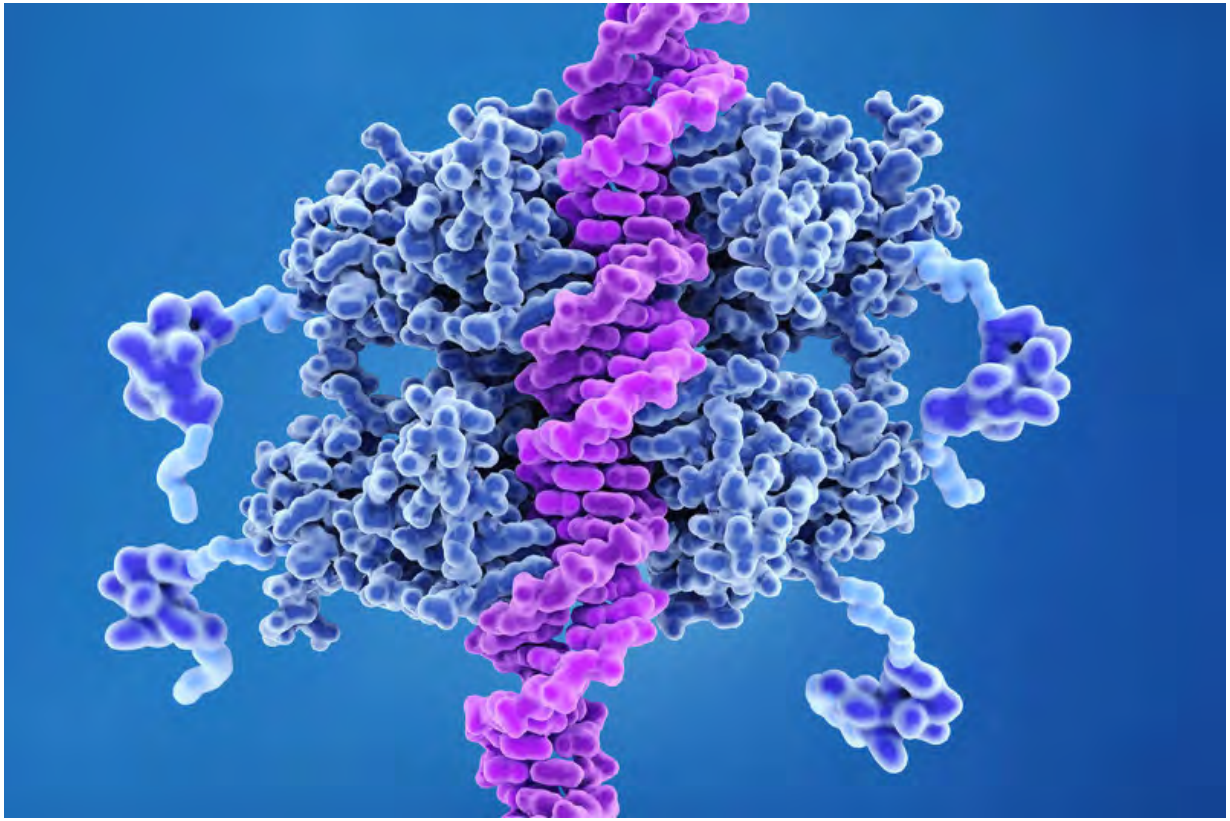
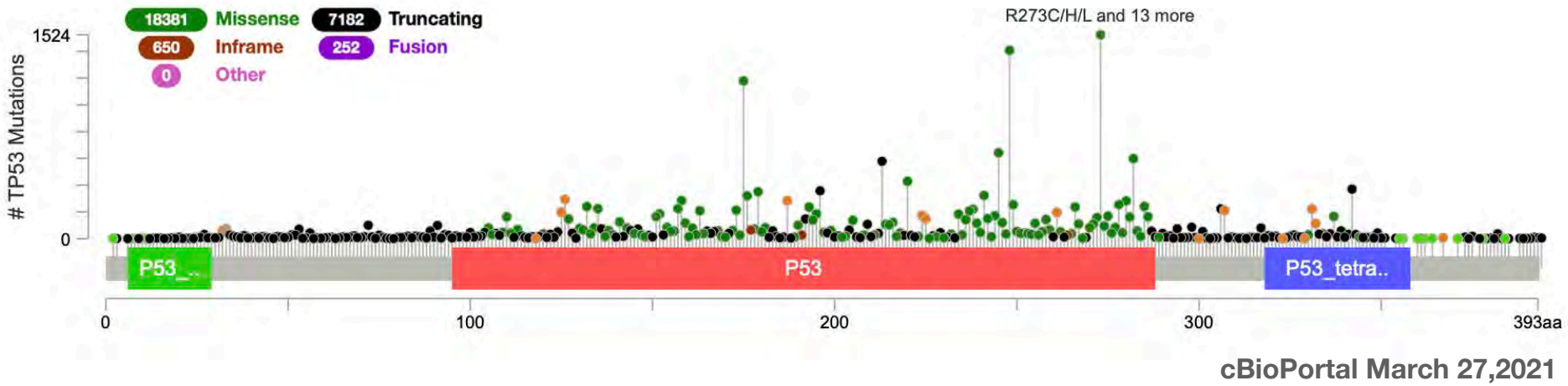


The p53 tumor suppressor

GSK Lecture

April 12, 2023

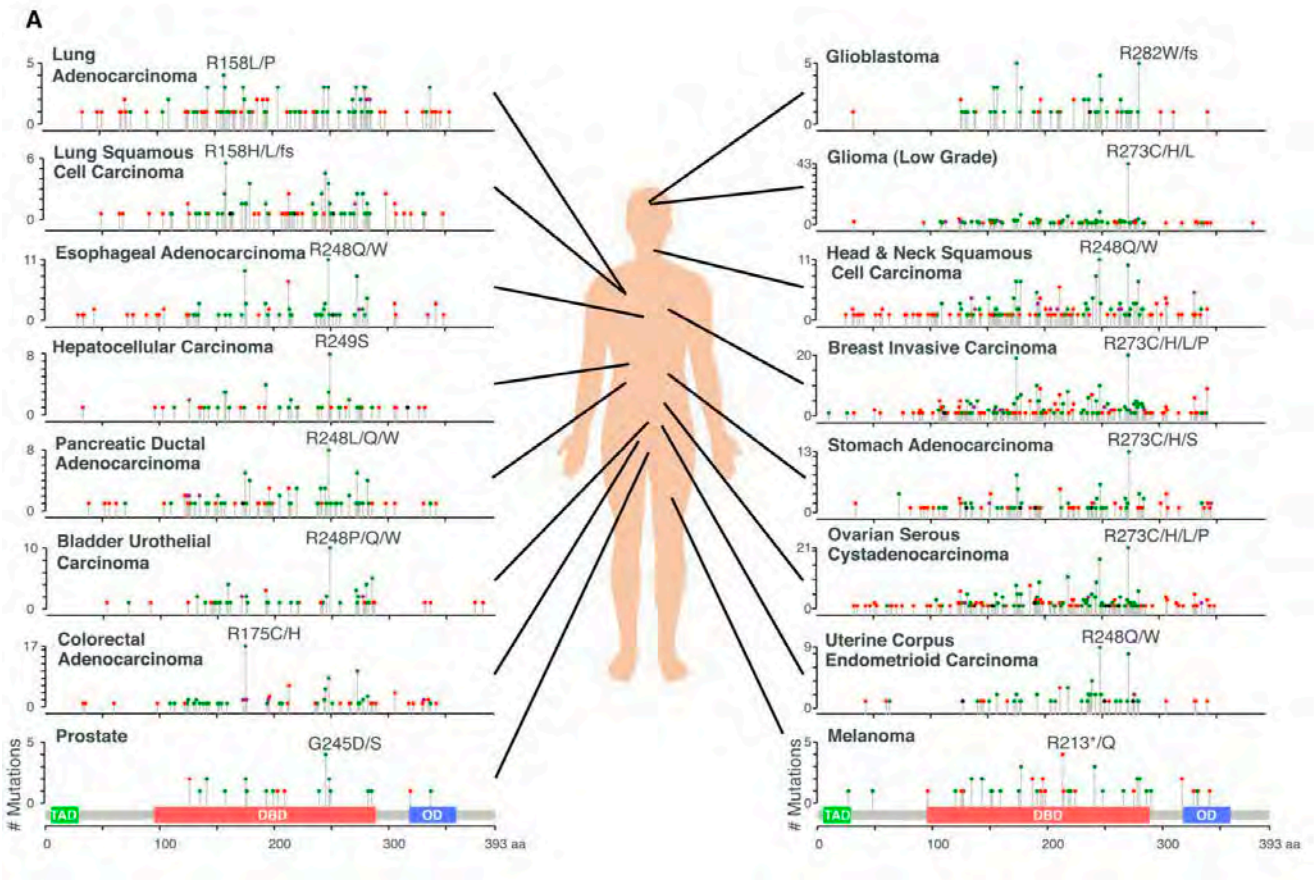
p53 mutations in human cancers



cBioPortal March 27,2021

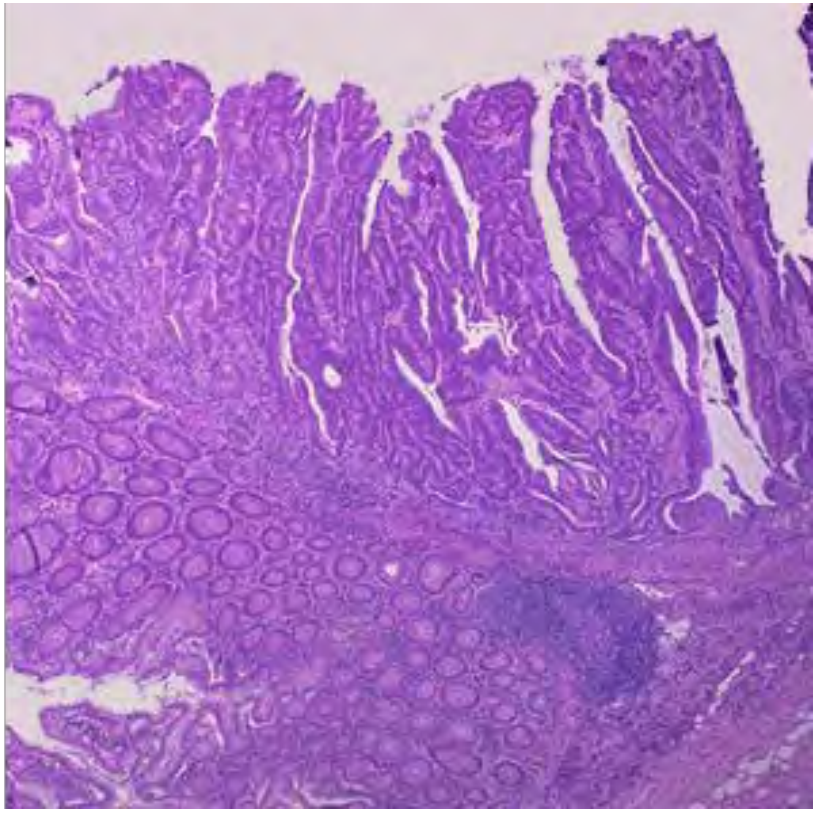
- p53 mutations are the most frequent event in human cancer
- Mutations can occur throughout the open reading frame

