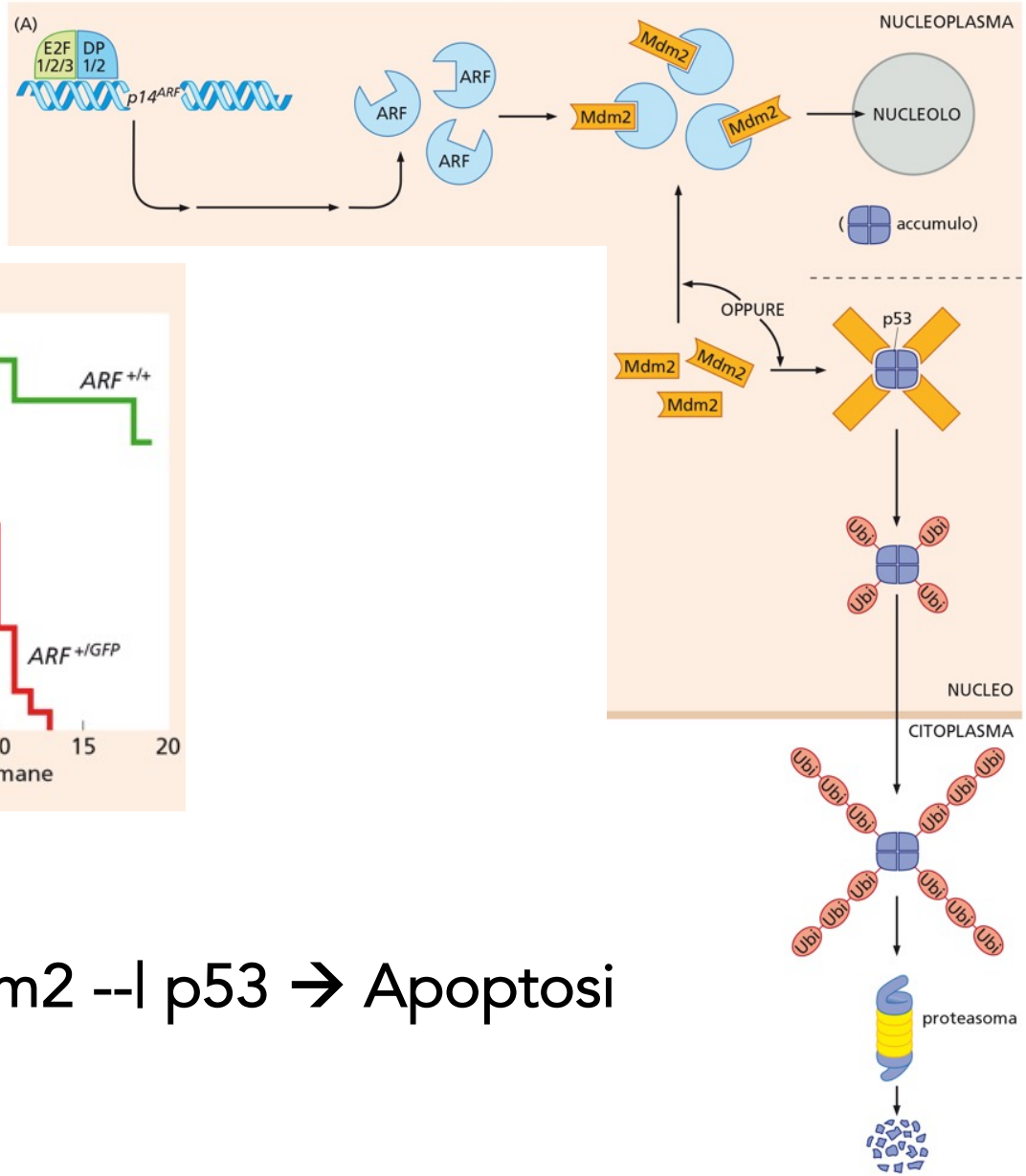
CDKN2A: ARF e p16^{INK4A}

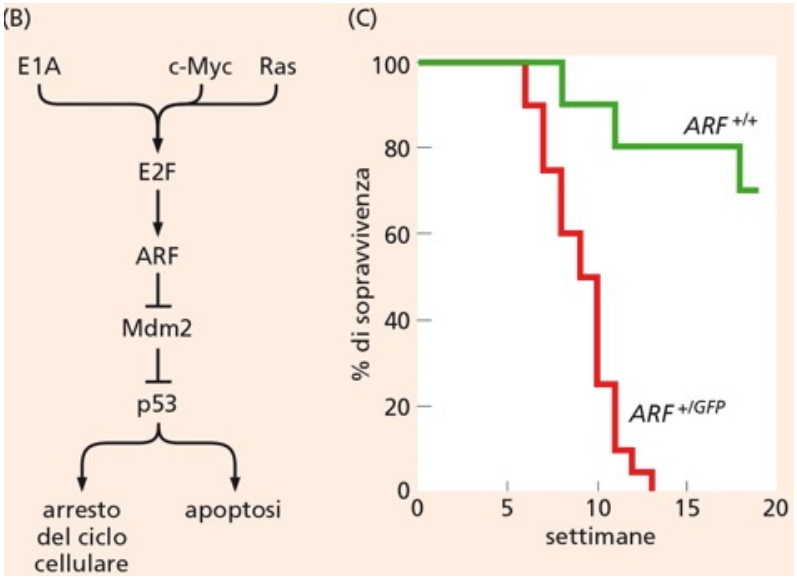


Kung and Weber, 2022

MDM2, ARF e p53

ARF regola p53, ma cosa regola ARF?





pRB --| E2F → ARF --| Mdm2 --| p53 → Apoptosi

Hershko and Ginsberg, 2004

E2F e apoptosi

Analisi livelli espressione geni proapoptotici in presenza di alti livelli di E2F

RT-PCR in cellule che esprimono alti livelli di E2F

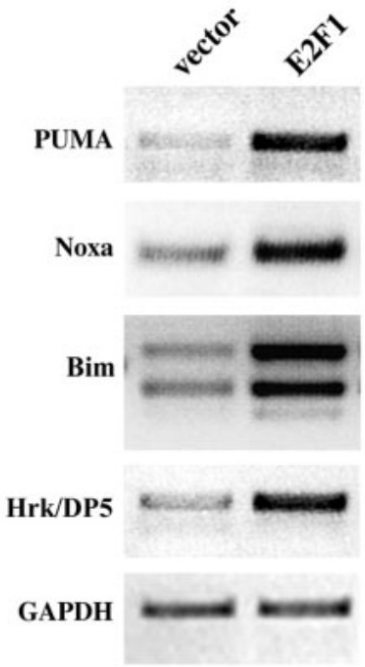


FIG. 1. **Over-expressed or deregulated E2F elevates levels of BH3-only genes.** *A*, NIH3T3 cells were infected with a pBabe-puro retroviral vector (*vector*) or with a retrovirus expressing E2F1 (*E2F1*). Total RNA was extracted from the cells, and RT-PCR was performed using specific primers for the *PUMA*, *Noxa*, *Bim*, *Hrk/DP5*, and *GAPDH*

Hershko and Ginsberg, 2004

E2F e apoptosi

Analisi livelli espressione geni proapoptotici in presenza di alti livelli di E2F

ChIP

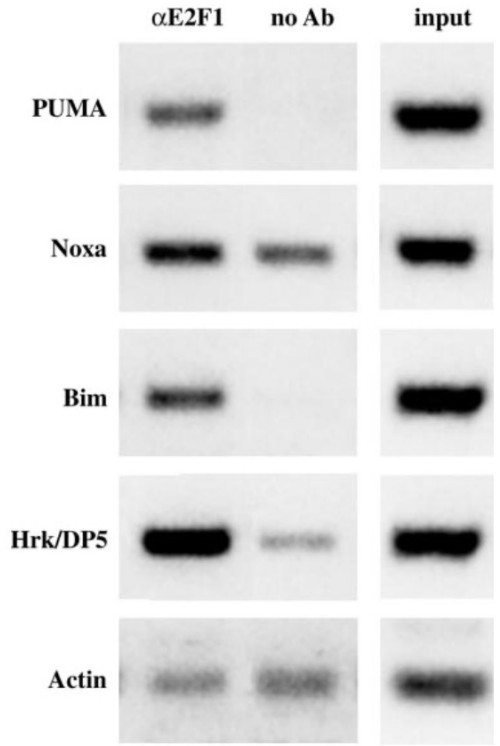


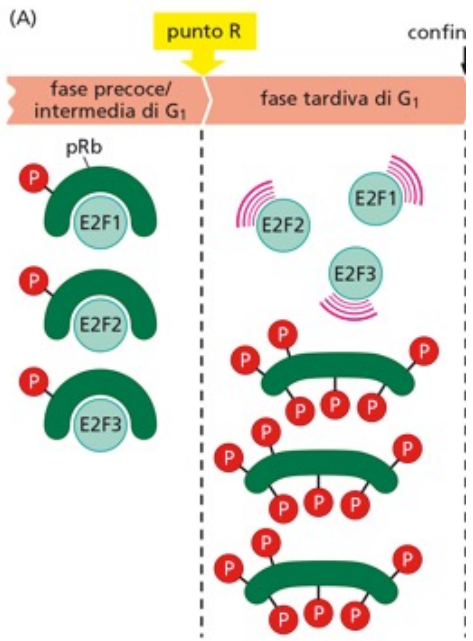
FIG. 6. **Endogenous E2F1 binds the promoters of the BH3-only genes.** A chromatin immunoprecipitation assay was performed using growing NIH3T3 cells. Cross-linked chromatin was incubated with an antibody against E2F1 (*αE2F1*) or without an antibody (*no Ab*). Immunoprecipitates from each sample were analyzed by PCR using primers specific for the *PUMA*, *Noxa*, *Bim*, and *Hrk/DP5* promoters and for the β-actin coding region (*Actin*). As a control, a sample representing 0.2% of the total chromatin used for immunoprecipitation reactions was included (*input*).

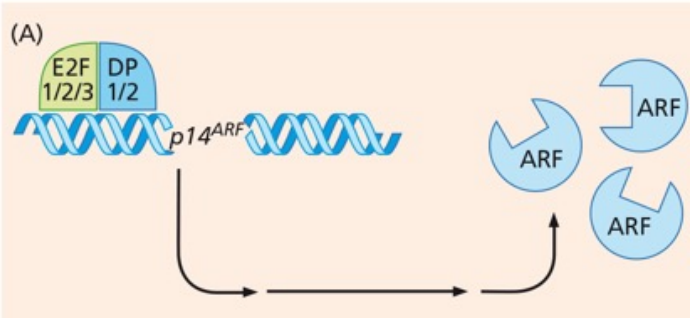
Hershko and Ginsberg, 2004

MDM2, ARF e p53

ARF monitora funzionamento via di Rb rilevando i livelli del fattore trascrizionale E2F

E2F ha come bersagli i promotori di molti geni proapoptotici (caspasi, Bim, Noxa, Puma) e anche quello di ARF