p53 mutations in human cancers



Kastenhuber Cell 2017

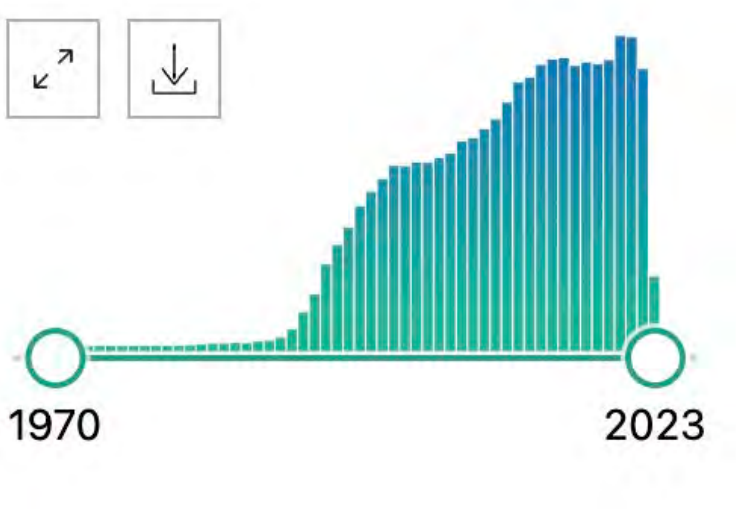
Features of p53 mutant tumors



- More aggressive histopathology and increased invasion
- Highly rearranged genomes
- Typically linked to poor patient prognosis
- Frequent in tumors with limited treatment options
- Often linked to poor therapy response

Challenges in navigating p53 research

- p53 is a highly studied gene
- ~ 115,000 papers published with the term ‘p53’ in the abstract (‘chromatin’ ~ 143,000)
- More information has not achieved clarity
- We still do not understand why p53 is so important in cancer nor its role (if any) in normal physiology



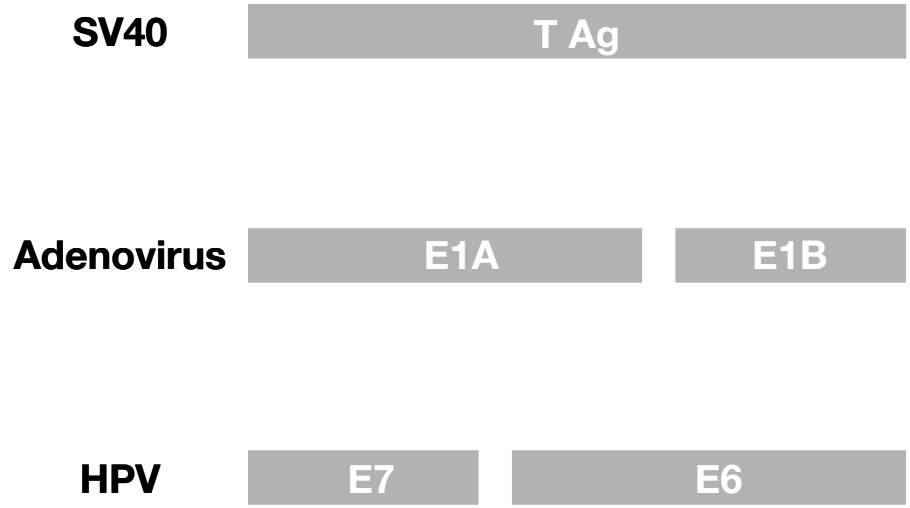
March 27, 2023: ~115,149 papers

Decade 1

The discovery of p53 and its oncogenic role

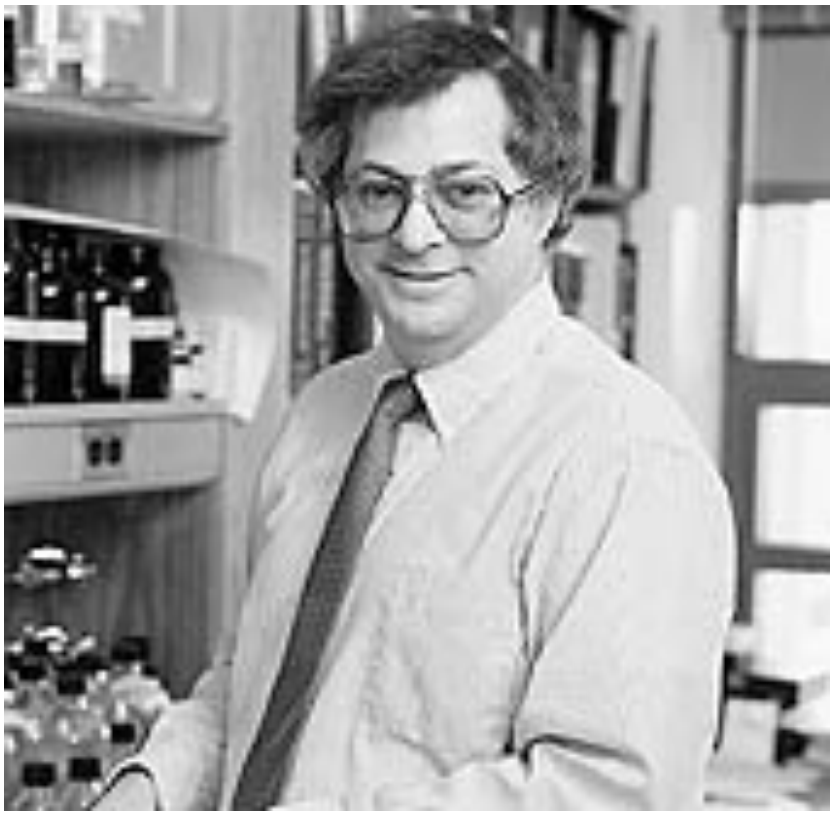
DNA tumor viruses

- RNA tumor viruses inadvertently pick up cellular DNA and were essential for the identification of oncogenes
- Tumor viruses a tool to unlock molecular events linked to oncogenic “transformation”
- DNA tumor viruses occasionally integrate into the host genome leading to transformation
- DNATV oncoprotein were used to identify cellular processes linked to transformation