Weinberg, la Biologia del Cancro

P53 e origine del retinoblastoma

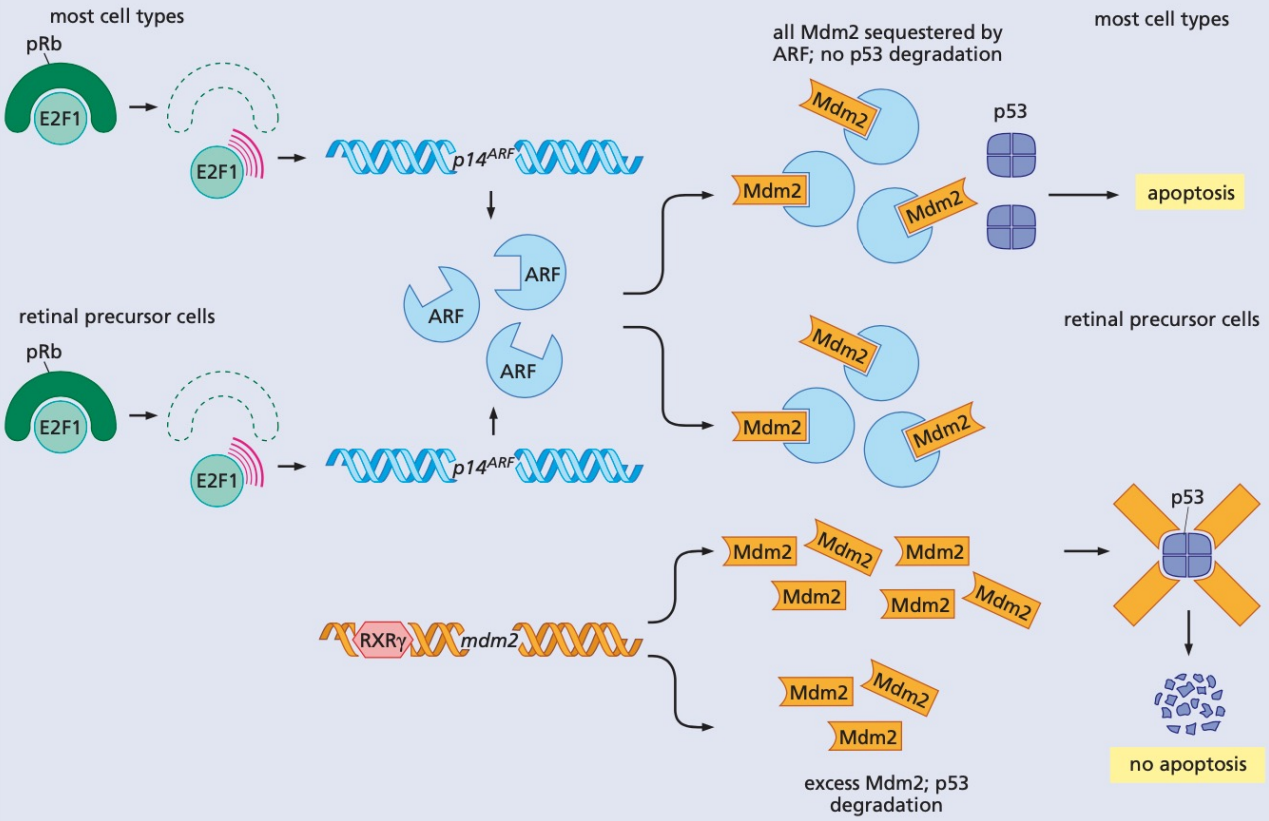
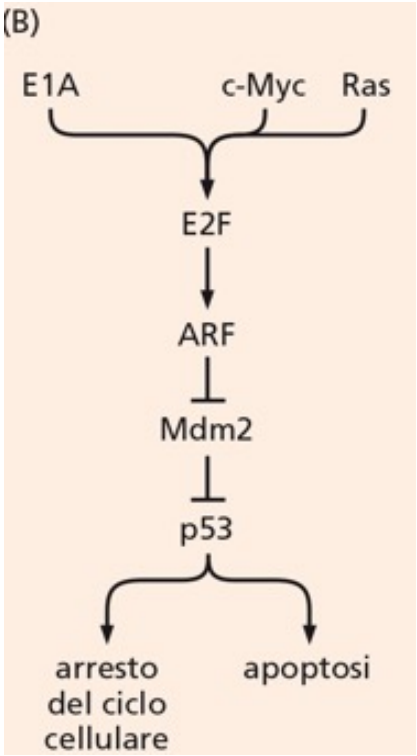


Figure S8.5 Origin of retinoblastomas Retinoblastomas appear to arise from a cell type that is closely related to the precursors of the cone cells in the eye that give us color vision. The reason *Rb* gene inactivation leads specifically to retinal tumors in young children has been elusive, since this gene and its encoded protein are responsible for regulating cell cycle progression in many cell types throughout the body. One important contributing factor derives from the responses that many cell types throughout the body mount following inactivation of the *Rb* gene or its product, pRb. As described in Sections 9.7 and 9.8, the resulting deregulated activation of the E2F transcription factors (E2F1 is shown here) often leads to expression of $p14^{ARF}$ ($p19^{ARF}$ in mice). $p14^{ARF}$ then sequesters Mdm2, allowing p53 to escape Mdm2-driven degradation and to accumulate to high levels and trigger apoptosis. In cone cell precursors, the first steps of this process proceed identically, i.e., *Rb* inactivation leads, once again, to production of $p14^{ARF}$. However, in these cells, a relative of the retinoic acid receptor, another nuclear receptor (see Section 5.8) termed RXRγ, drives high levels of *Mdm2* transcription, leading to high concentrations of Mdm2 protein. These high levels of Mdm2 overwhelm $p14^{ARF}$ and thereby succeed in triggering p53 degradation, allowing these retinal cells to evade p53-initiated apoptosis. As a consequence, *Rb* nullizygous retinal cells apparently can survive and proliferate, leading to retinal tumors.

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

ARF è attivata da segnali diversi

ARF attivata come conseguenza attivazione oncogeni



Arf tumor suppressor promoter monitors latent oncogenic signals *in vivo*

Frederique Zindy*, Richard T. Williams†, Troy A. Baudino‡, Jerold E. Rehg§, Stephen X. Skapek†, John L. Cleveland‡, Martine F. Roussel*, and Charles J. Sherr*¶‖

Departments of *Genetics and Tumor Cell Biology, †Hematology–Oncology, ‡Biochemistry, and §Pathology and ¶Howard Hughes Medical Institute, St. Jude Children's Research Hospital, 332 North Lauderdale Street, Memphis, TN 38105

Contributed by Charles J. Sherr, October 21, 2003

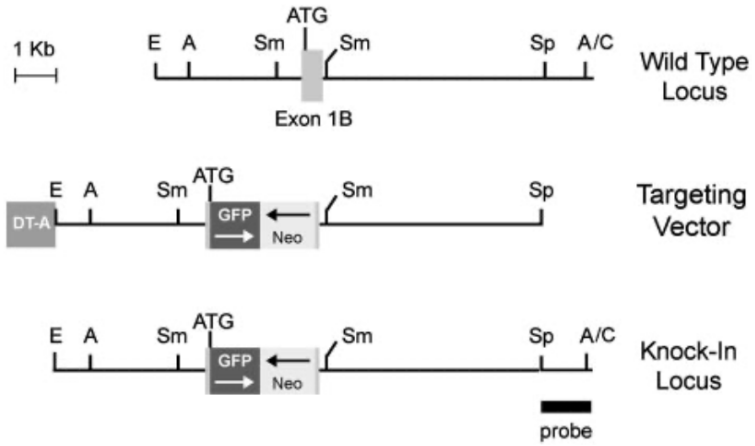


Fig. 1. Targeting of the *Arf* locus surrounding exon 1β. A schematic map of the region flanking exon 1β (*Top*), relevant sequences in the targeting vector (*Middle*), and the knock-in allele (*Bottom*) are illustrated. *Arf* coding sequences were replaced by a cassette encoding enhanced GFP and the neomycin-resistance gene (*neo*) in opposite orientations (arrows). The *neo* gene includes its own 5′ promoter, whereas GFP expression is driven by the *Arf* promoter; both *neo* and *GFP* terminate with 3′ polyadenylation signals. The targeting vector contains a gene encoding the diphtheria toxin A chain (DT-A), which is toxic unless eliminated and therefore selects against nonhomologous recombination of the targeting vector elsewhere in the mouse genome. The probe used to score the different alleles is illustrated at the bottom right. ATG refers to the position of the GFP initiation codon. Restriction sites for *EcoR1* (E), *Afl*II (A), *Sma*I (Sm), *Spe*I (Sp), and *Cla*I (C) are indicated.

Zindy et al., 2003

ARF è attivata da segnali diversi

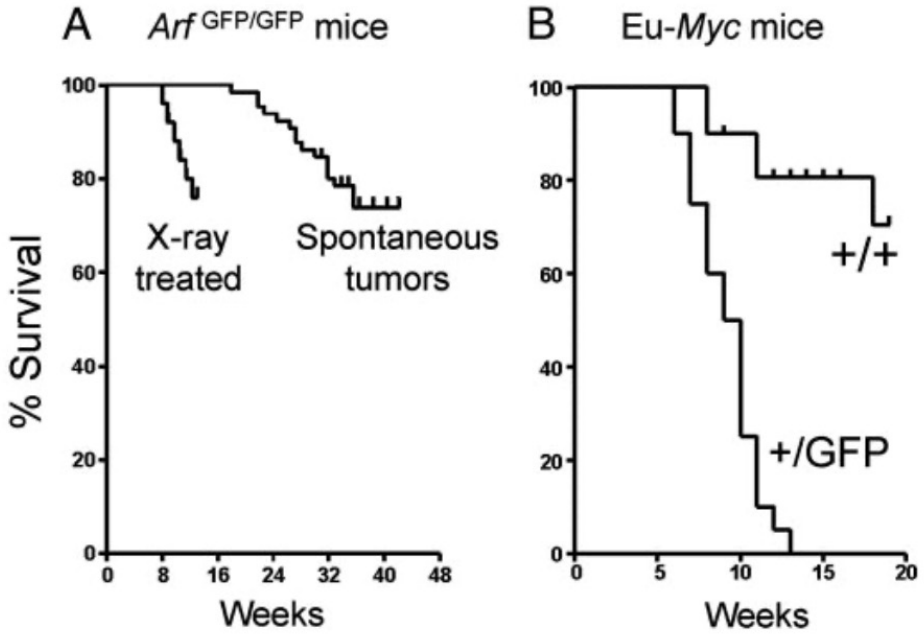


Fig. 3. Mouse survival curves. (*A*) Rates of spontaneous tumor development in *Arf* $^{GFP/GFP}$ mice observed for up to 44 weeks after birth, as compared with those arising in animals that received a single sublethal dose of ionizing irradiation at 5 days of age. (*B*) Rates of lymphoma development in Eμ-*Myc* transgenic mice contrasted on an *Arf*$^{+/+}$ or *Arf*$^{+/GFP}$ genetic background.

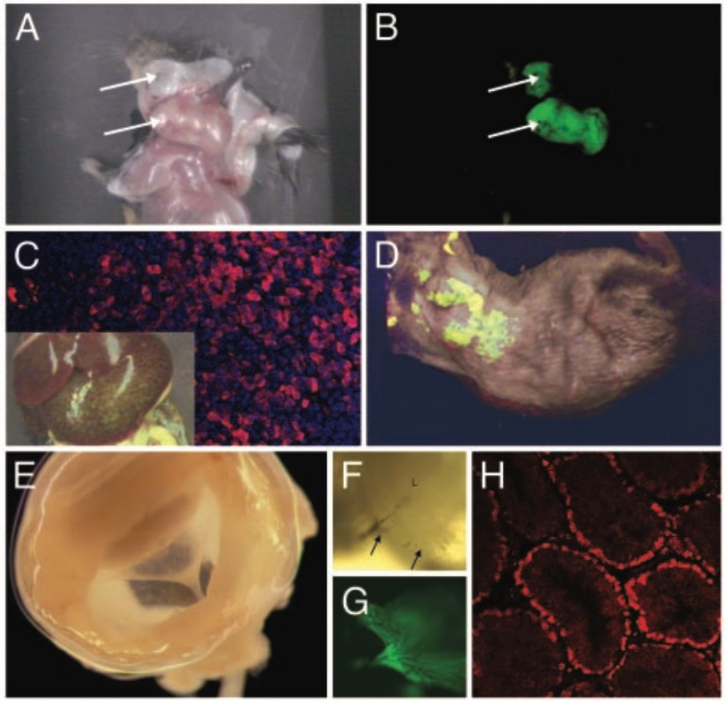


Fig. 4. GFP expression in mouse tissues. (*A* and *B*) *Arf* $^{GFP/GFP}$ mouse with a green fluorescent sarcoma (arrows) in the neck region. (*C*) Illustration of macroscopic foci of GFP-positive lymphoma cells that metastasized to liver (*Inset*) and microscopic foci visualized by immunofluorescence (red) and counterstained with 4′,6-diamidino-2-phenylindole (blue). (*D*) Whole-body imaging of a shaved *Arf* $^{+/GFP}$, Eμ-*Myc* mouse with lymphoma. A whole mount of a dissected eye from an *Arf*$^{GFP/GFP}$ mouse (*E*) illustrates a funnel-shaped mass stretching from the lens (top left) toward the optic cup at the rear. A closer view (*F*) illustrates elements of the hyaloid vasculature (arrows) within the green fluorescent mass (*G*). (*H*) Immunohistochemical staining of GFP (red) in the testis of an 8-month-old mouse. The position of stained cells within the tubules closely corresponds to regions containing spermatogonia and immature (leptotene) spermatocytes in meiosis I.

Zindy et al., 2003

Cosa fa p53?

Regola trascrizione

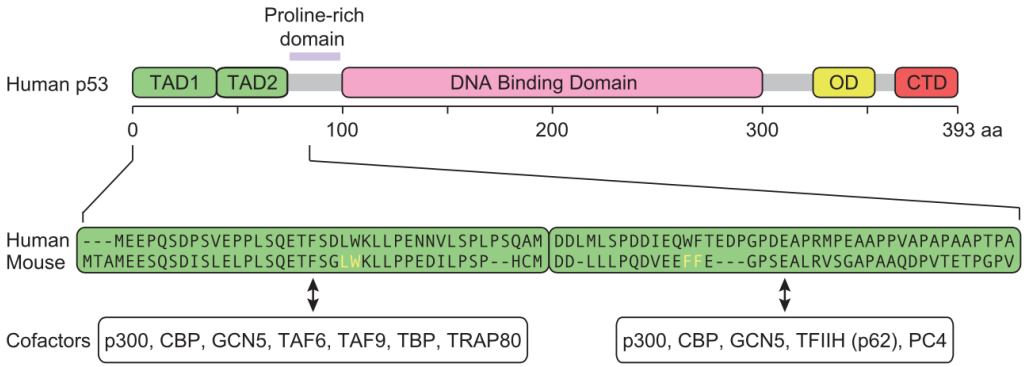


Figure 1 Schematic of p53 protein domain organization. (Top) Transactivation domains (TADs) 1 and 2 are indicated in green, DNA binding domain in pink, oligomerization domain (OD) in yellow, and C-terminal domain (CTD) in red. (Bottom) Primary amino acid sequences for TAD1 and TAD2 in both human and mouse. Residues altered in mouse models of TAD inactivation indicated in yellow. Known transcriptional cofactors are listed below the TAD with which they associate

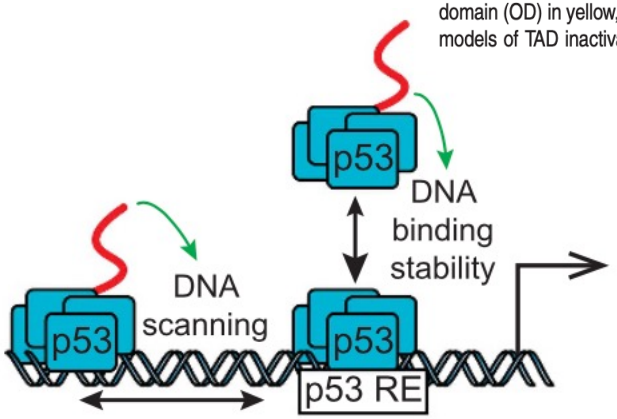
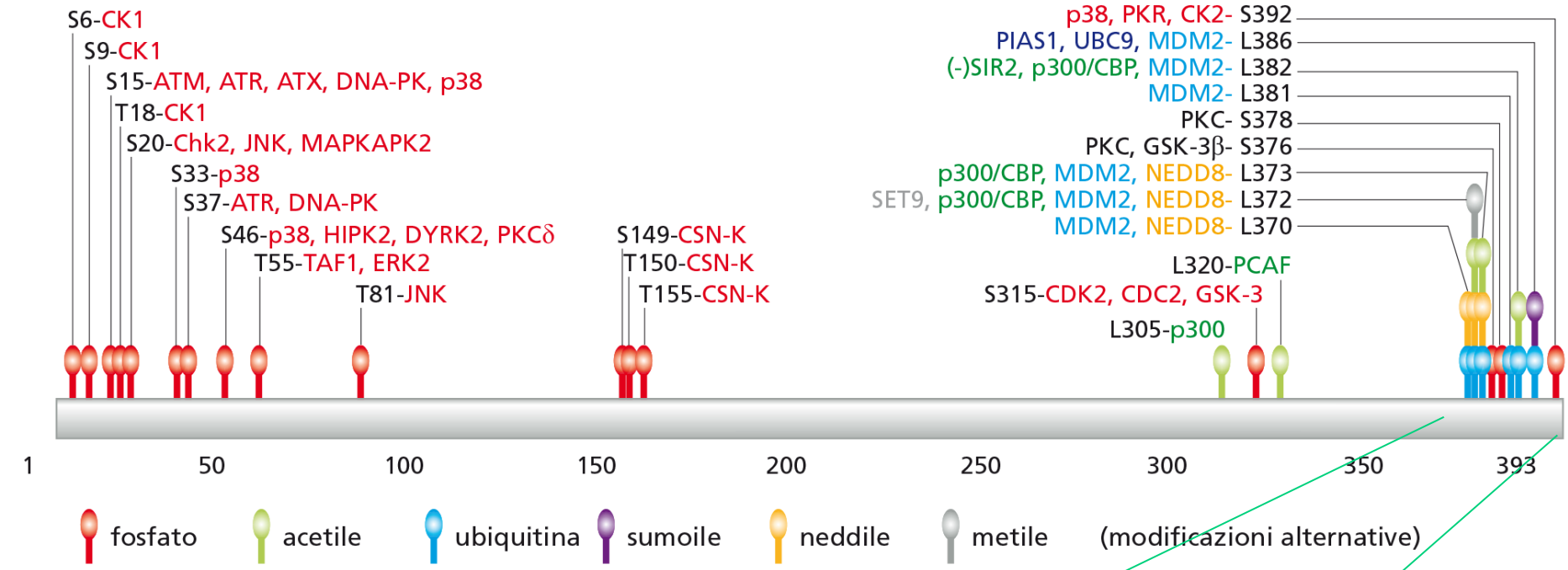


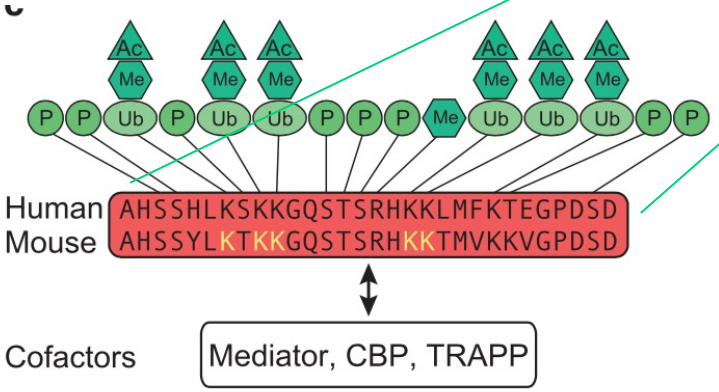
Figure 2 Functions of the p53 C-terminal domain (CTD). (**a**) The p53 CTD is important for DNA binding. The cartoon depicts the dual roles of the CTD (red) in recognition of the p53 response element (p53RE), by positively influencing scanning along DNA and stability of binding.[39,49] (**b**) The p53 CTD is structurally flexible. Representative ribbon structures of alternative conformations adopted by the CTD (red) upon binding to the different partners (gray) S100ß,[56] the bromodomain of CBP,[57] and the tandem tudor domain of 53BP1.[58] (**c**) The p53 CTD is postranslationally modified. Schematic depicts the primary structure of the CTD with known modifications and sites indicated. Lysine residues altered in mouse models of CTD inactivation highlighted in yellow. CTD-interacting transcriptional cofactors are listed at the bottom. (**d**) Model of p53 CTD (red) intrinsically disordered domain-mediated aggregation at RNA factories (light green cloud) along with RNA polymerase II (RNAPII, gray). Ac, acetylation; Me, methylation; P, phosphorylation; Ub, ubiquitination

Sullivan et al., 2018

Cosa fa p53?

Regolazione post-traduzionale di p53 ne modifica l'attività





Sullivan et al., 2018

Cosa fa p53?

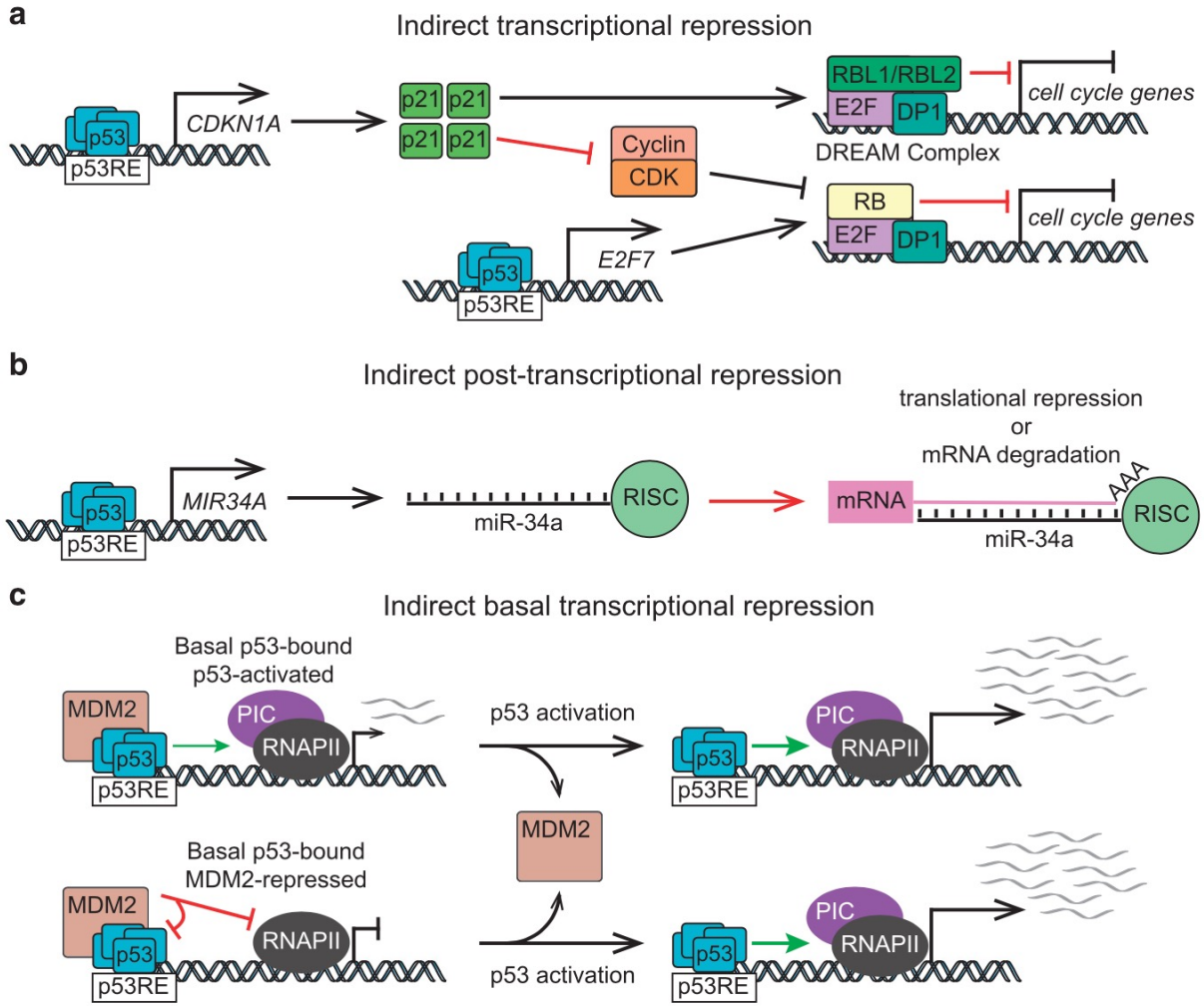


Figure 3 Mechanisms of p53-dependent repression of gene expression. (**a**) p53 indirectly represses E2F target genes via transactivation of *CDKN1A* that encodes p21, a CDK inhibitor, leading to transcriptional repression of cell cycle genes by the RB-E2F4 complex and the DREAM complex.[79–82] In addition, p53 directly transactivates E2F7, a member of the repressive subfamily of E2F transcription factors.[83,84] (**b**) p53 post-transcriptionally represses gene expression via microRNAs (miRs) such as miR-34a that can target mRNAs for degradation or translational repression via the RNA-induced silencing complex (RISC). (**c**) Under basal (non-activated) conditions, even when bound to MDM2, p53 can bind to target genes. Some of these genes are activated by basal p53 (top), while others are repressed by MDM2 (bottom).[70] p53RE, p53 response element; CDK, cyclin-dependent kinase; PIC, preinitiation complex; RNAPII, RNA polymerase II