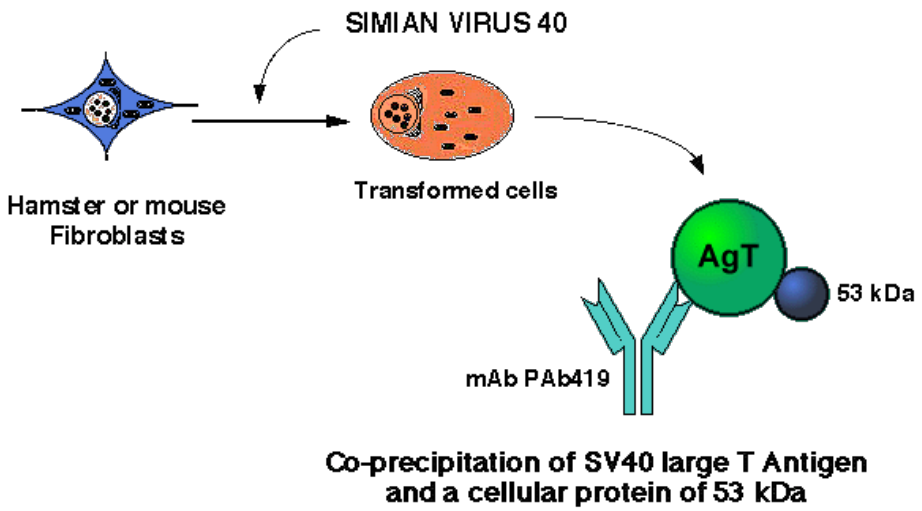
Discovery of p53

David Lane



Arnold Levine

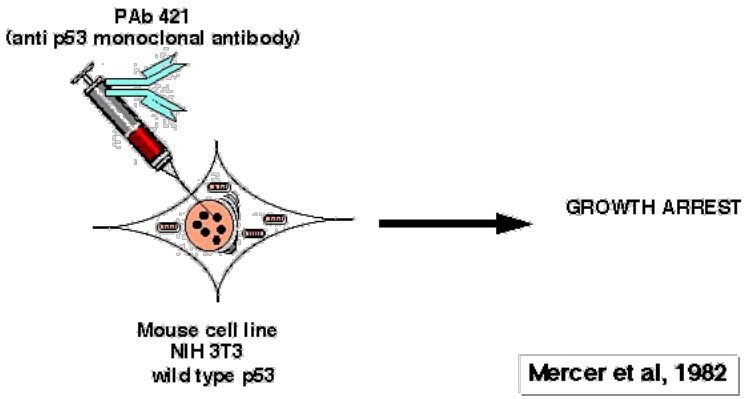
Discovery of p53



Co-precipitation of SV40 large T Antigen and a cellular protein of 53 kDa

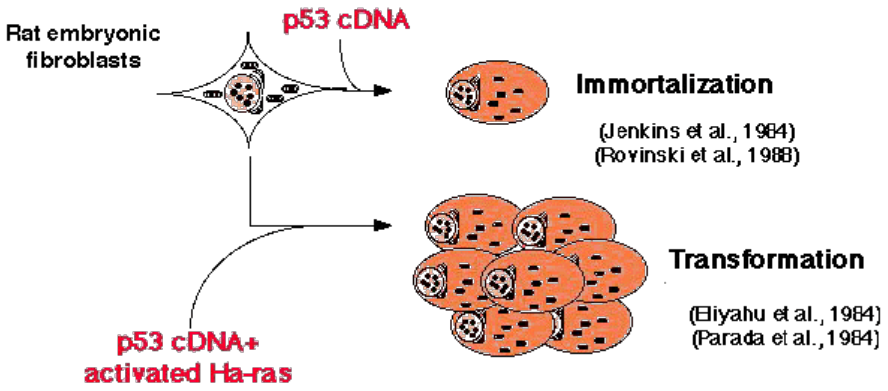
- David Lane and Arnold Levine were independently studying SV40 t antigen and noticed it bound a 53kD cellular protein
- Protein was highly expressed in SV40 transformed cells
- Hypothesized it might mediate action of T Ag on transformation (i.e. pro-oncogenic)

p53 has properties of an oncogene



- p53 protein is highly expressed in certain cancers
- Microinjection of antibodies against p53 protein triggers proliferative arrest

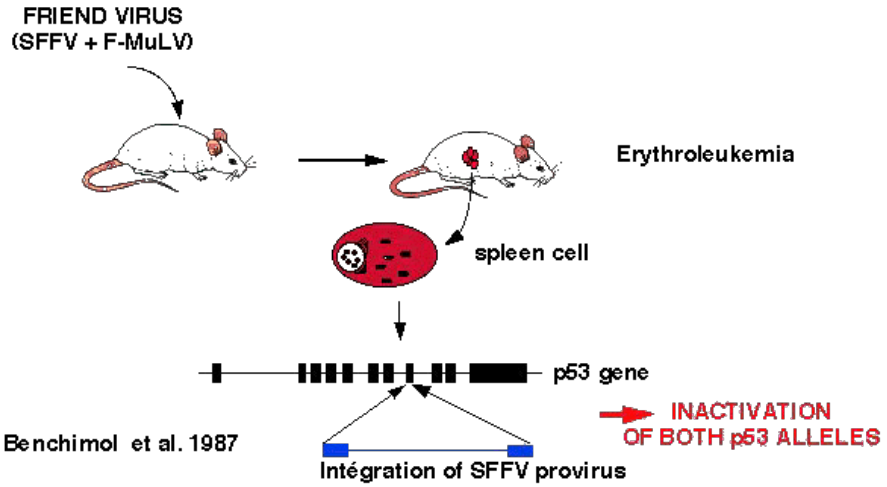
p53 has properties of an oncogene



- p53 protein is highly expressed in certain cancers
- Microinjection of antibodies against p53 protein triggers proliferative arrest
- A cloned p53 cDNA facilitates immortalization of primary fibroblasts and cooperates with oncogenic was to transform

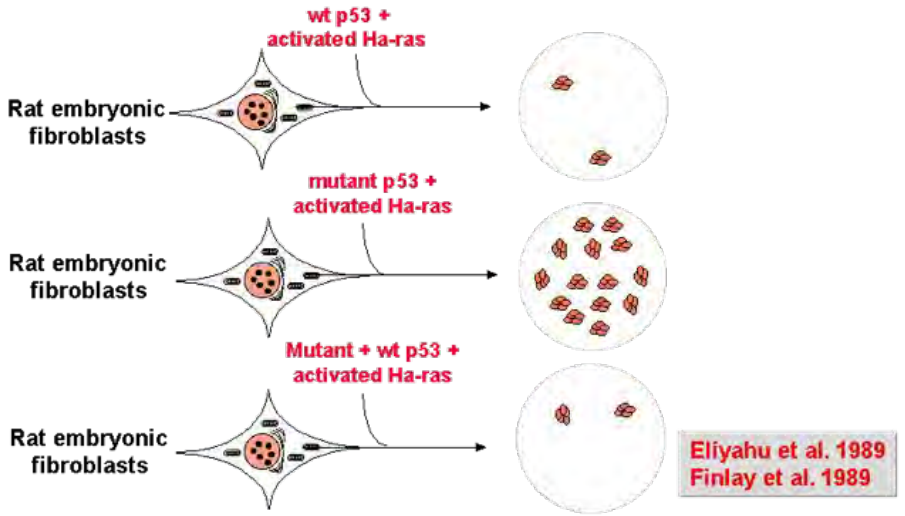
Emerging inconsistencies in oncogenic role

- Friend murine leukemia virus thought to promote cancer through insertional mutagenesis
- Some leukemia harbored integrations with two integrations in the p53 gene
- The dogma of “oncogenes” prevented a full understanding of the potential implications



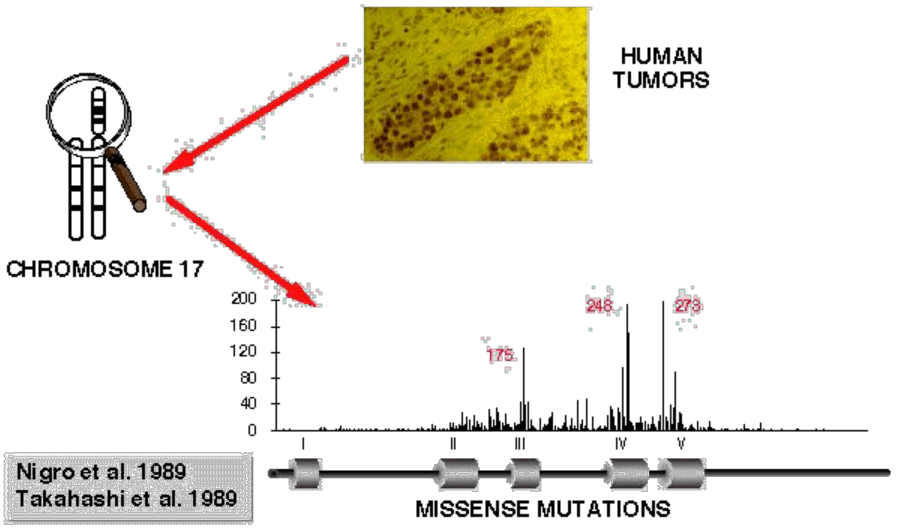
p53 is a tumor suppressor gene

- Different p53 cDNA clones have different sequences
- Different clones had different behaviors in cellular transformation assays
- So-called ‘wild-type’ clones inhibited proliferation and could suppress transformation (Oren and Levine)



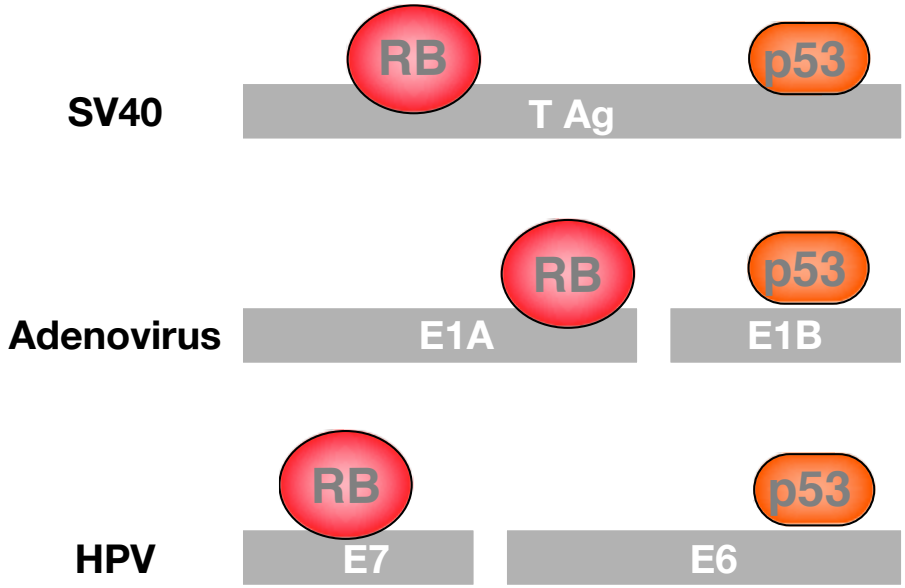
p53 is a tumor suppressor gene

- Different cloned p53 cDNAs and different sequences
- Different clones had different behaviors in cell transformation assays
- So-called ‘wild-type’ clones inhibited proliferation and could suppress transformation (Oren and Levine)
- Apparent loss of function mutations observed at high frequency in human cancers (Vogelstein)



What went wrong?

- TAg actively counters p53 stabilization to prevent proliferative arrest
- p53 mutations in tumors are dysfunctional but stablized owing to lack of degradation
- PAb421 anti-p53 antibody changes p53 conformation to an active form
- Initial p53 cDNAs obtained from cancer cells
- p53 functions as a tetramer and mutant proteins can function as dominant negatives when expressed at high levels



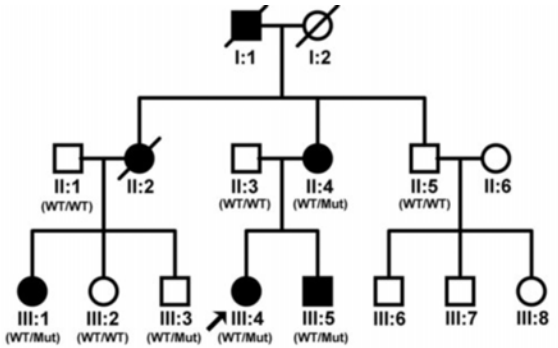
Decade 2

p53 activity and regulation

Li-Fraumeni syndrome linked to p53

- Li-Fraumeni is an autosomal dominant familial cancer syndrome characterized by early onset cancer (soft tissue sarcoma, breast, other)
- A substantial subset of patients have p53 mutations
- Tumors display ‘loss of heterozygosity’ at the p53 locus

A



B

ID	Age in 2014	Tumors
I:1	Deceased	Liver mass at 46 y
II:1	40 y	None
II:2	Deceased	Breast cancer at 32 y
II:3	35 y	None
II:4	35 y	Breast cancer at 34 y
II:5	30 y	None
III:1	13 y	Adrenal pheochromocytoma at 3 y, and kidney cyst at 12 y
III:2	10 y	None
III:3	6 y	No
III:4	5 y	Medulloblastoma at 5 y
III:5	3 y	Choroid plexus papilloma at 3 y

Li-Fraumeni Association

p53 mutations linked to carcinogens

- The high frequency of p53 mutations lent itself to the study of mutational signatures/ mechanisms early on.
- Different tumor types display different types of mutations at the gene level (Curt Harris, others)
- Often these could be traced to carcinogenic factors pre-disposing to cancer (aflatoxin, UV, cigarette smoke)

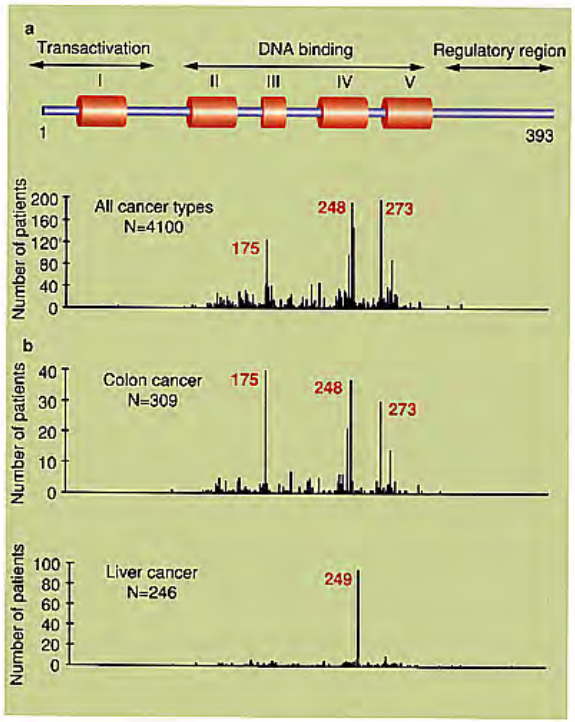


Figure 4. Distribution of p53 mutations in various types of cancers. (a) Schematic diagram of p53. Human p53 consists of 393 amino acids with five evolutionarily conserved domains (I–V). Domains II–V correspond to the DNA-binding domain, which is the target for p53 mutations[35]. (b) In each type of cancer surveyed, mutations are scattered in this central region, although a hot-spot exists at codons 175, 248 and 273. The only exception is hepatocellular carcinoma, which shows a mutational hot-spot at codon 249 (data for hepatocellular carcinoma also include cancers from countries not exposed to aflatoxin B1).

Hollstein Science 1991

p53 knockout mouse develops tumors

- p53 constitutive knockout mice develop normally
- Mice highly predisposed to tumors (mainly thymic lymphoma and certain sarcomas)
- p53+/- develop tumors at a longer latency of typically lose the remaining wild-type p53 allele
- Superficially, p53 functions as a ‘pure’ tumor suppressor

nature 19 March 1992

Article

Nature **356**, 215 - 221 (1992); doi:10.1038/356215a0

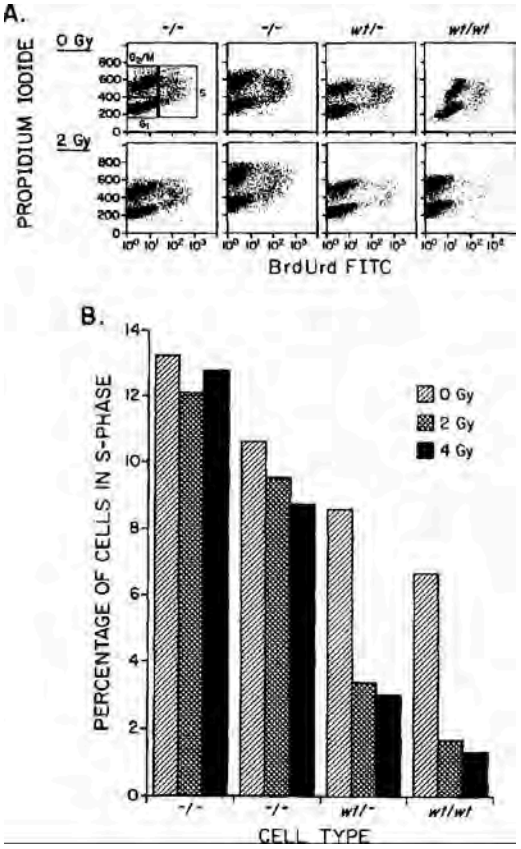
Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours

Lawrence A. Donehower, Michele Harvey, Betty L. Slagle, Mark J. McArthur, Charles A. Montgomery, Janet S. ButelAllanBradley

Mutations in the p53 tumour-suppressor gene are the most frequently observed genetic lesions in human cancers. To investigate the role of the p53 gene in mammalian development and tumori-genesis, a null mutation was introduced into the gene by homologous recombination in murine embryonic stem cells. Mice homozygous for the null allele appear normal but are prone to the spontaneous development of a variety of neoplasms by 6 months of age. These observations indicate that a normal p53 gene is dispensable for embryonic development, that its absence predisposes the animal to neoplastic disease, and that an oncogenic mutant form of p53 is not obligatory for the genesis of many types of tumours.

p53 as ‘the guardian of the genome’

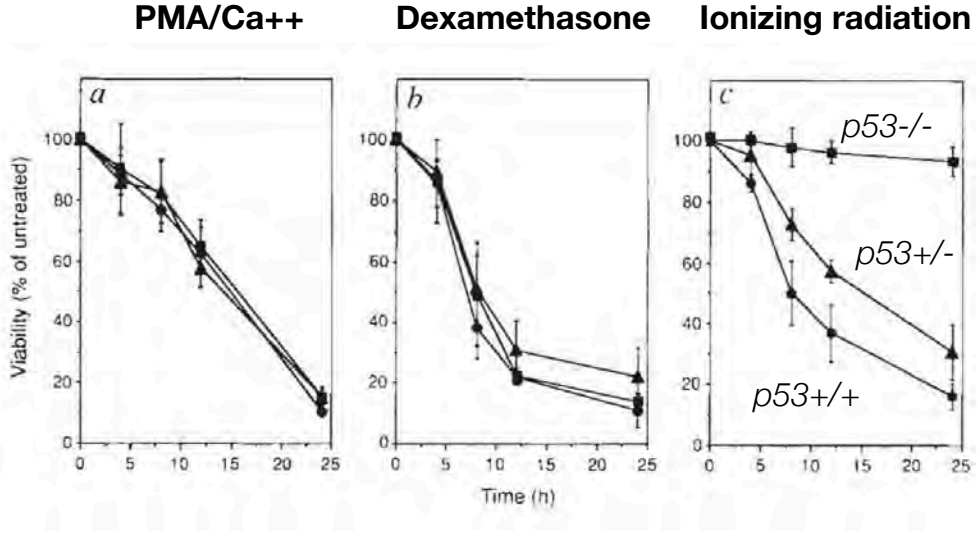
- p53 facilitates cell cycle arrest (G1 checkpoint) following DNA damage (Kastan)
- p53 loss triggers genomic instability (Tlsty, Wahl)
- David Lane coins the phase ‘p53, guardian of the genome’



Kastan Cell 1992

p53 can promote apoptosis

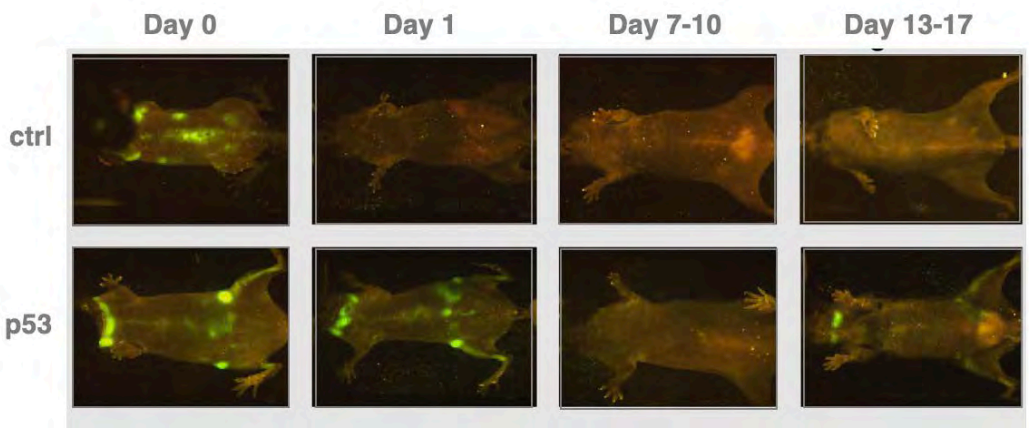
- p53 over expression promotes apoptosis in leukemia cell line (Oren)
- Studies using p53 knockout mice indicates that p53 promotes apoptosis following DNA damage (but not other stimuli) in thymocytes
- p53 is stabilized by certain oncogenes, which promote apoptosis