Sullivan et al., 2018

Funzioni di p53

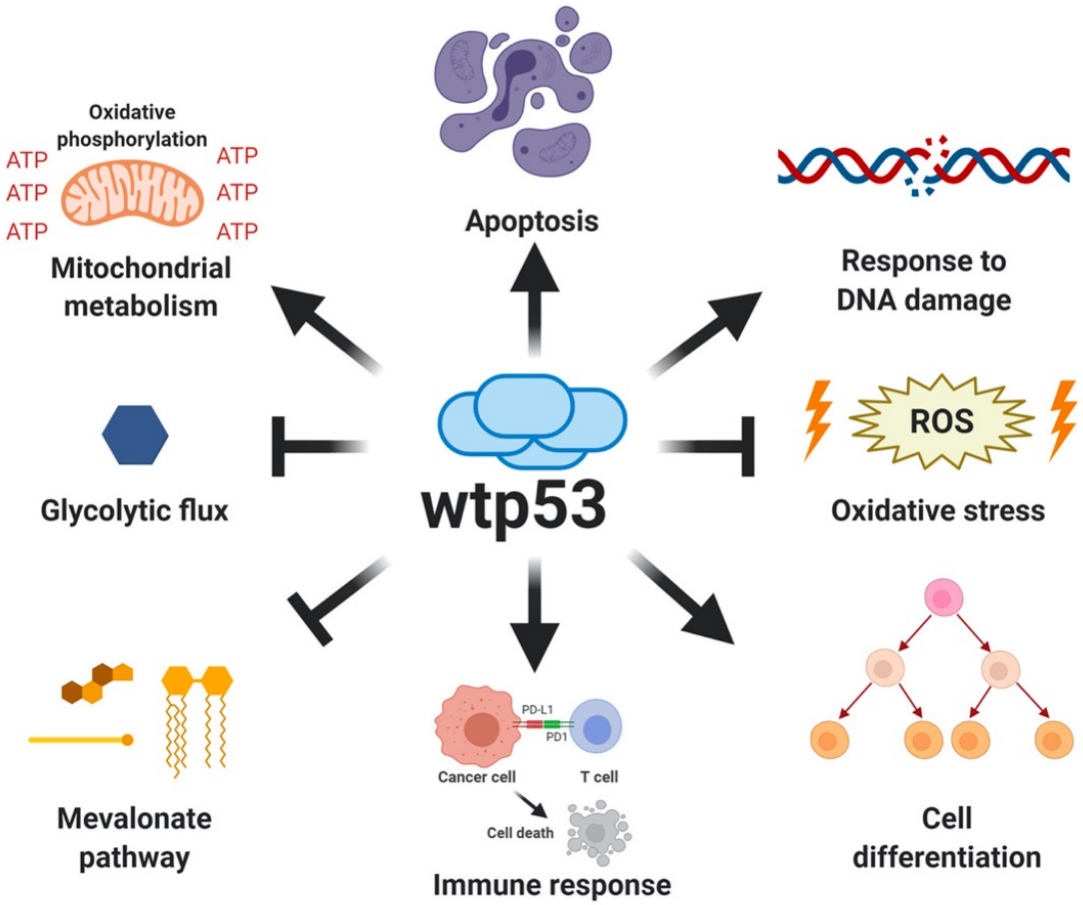
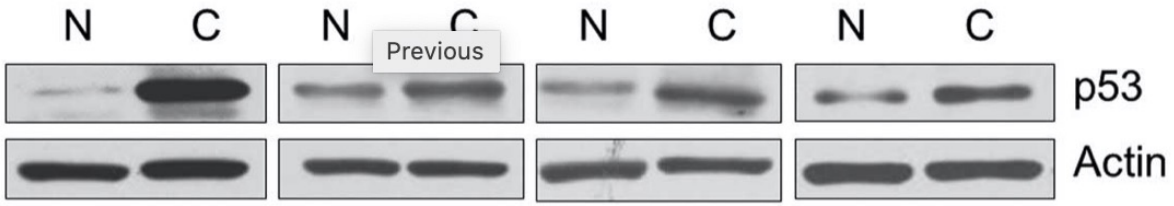


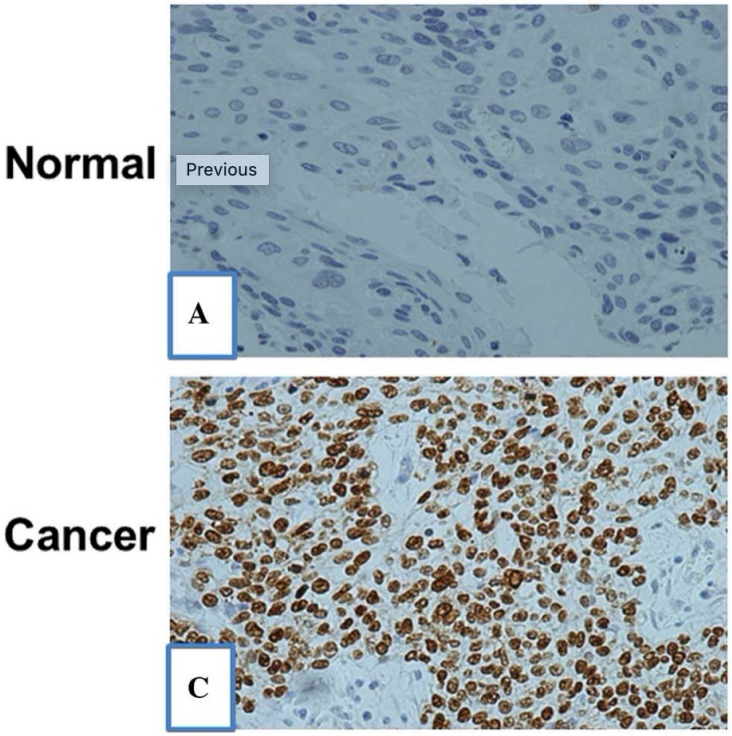
FIGURE 2 | Canonical functions of wild type p53. Wild type p53 is a major tumor suppressor whose functions are critical for protection against cancer. The canonical functions of wild type p53 include the induction of apoptosis, regulation of oxidative metabolism, and inhibition of glycolytic flux, as well as the response to DNA damage, increased antioxidant capabilities, regulation of immune response and differentiation processes.

Alvarado-Ortiz et al., 2021

P53 in tumori

Molti tumori hanno livelli elevati di p53 → perché proliferano?





p53 protein expression in esophageal squamous cell carcinoma (ESCC) patient tissues. (A) Western blot analysis of p53 protein expression in ESCC patient tumor tissue (denoted as C) and matched tumor-adjacent non-neoplastic tissue (denoted as N)

Huang et al., 2014

P53 in tumori

Mdm2 è un bersaglio di p53

Cosa succede se p53 mutata non attiva Mdm2?