Lowe Nature 1993

p53 mutations and chemoresistance

Treatment response of murine lymphomas to cyclophosphamide

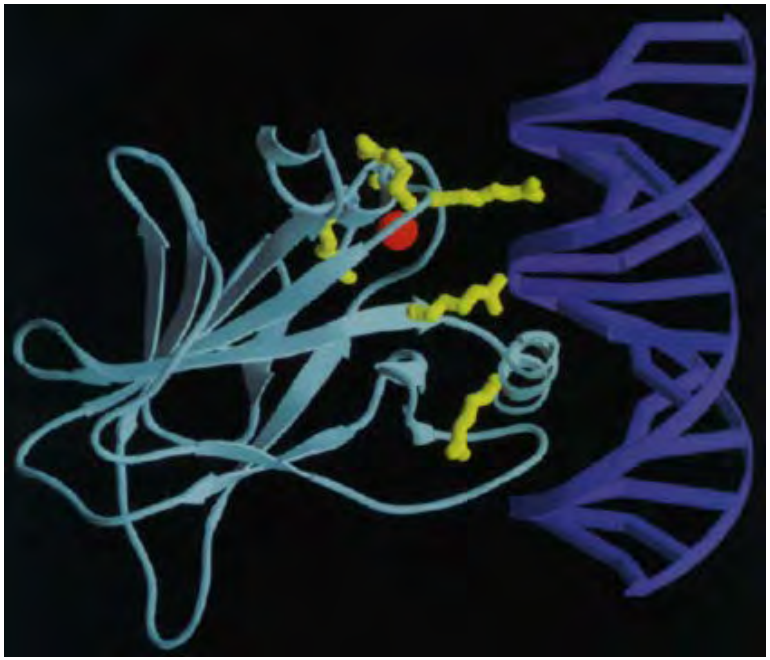


Schmitt Cell 2002

- Disruption of p53 apoptotic function reduces killing by radiation and cytotoxic drugs
- Loss of p53 is linked to chemoresistance in vivo
- Some human tumors show clear links between p53 mutation and therapy resistance, though whether it is due to an apoptotic defect remains unclear
- NEW IDEA: genotype of cancer cell dictates treatment outcome

p53 is a transcription factor

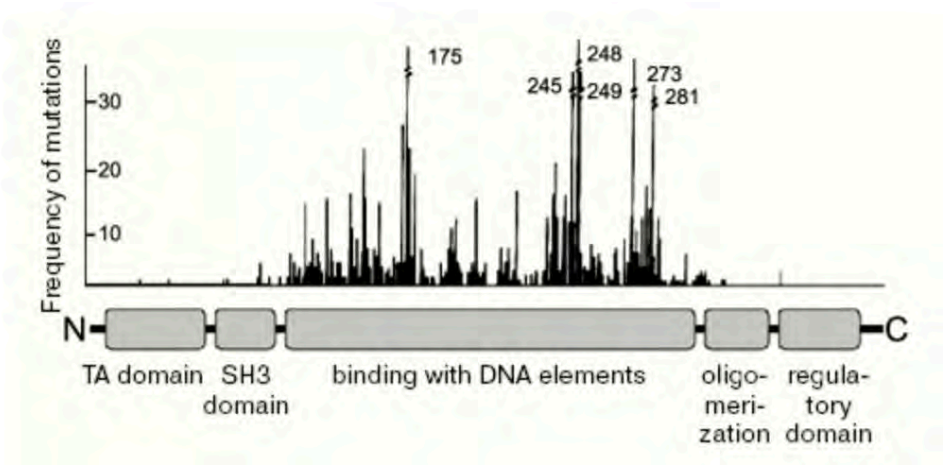
- p53 has transcriptional activation capabilities
- p53 is a sequence specific DNA binding protein
- Crystal structure of the core domain suggests mutations disrupt this activity



Nikola Pavletich (MSK)

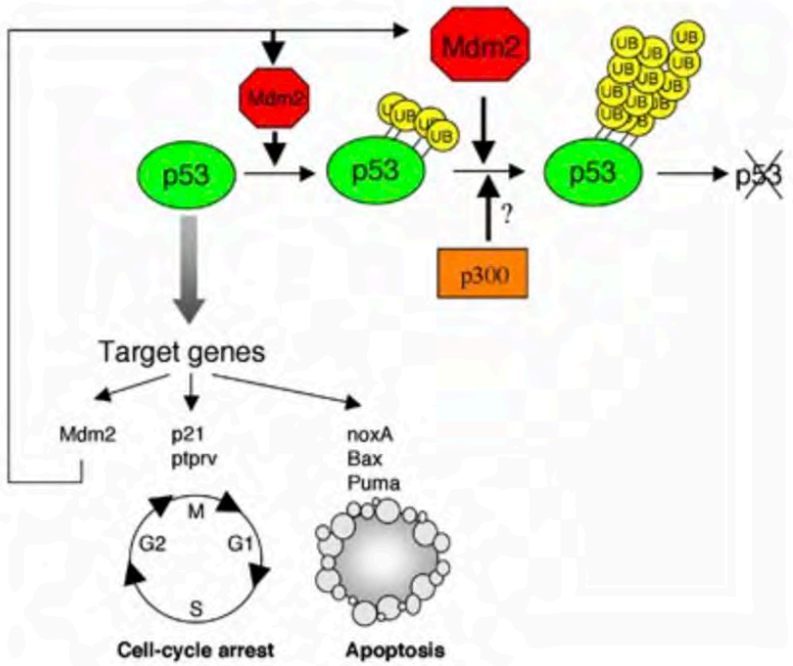
p53 is a transcription factor

- p53 has transcriptional activation capabilities
- p53 is a sequence specific DNA binding protein
- Crystal structure of the core domain suggests mutations disrupt this activity
- The biochemical function of p53 as a transcription factor appears important for its tumor suppressive role



Peter Chumkov

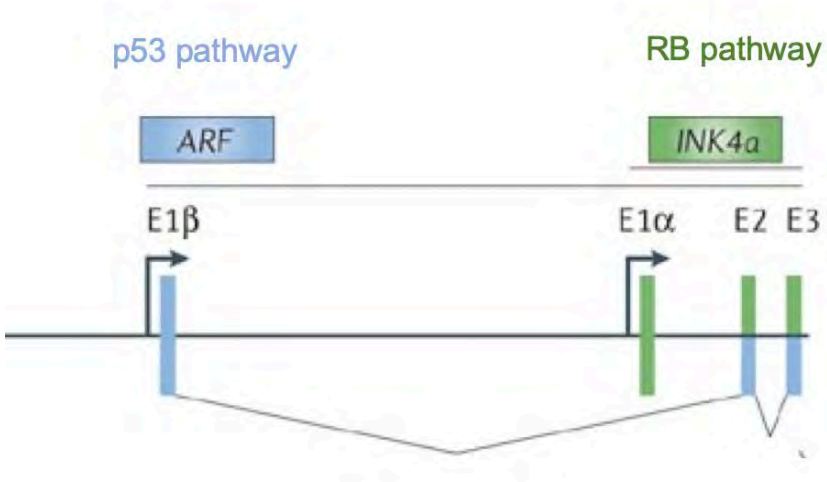
Negative regulation of p53 via Mdm2



JC Marine and G Lozano

- 'Murine double minute 2' (aka Mdm2) is amplified in murine and human cancers
- Mdm2/Hdm2 protein degrades p53
- p53 is phosphorylated by the DNA damage kinase ATM which disrupts Mdm2-mediated degradation
- Mdm2 is a p53 target gene
- Mdm2 functions in a feedback mechanism to blunt p53 activity
- Mutant p53 proteins do not induce Mdm2 and thus are stabilized

ARF and oncogene signaling to p53

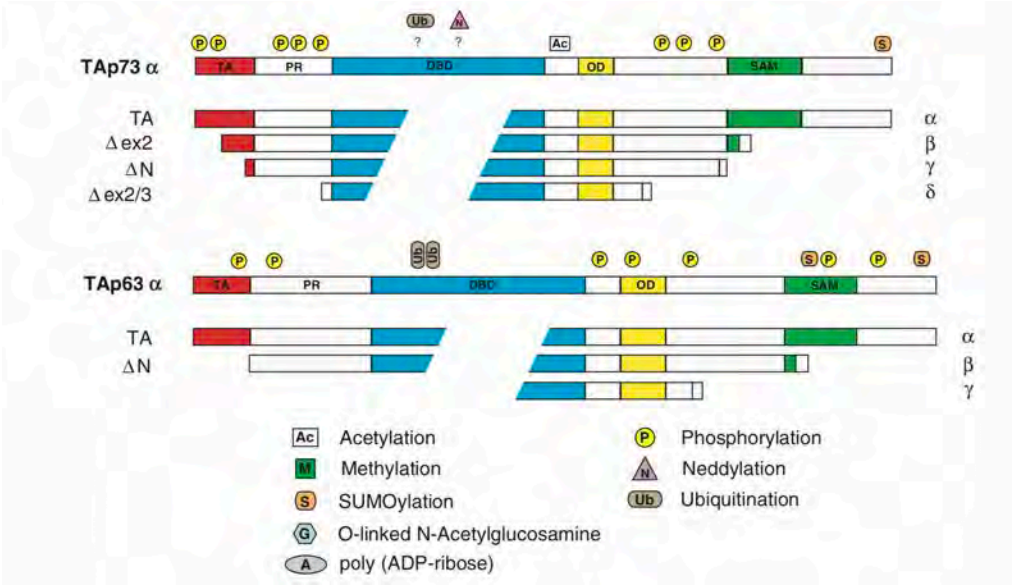


Charles Sherr (St. Jude)