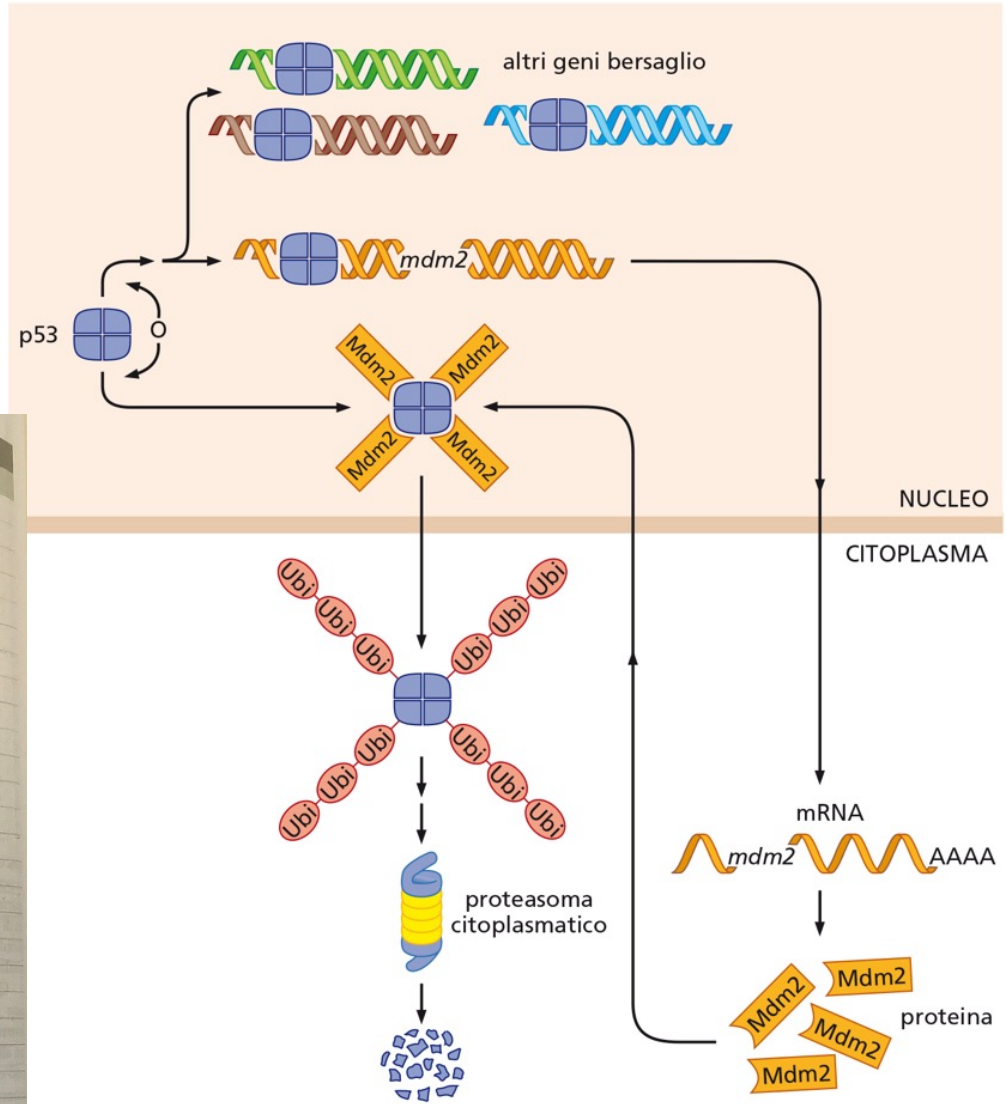


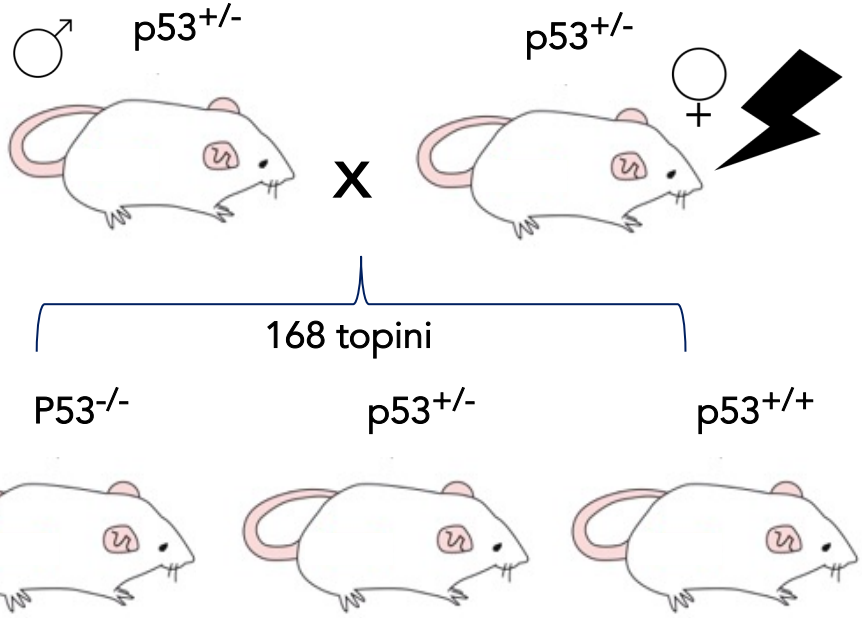
TABELLA 9.2

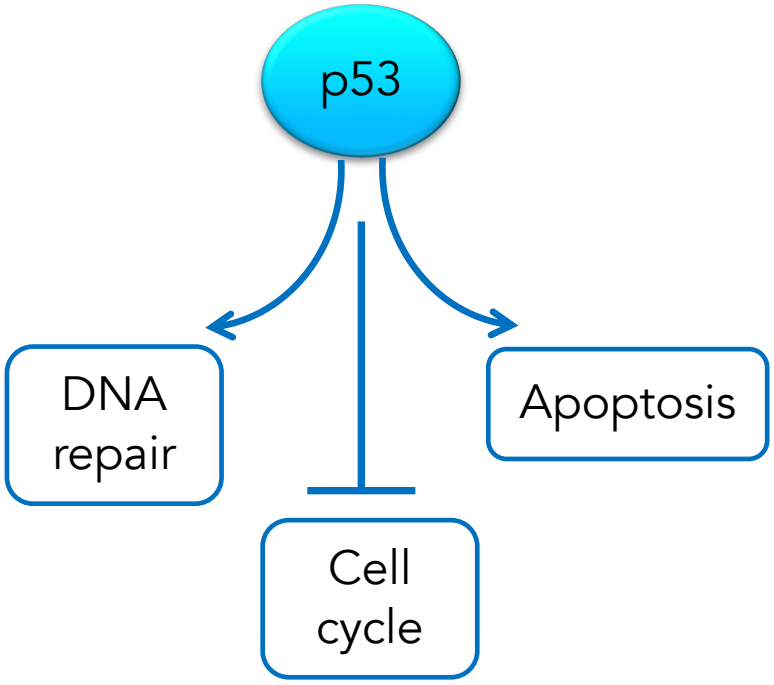
Esempi di geni bersaglio di p53 a seconda della loro funzione[a]

Classe di geni	Nome del gene	Funzione del prodotto genico
Antagonista di p53	*MDM2/HDM2*	induce l'ubiquitinazione di p53
Geni di arresto della crescita	*$p21^{Cip1}$*	inibitore di CDK, DNA polimerasi
	Siah-1	coopera nella degradazione della β-catenina
	14-3-3σ	sequestra ciclina B-CDC2 nel citoplasma
	Reprimo	arresto in G_2
Geni di riparazione del DNA	*p53R2*	ribonucleotide reduttasi – biosintesi di precursori del DNA
	XPE/DDB2	riparazione globale dell'escissione di nucleotidi
	XPC	riparazione globale dell'escissione di nucleotidi
	XPG	riparazione globale dell'escissione di nucleotidi
	GADD45	riparazione globale dell'escissione di nucleotidi [?]
	DNA pol κ	DNA polimerasi infedele
Regolatori dell'apoptosi	*BAX*	proteina dei pori mitocondriali
	PUMA	proteina dei pori mitocondriali *BH3-only*
	NOXA	proteina dei pori mitocondriali *BH3-only*
	p53A/P1	dissipa il potenziale di membrana mitocondriale
	Killer/DR5	recettore di morte sulla superficie cellulare
	PIDD	proteina con un dominio di morte
	PERP	proteina proapoptotica transmembrana
	APAF1	attivatore di caspasi 9
	NF-κB	fattore di trascrizione, mediatore della trasduzione di segnale del TNF
	FAS/APO1	recettore di morte
	PIG3	controllo dell'ossidazione/riduzione mitocondriale
	PTEN	riduce i livelli di PIP3 antiapoptotico
	Bcl-2	repressione dell'espressione di proteine antiapoptotiche
	IGF-1R	repressione dell'espressione di proteine antiapoptotiche
	IGFBP-3	proteina che sequestra IGF-1
Proteine antiangiogeniche	*TSP-1* (trombospondina)	antagonista dell'angiogenesi

Weinberg, la Biologia del Cancro

P53 in tumori





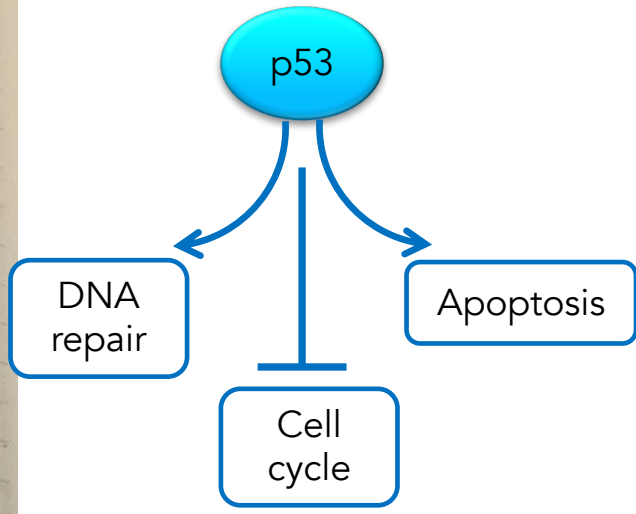
Tumori cerebrali	70%	3,6%	0

Bersagli di p53

TABELLA 9.2

Esempi di geni bersaglio di p53 a seconda della loro funzione[a]

Classe di geni	Nome del gene	Funzione del prodotto genico
Antagonista di p53	*MDM2/HDM2*	induce l'ubiquitinazione di p53
Geni di arresto della crescita	*$p21^{Cip1}$*	inibitore di CDK, DNA polimerasi
	Siah-1	coopera nella degradazione della β-catenina
	14-3-3σ	sequestra ciclina B-CDC2 nel citoplasma
	Reprimo	arresto in G_2
Geni di riparazione del DNA	*p53R2*	ribonucleotide reduttasi – biosintesi di precursori del DNA
	XPE/DDB2	riparazione globale dell'escissione di nucleotidi
	XPC	riparazione globale dell'escissione di nucleotidi
	XPG	riparazione globale dell'escissione di nucleotidi
	GADD45	riparazione globale dell'escissione di nucleotidi [?]
	DNA pol κ	DNA polimerasi infedele
Regolatori dell'apoptosi	*BAX*	proteina dei pori mitocondriali
	PUMA	proteina dei pori mitocondriali *BH3-only*
	NOXA	proteina dei pori mitocondriali *BH3-only*
	p53A/P1	dissipa il potenziale di membrana mitocondriale
	Killer/DR5	recettore di morte sulla superficie cellulare
	PIDD	proteina con un dominio di morte
	PERP	proteina proapoptotica transmembrana
	APAF1	attivatore di caspasi 9
	NF-κB	fattore di trascrizione, mediatore della trasduzione di segnale del TNF
	FAS/APO1	recettore di morte
	PIG3	controllo dell'ossidazione/riduzione mitocondriale
	PTEN	riduce i livelli di PIP3 antiapoptotico
	Bcl-2	repressione dell'espressione di proteine antiapoptotiche
	IGF-1R	repressione dell'espressione di proteine antiapoptotiche
	IGFBP-3	proteina che sequestra IGF-1
Proteine antiangiogeniche	*TSP-1* (trombospondina)	antagonista dell'angiogenesi



Weinberg, la Biologia del Cancro

Mutazioni ereditarie in p53

Journal of Medical Genetics 1982, **19**, 362–365

Two families with the Li-Fraumeni cancer family syndrome

A D J PEARSON,* A W CRAFT,* J M RATCLIFFE,† J M BIRCH,† P MORRIS-JONES,† AND D F ROBERTS*

*From *the Departments of Child Health and Human Genetics, University of Newcastle upon Tyne and Royal Victoria Infirmary, Newcastle upon Tyne; and †the Departments of Child Health and Epidemiology and Social Research, University of Manchester, Manchester.*

SUMMARY The first two families to be identified in the United Kingdom with the Li-Fraumeni syndrome of familial cancer are reported. The first family comprises breast carcinoma in the mother and adrenocortical carcinoma, medulloblastoma, and rhabdomyosarcoma in three of her four children, and the second family comprises breast carcinoma in the mother and adrenocortical carcinoma and rhabdomyosarcoma in two of her three children. All three of the surviving children with malignancy, and one other who has recently died, possess the tissue type antigen B12.

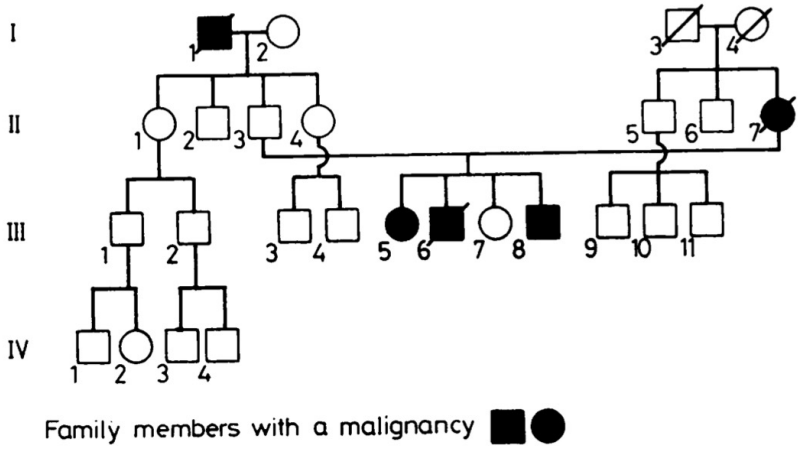


FIG 1 *Pedigree of family A. I.1 gastric carcinoma, II.7 breast carcinoma, III.5 adrenocortical carcinoma, III.6 medulloblastoma, III.8 rhabdomyosarcoma.*