- Chromosome 9 deletions frequently occur in human cancers
- The locus encodes (at least) two tumor suppressors - p16 and ARF - that have an unusual organization
- ARF inhibits Mdm2 interactions with p53 (DePinho, Sherr, others)
- ARF is not induced by DNA damage but is by oncogenes (Sherr)
- ARF is required for oncogene induced apoptosis and senescence (Sherr, Serrano, Lowe, Vousden)

p53 is a member of a gene family

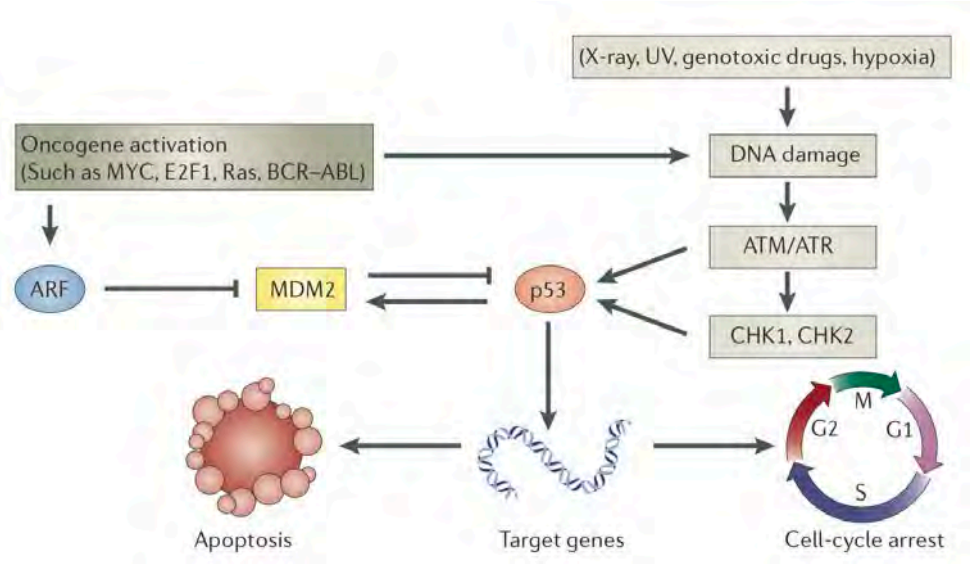
- p53 is a member of a gene family that includes p63 and p73
- p63 and p73 KO have developmental phenotypes
- p63 and p73 have splice variants that influence function (TA and DN)
- Re-evaluation of p53 indicates it also has splice variants of unclear function
- Neither p63 or p73 are potent tumor suppressors



del Sal CDD 2010

Textbook view of p53

- p53 is a transcription factor that can be activated by cellular stress to drive distinct anti proliferative processes
- p53 activity is regulated upstream by DNA damage or ARF which prevent Mdm2 inhibition and stabilize p53
- p53 mutations disable transcription functions allowing aberrant proliferation
- p53 regulators are also mutated in cancer



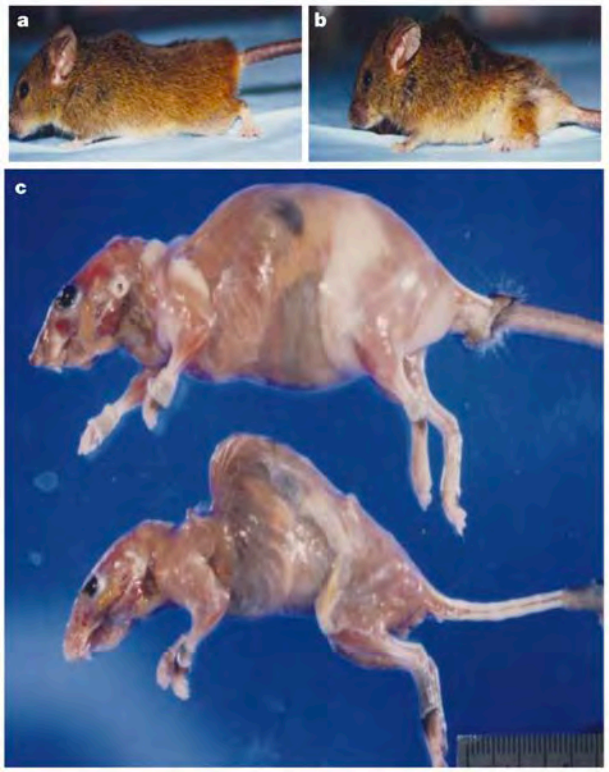
Charles Sherr Nat Rev Cancer

Decade 3

New p53 biology and emerging confusion

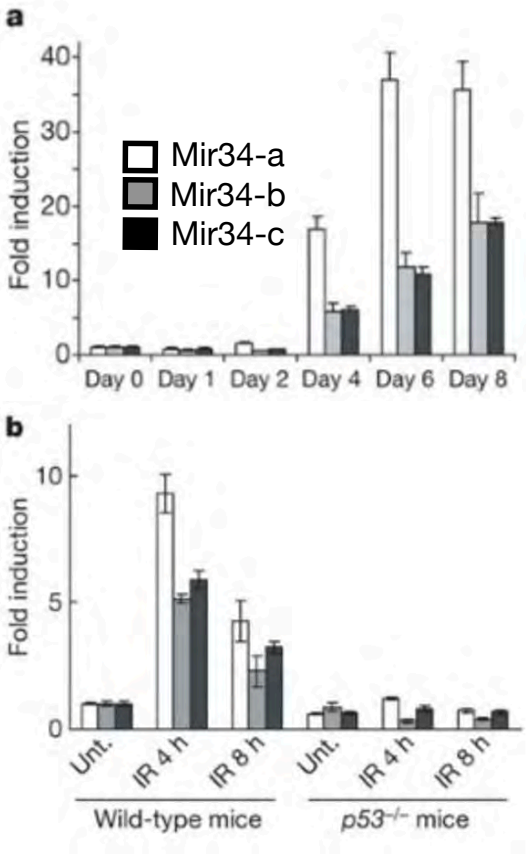
p53 and organismal aging

- A fortuitously engineered p53 mutant mouse has p53 hyper activation and displays premature aging (Donehower)
- Suggests that too much of p53 is a bad thing
- Mice with an extra copy of wt p53 (‘super p53 mice’) are cancer resistant but do not show aging phenotypes (Serrano)



Donehower Nature 2002

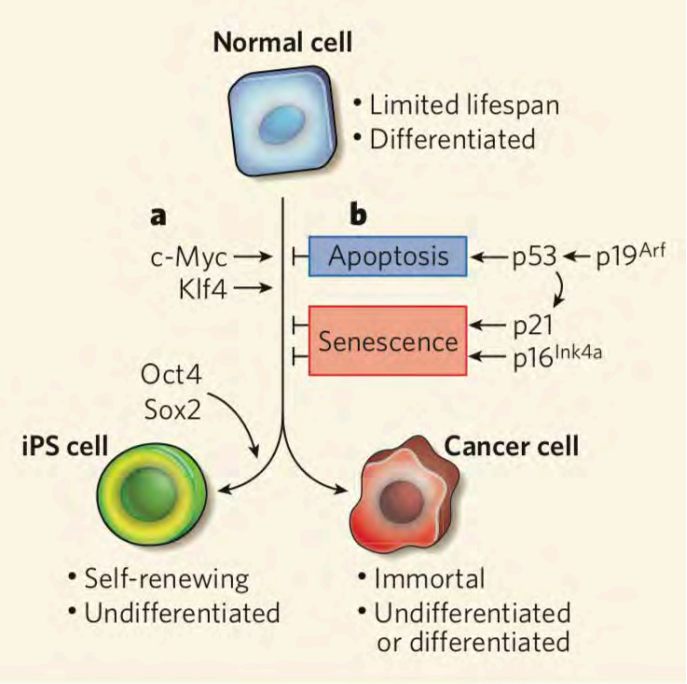
Hot topic: p53 regulates microRNAs



He, Nature 2007

- microRNAs can be direct transcriptional targets of p53 (Hannon, Ventura)
- Induction of mir34 family is linked to repression of genes involved in cell cycle arrest
- Provides one mechanism for how p53 represses gene expression

Hot topic: p53 and stem cell biology

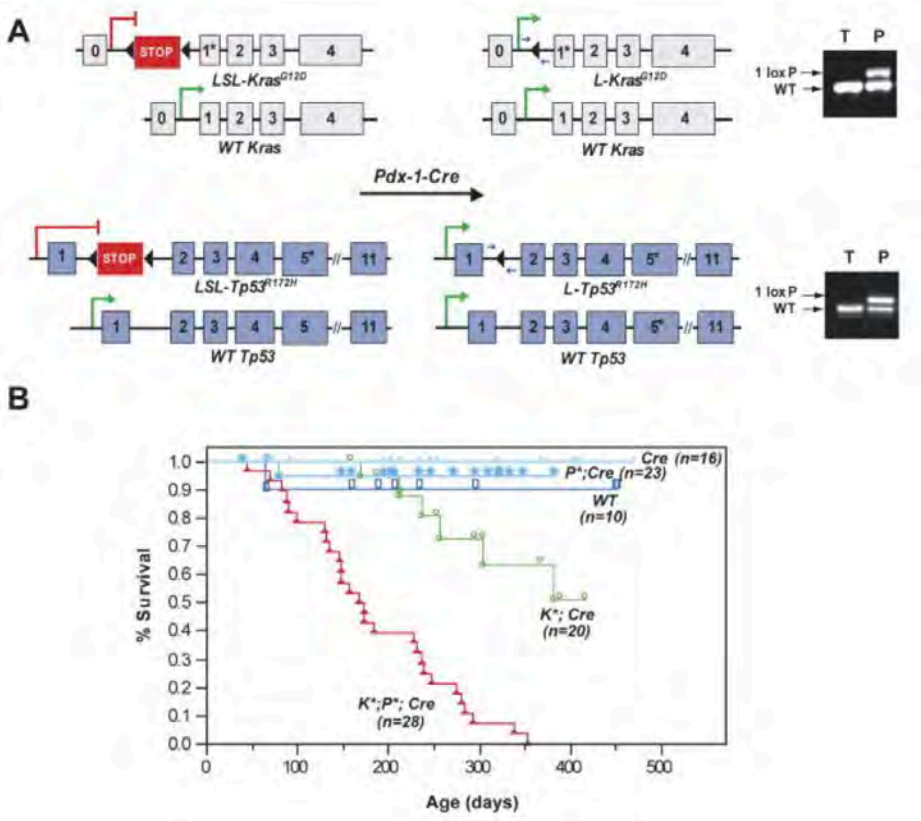


Krizhanovsky and Lowe, News and Views

- p53 restricts the ability of Yamanaka factors to produce induced pluripotent stem cells (5 Nature papers)
- Loss of p53 increases iPS frequency ~100 fold
- Senescence is a p53 output that prevents iPS formation
- Suggests a role for p53 in restricting lineage plasticity but role in cancer undefined.

More sophisticated models expand capabilities

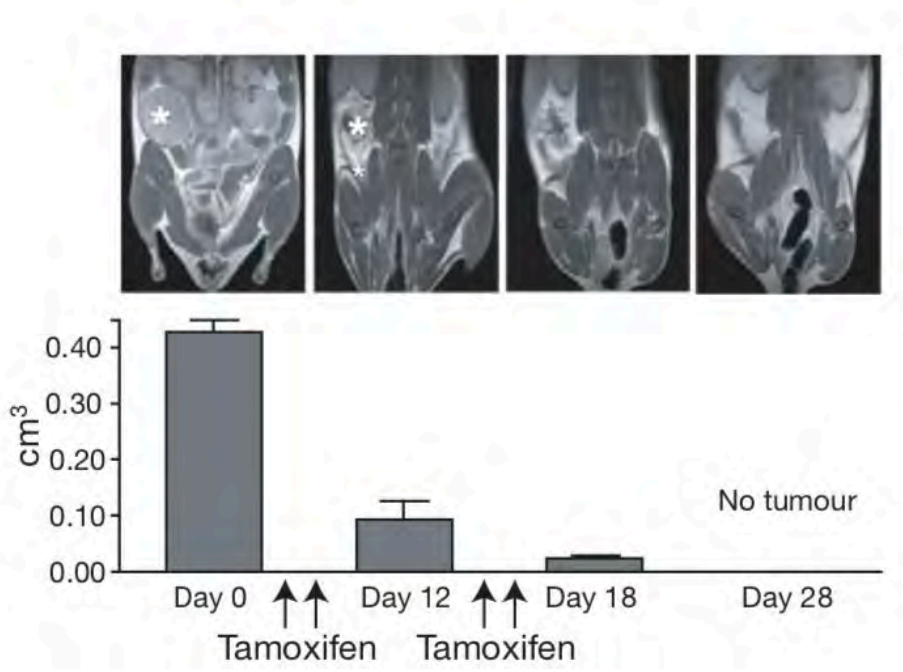
- More sophisticated mouse models allow for tissue specific p53 inactivation together with other oncogenic events
- KP lung cancer model and KPC pancreas cancer models become work horse tools that genetically and histologically model human carcinomas



Hingorani Cancer Cell 2005

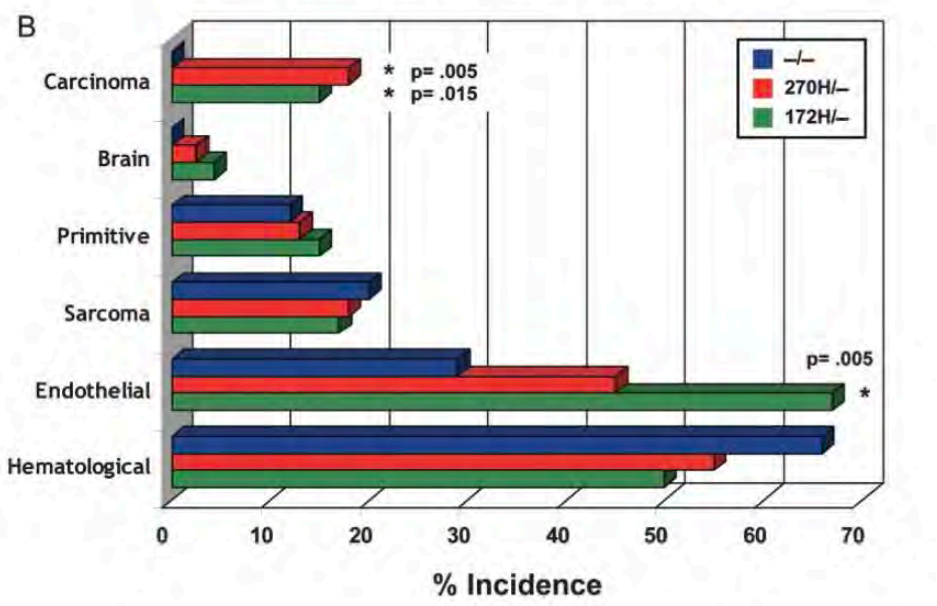
p53 restoration in tumors

- Mouse models with conditional (inducible) wt p53 alleles are produced
- p53 reactivation triggers tumor regression (Evans, Ventura/Jacks, Lowe)
- Loss of p53 is required to maintain cancer
- Targeting the p53 network would be therapeutically viable



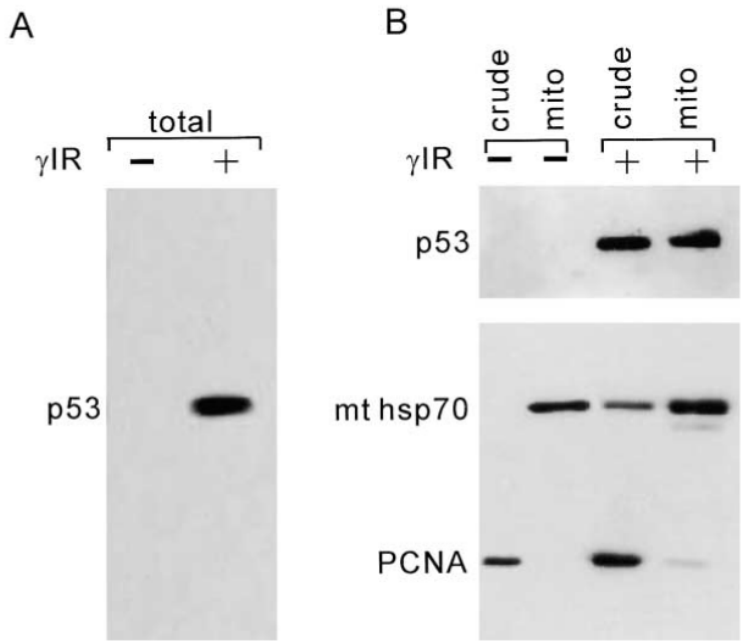
Ventura Nature 2007

Does p53 have oncogenic activities?



- Mice harboring p53 hotspot mutants develop tumors with a different spectrum than nulls (Jacks, Lozano)
- p53 mutant strains display more metastases
- p53 mutant proteins can have ‘gain of function’ activities
- Mechanism appears to involve mutant p53 protein binding to p53 family members

Is p53 just a transcription factor, or more?



- p53 can associate with mitochondria and this may be linked to apoptosis (Moll, Green)
- Mitochondrial p53 binds Puma and promotes apoptosis (Green)
- p53 mutants defective in puma binding…. So could it be linked to tumor suppression? (Green)
- No one talks about this anymore…