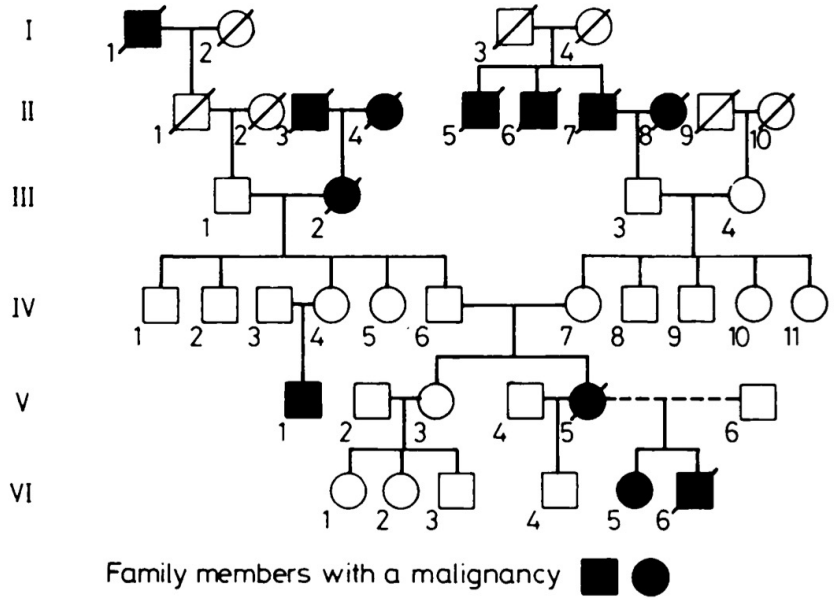
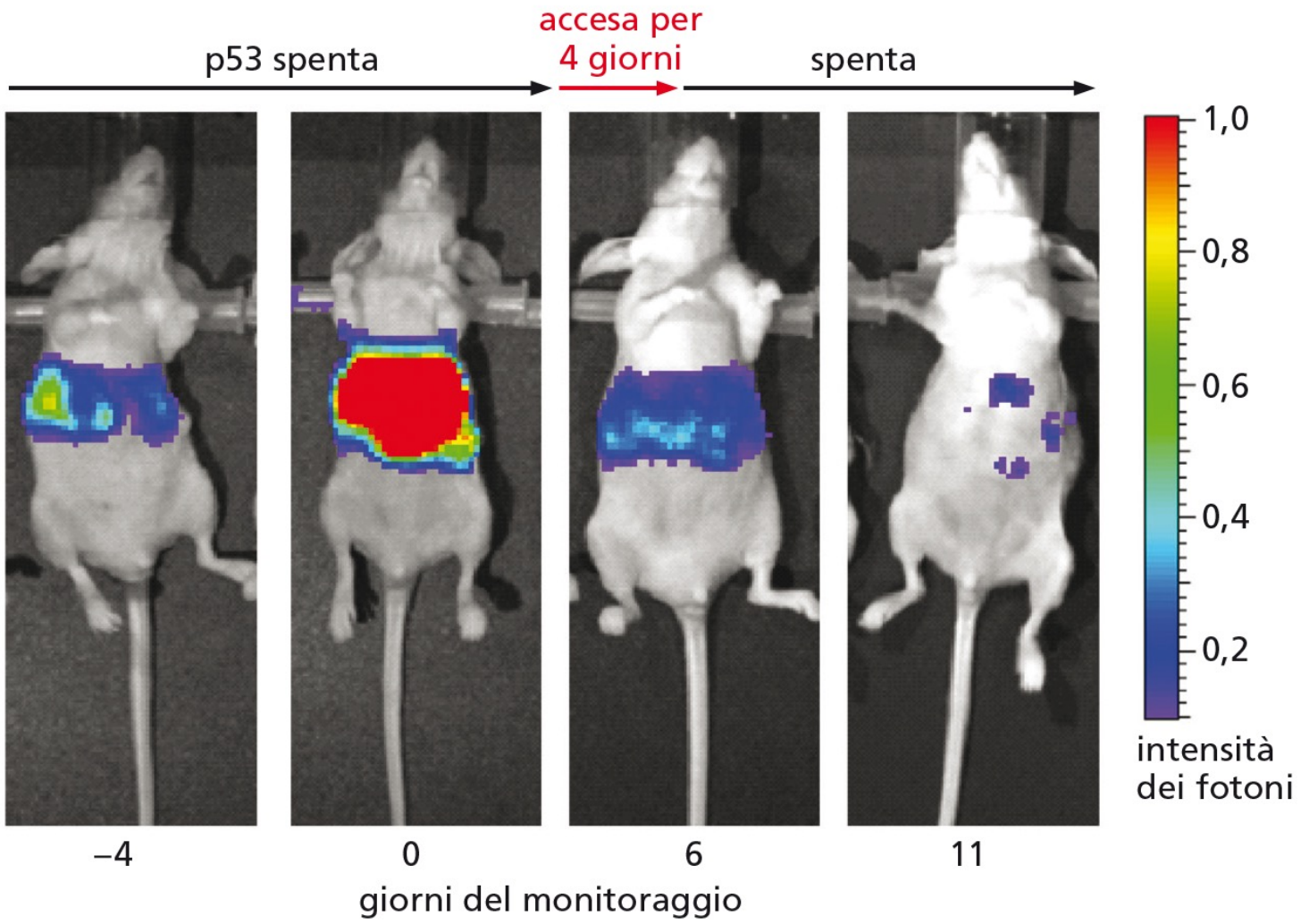


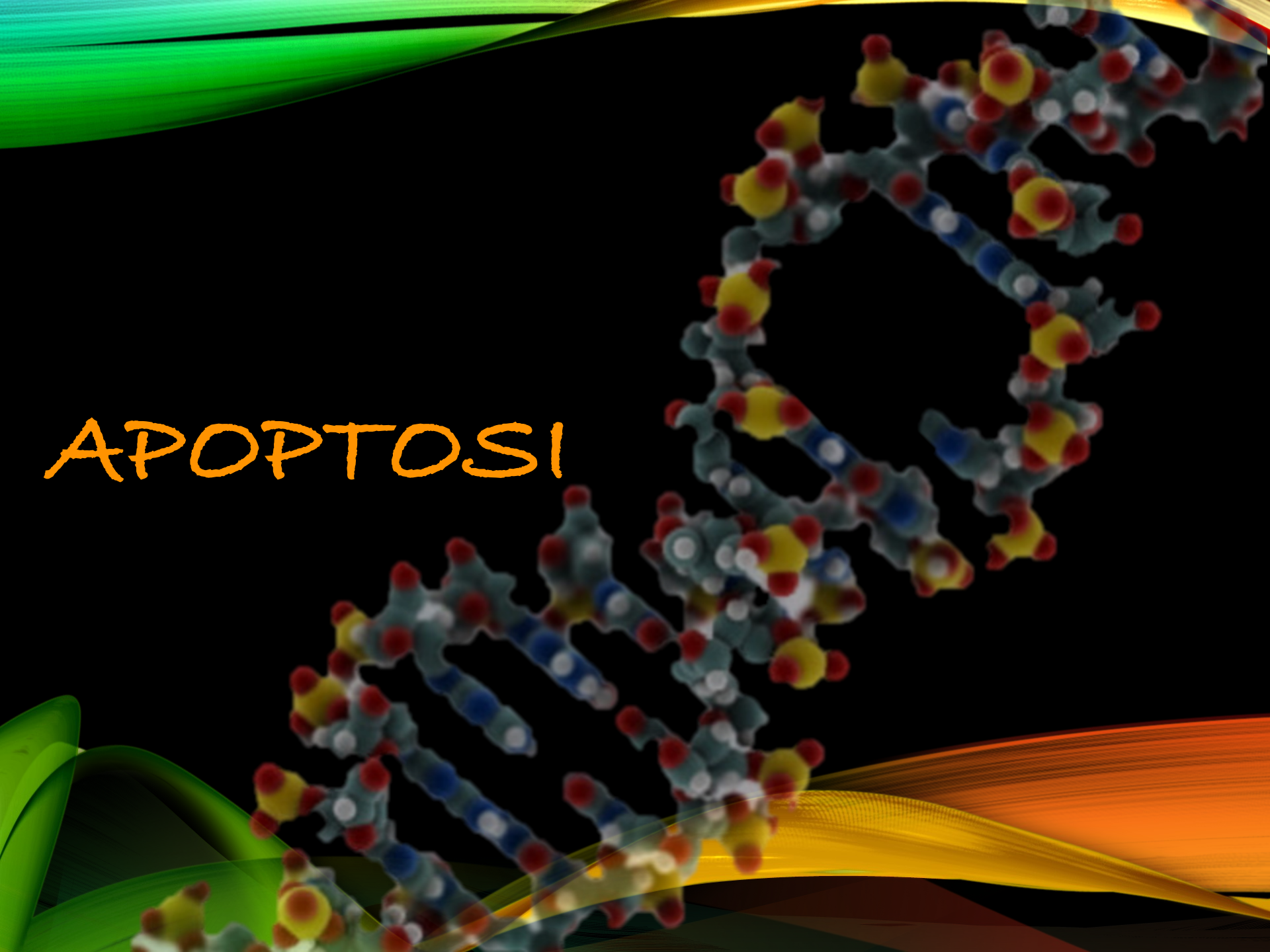
FIG 2 *Pedigree of family B. I.1 carcinoma of lung, II.3 carcinoma of lung, II.4 carcinoma unknown site, II.5 carcinoma of colon, II.6 carcinoma of stomach, II.7 carcinoma of lung, II.8 carcinoma of stomach, III.2 carcinoma of breast, V.1 testicular seminoma, V.6 carcinoma of breast, VI.5 adrenocortical carcinoma, VI.6 rhabdomyosarcoma.*

Riattivazione p53 nei tumori

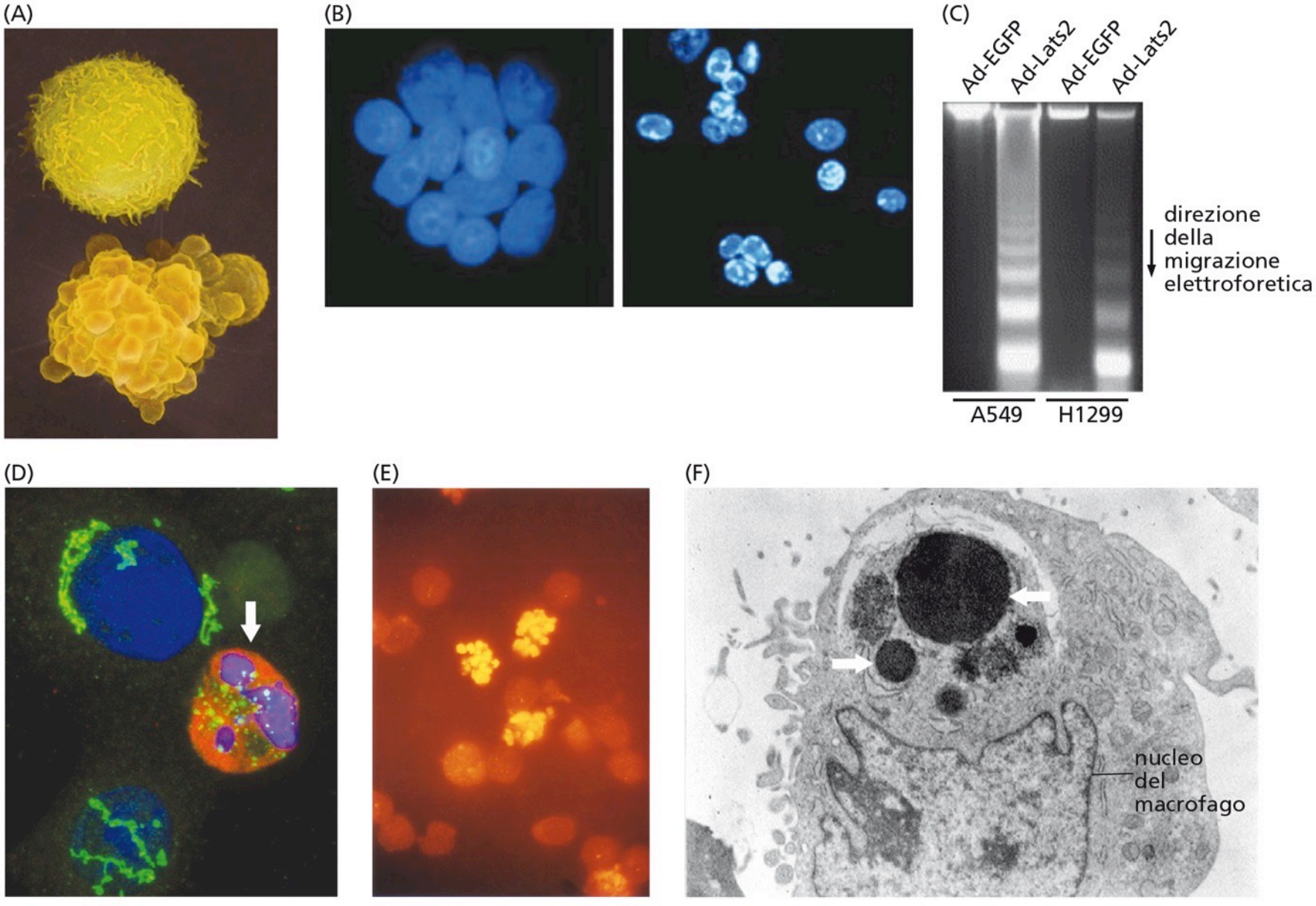


Weinberg, la Biologia del Cancro

APOPTOSI



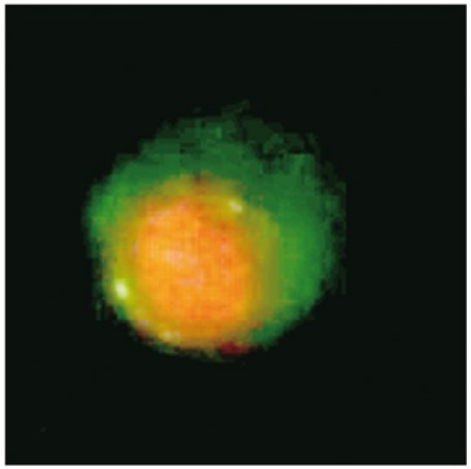
Il programma apoptotico



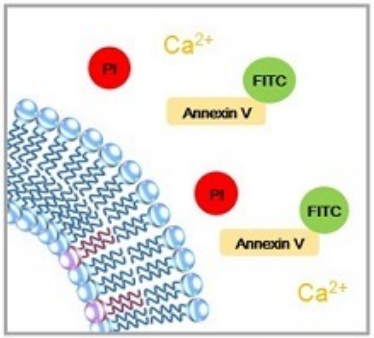
Weinberg, la Biologia del Cancro

Identificare cellule in apoptosi

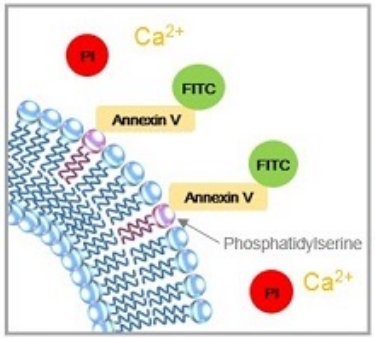
- Apoptosi è fisiologica
- Apoptosi fisiologica non dipende da p53



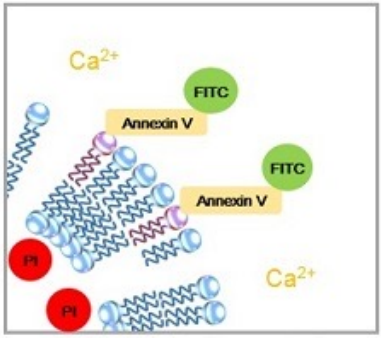
(A)



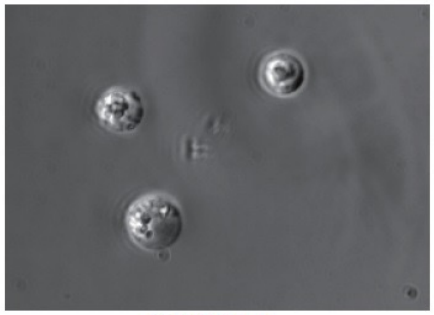
Normal Cell



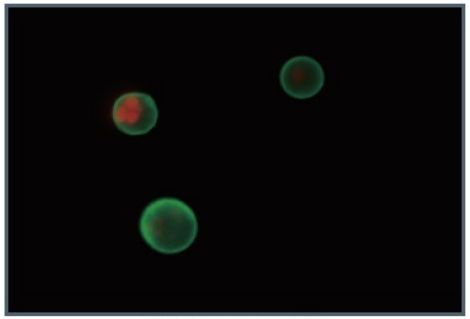
Early stages of apoptosis



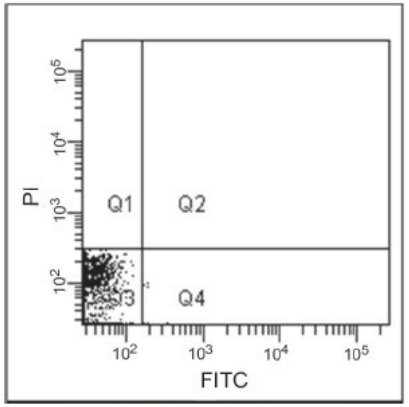
Late stages of apoptosis



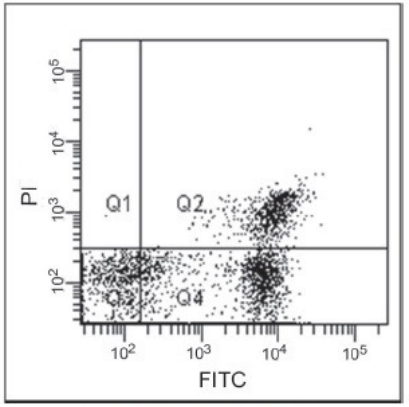
A: Bright Field



B: Fluorescent Image



A: Control (non-treated cells)

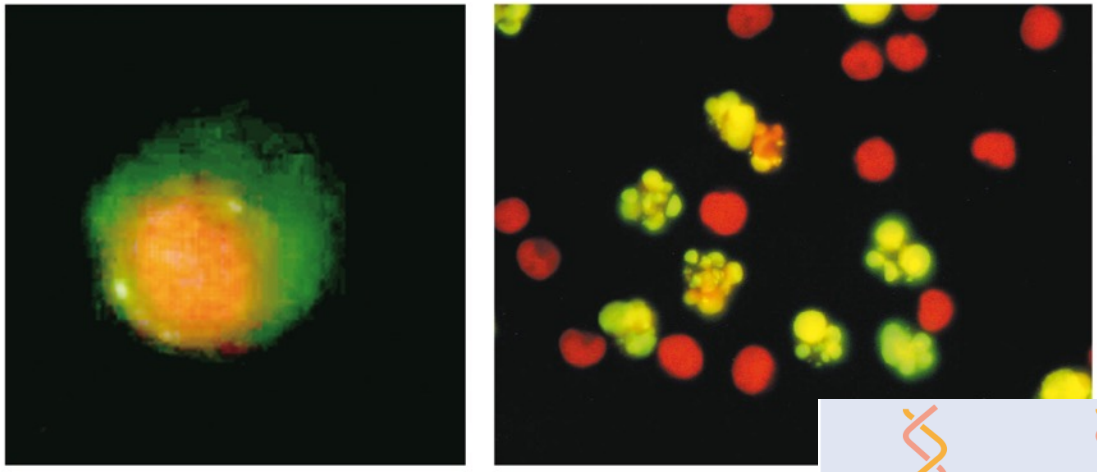


B: Apoptosis induced cells

Weinberg, la Biologia del Cancro

Identificare cellule in apoptosi

- Apoptosi è fisiologica
- Apoptosi fisiologica non dipende da p53



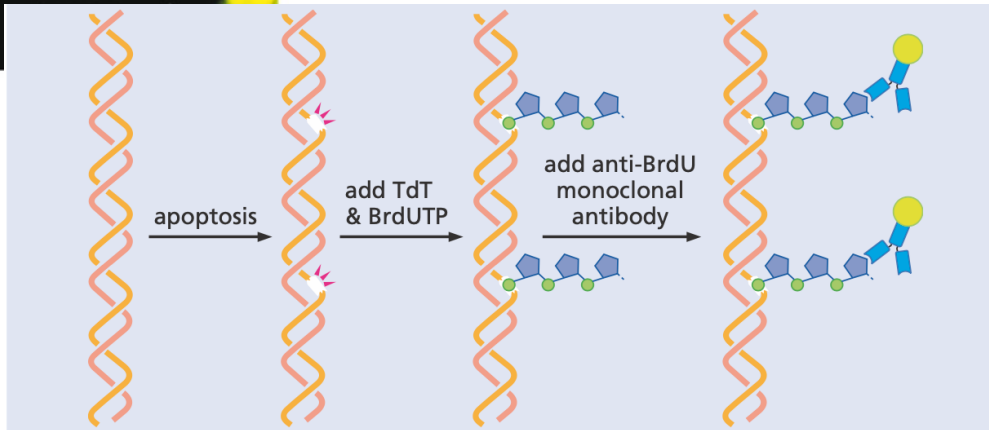
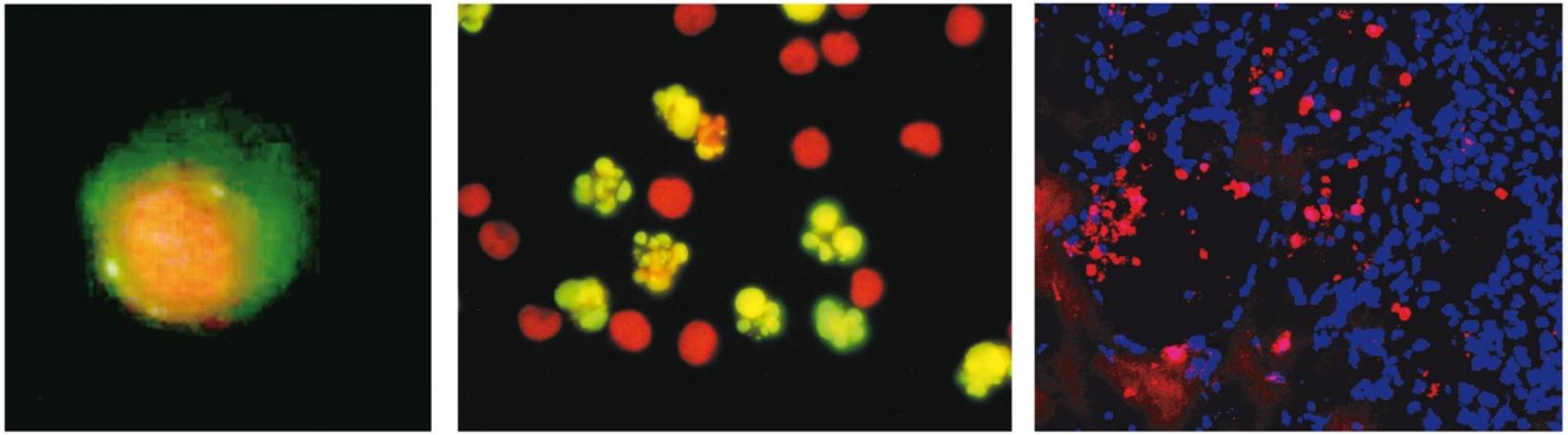


Figure S9.2 The TUNEL procedure Apoptotic cells can be detected because their chromosomal DNA has become fragmented (see Figure 9.18C), exposing 3′-OH DNA ends. The latter can be extended by the terminal deoxyribonucleotide transferase (TdT) enzyme, which acts processively to generate long tails from these ends, in this case doing so using bromodeoxyuridine triphosphate (BrdUTP) as substrate. The resulting BrdU-incorporated oligonucleotide tails can be detected with an anti-BrdU monoclonal antibody that has been coupled to a dye molecule (*yellow green*).

Identificare cellule in apoptosi

- Apoptosi è fisiologica
- Apoptosi fisiologica non dipende da p53

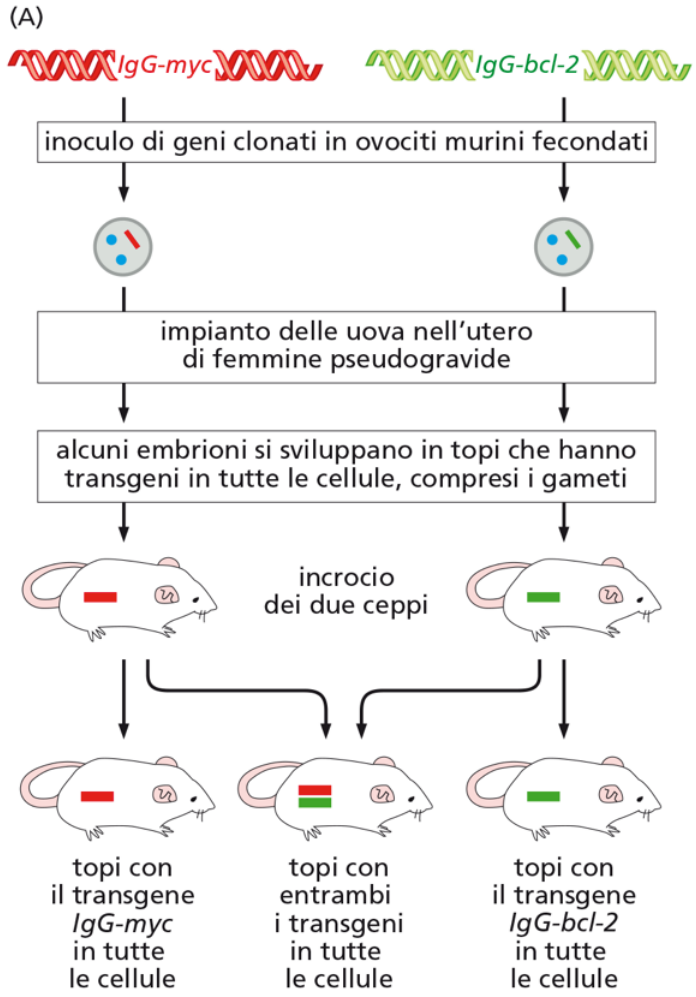


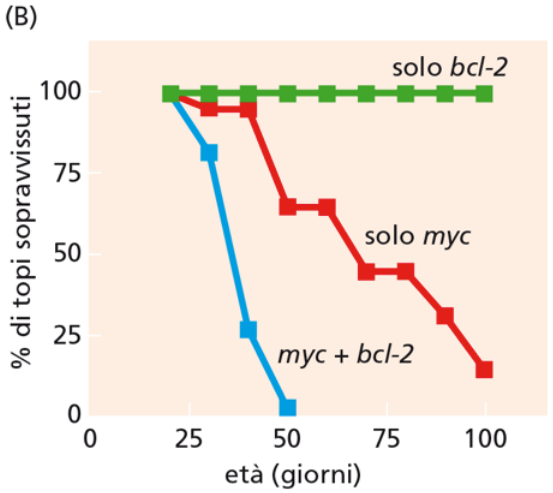
(A) (B) (C)

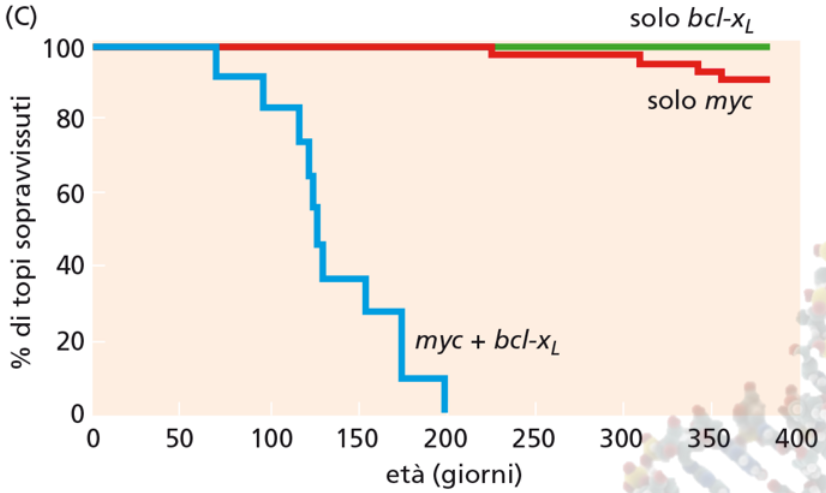
Weinberg, la Biologia del Cancro

Mitocondri e apoptosi

Altre proteine coinvolte in regolazione apoptosi: Bcl-2 (prot con funzioni mitocondriali)

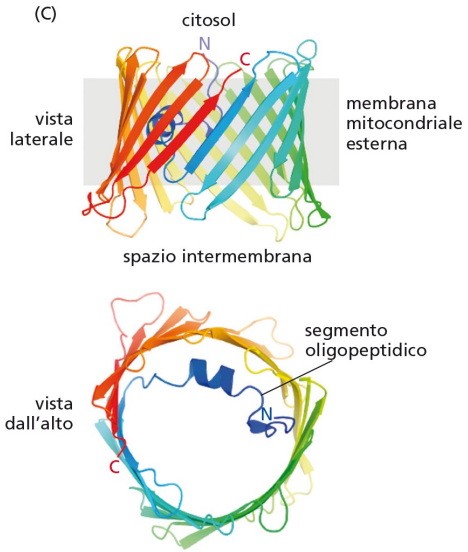


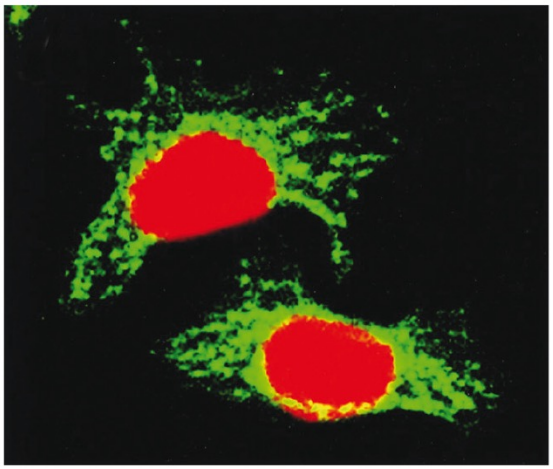


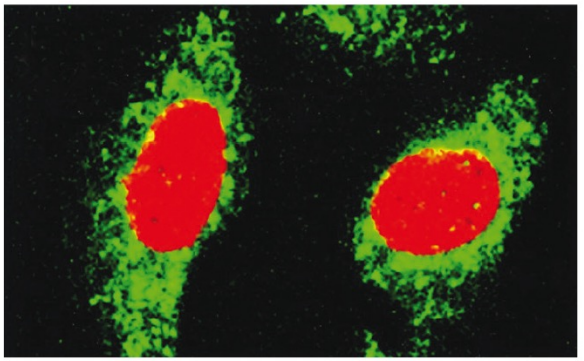


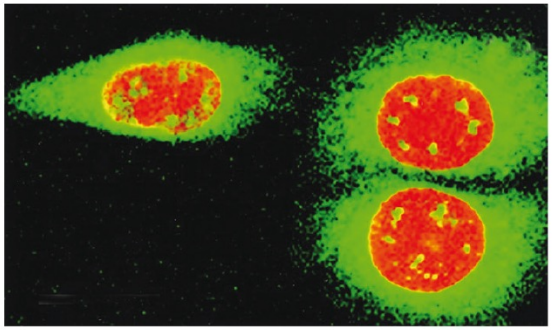
Weinberg, la Biologia del Cancro

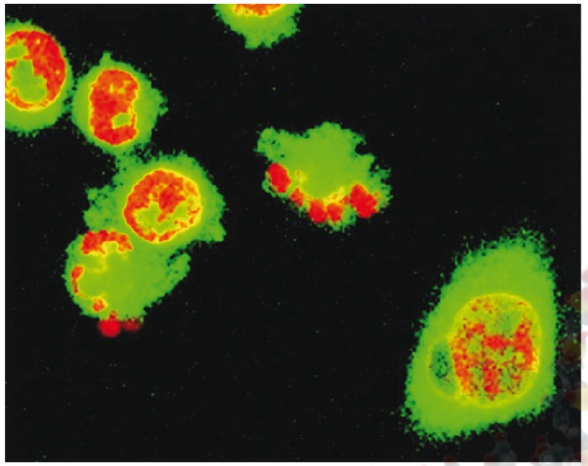
Liberazione del citocromo c nel citoplasma




(A)

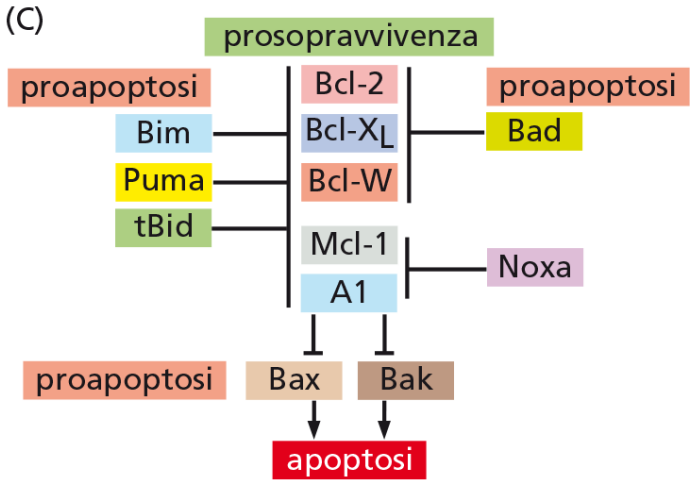

(B)

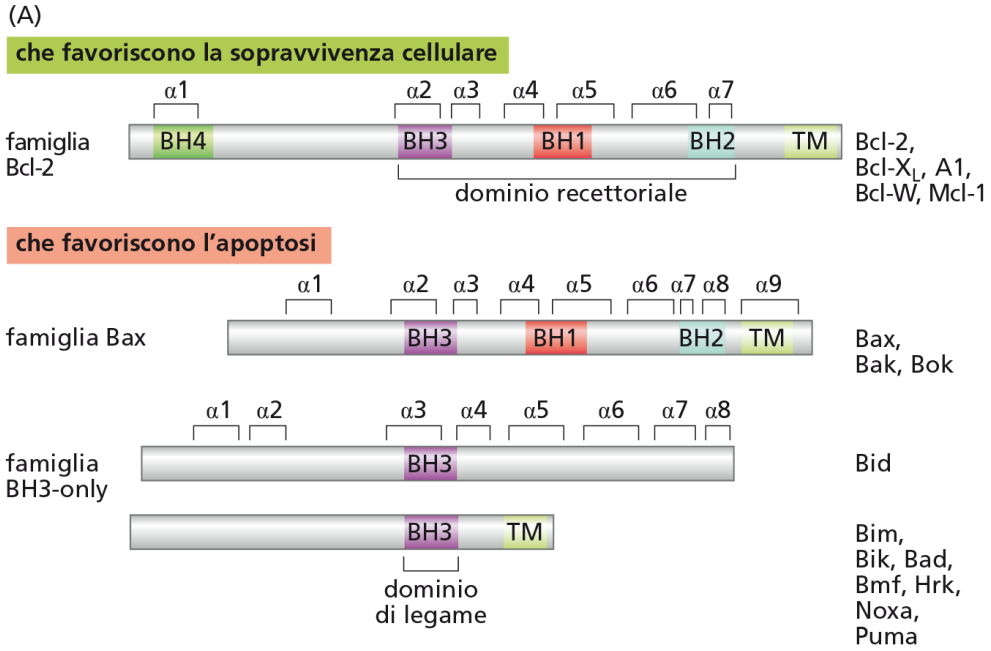

(C)

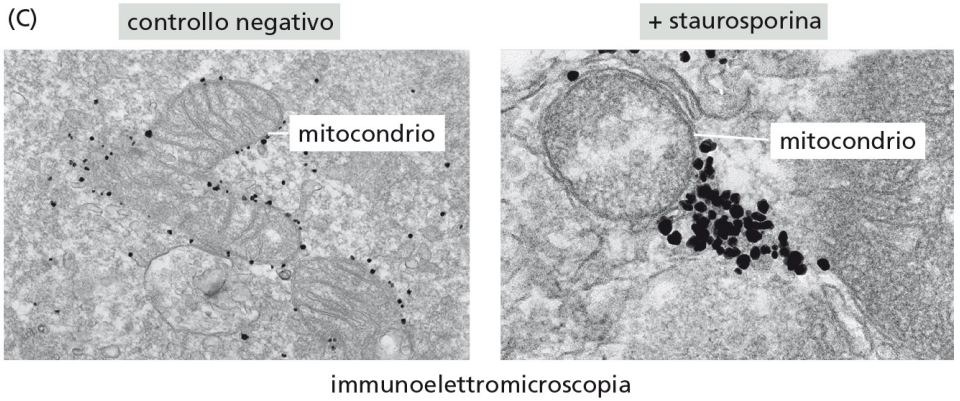

(D)

Proteine della famiglia Bcl-2 e apoptosi

Proteine pro e anti aptotiche regolano flusso citocromo c attraverso il canale mitocondriale







Weinberg, la Biologia del Cancro