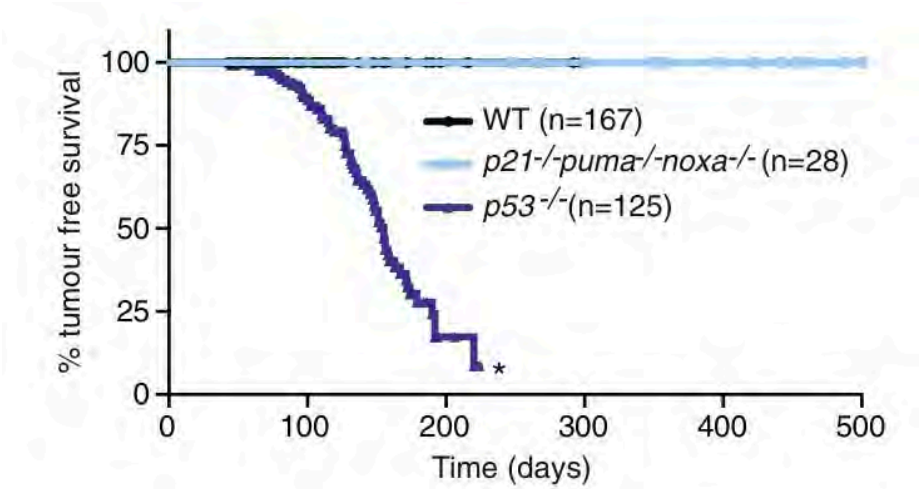
Mihara et al Mol Cell 2003

Decade 4

Increasing confusion yet notable advances

Emerging debate on what's important

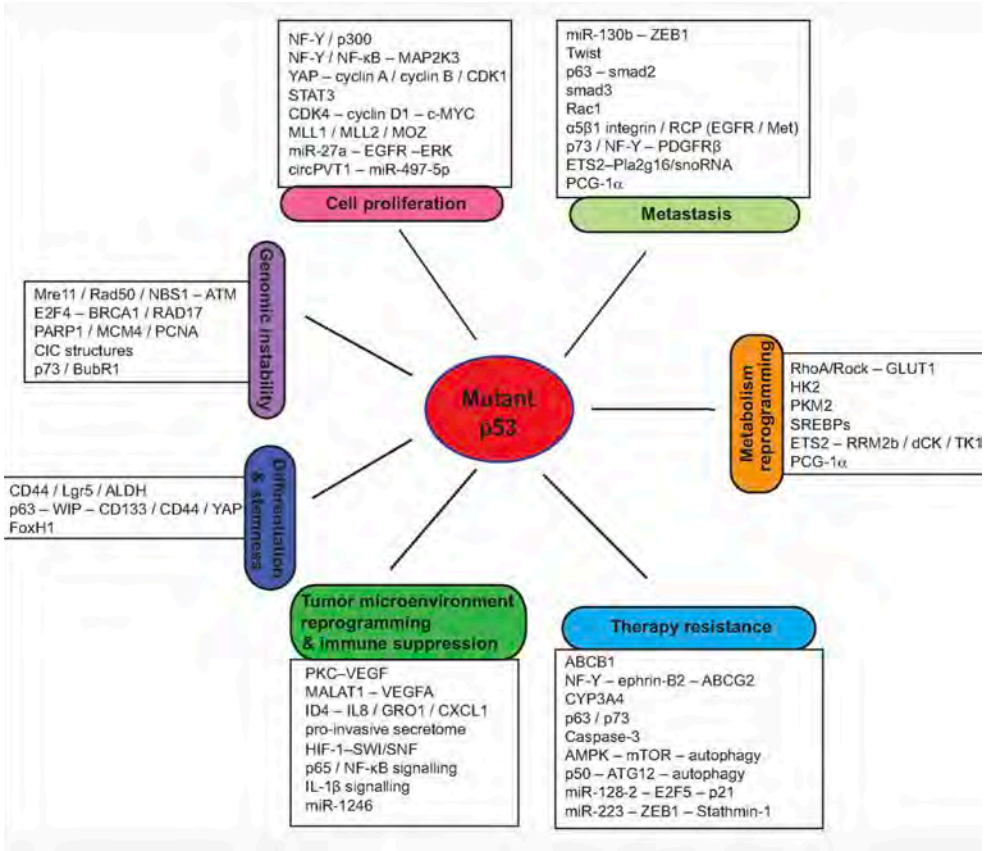
- There has been substantial debate regarding which p53 effector functions are most important for tumor suppression
- Studies demonstrate that disruption of key p53 effectors in cell cycle arrest and apoptosis do not form tumors as do p53 null
- Separation of function p53 mutations point to p53 and cancer metabolism or other p53 functions as key
- There are caveats…



Valente Cell Rep 2016

More gain of function mechanisms identified

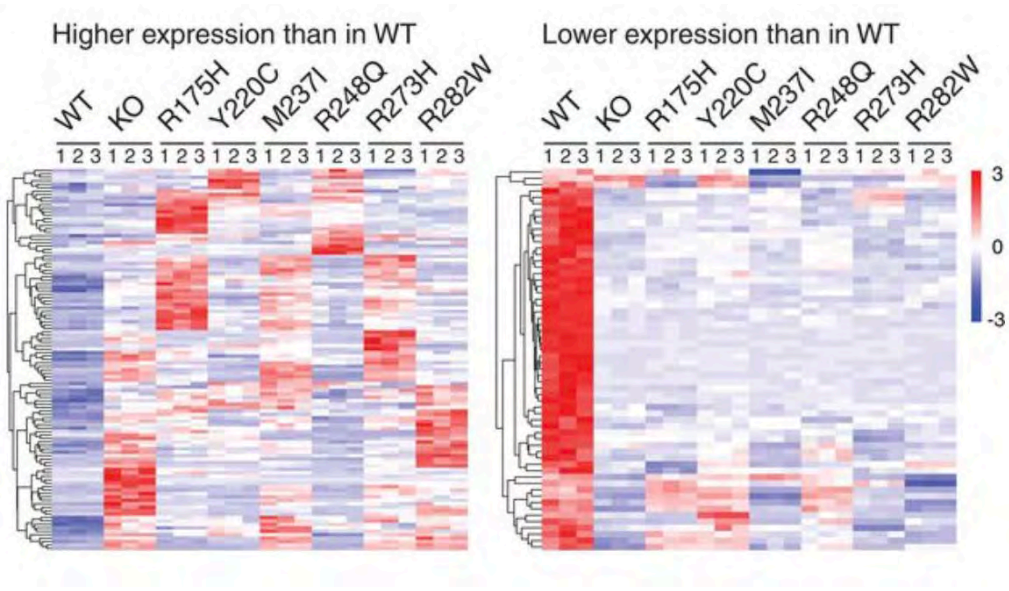
- While increased metastasis is the most common p53 ‘gain of function’ output identified, there are many other effects (stem cell biology, cell plasticity, epigenetics)
- Virtually every study reveals a different mechanism underlying the biology
- Even the same mutant allele can have different gain of function activities depending on context
- How might it work?



Zhang JMCB 2020

p53 gain of function push back

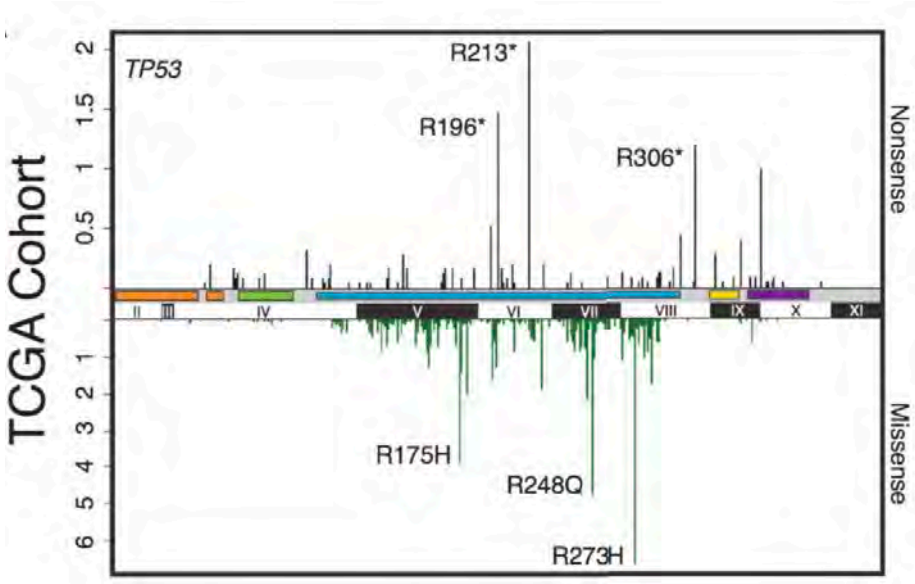
- Analysis of CRISPR screening data does not reveal a relationship between p53 mutation and p53 mutant dependency in cancer cell lines (Hahn)
- Inactivation of p53 by CRISPR in a range of cancer cell lines has no phenotype (Strasser)
- Precise generation of p53 mutants in AML lines appear strictly dominant negative (Ebert)
- BUT: analyses have been primarily (though not completely) in vitro



Boettcher Science 2019

Binary classification of tumors is simplistic

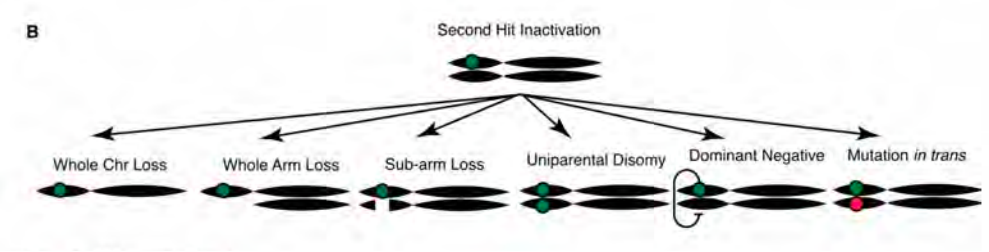
- p53 mutations show a range of distributions across cancers, including a large number of truncating alleles
- The most common p53 configuration is a missense mutations on one allele and a deletion event that targets the other and a series of adjacent genes
- Different p53 mutations have different biological activities (dominant negative, gain of function) and to different degrees
- Deletion events target haploinsufficient tumor suppressors linked to p53 deletions



Sordella eLife

A range of p53 mutational configurations

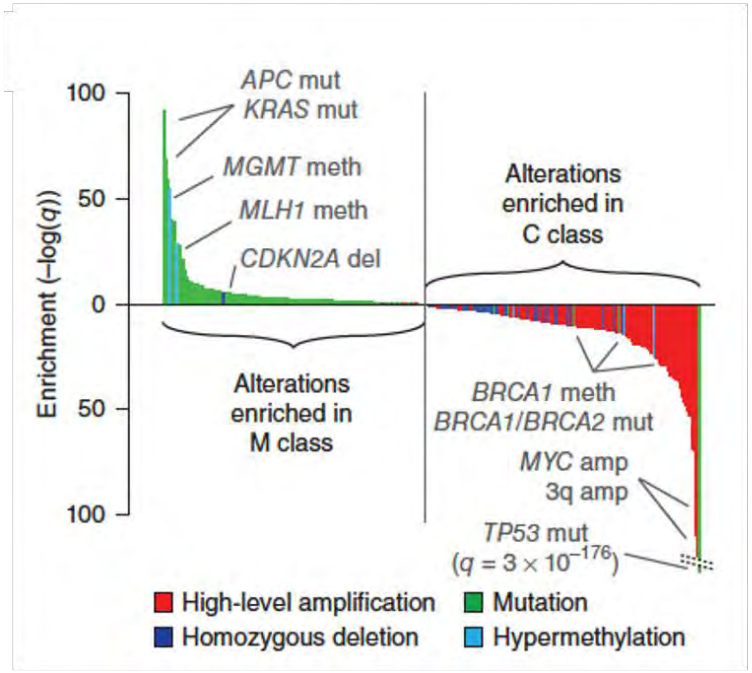
- p53 mutations show a range of distributions across cancers, including a large number of truncating alleles
- The most common p53 configuration is a missense mutations on one allele and a deletion event that targets the other and a series of adjacent genes
- Different p53 mutations have different biological activities (dominant negative, gain of function) and to different degrees
- Deletion events target haploinsufficient tumor suppressors linked to p53 deletions
- The classification of tumors as ‘wt’ or ‘mutant’ for p53 is likely simplistic



Kastenhuber Cell 2017

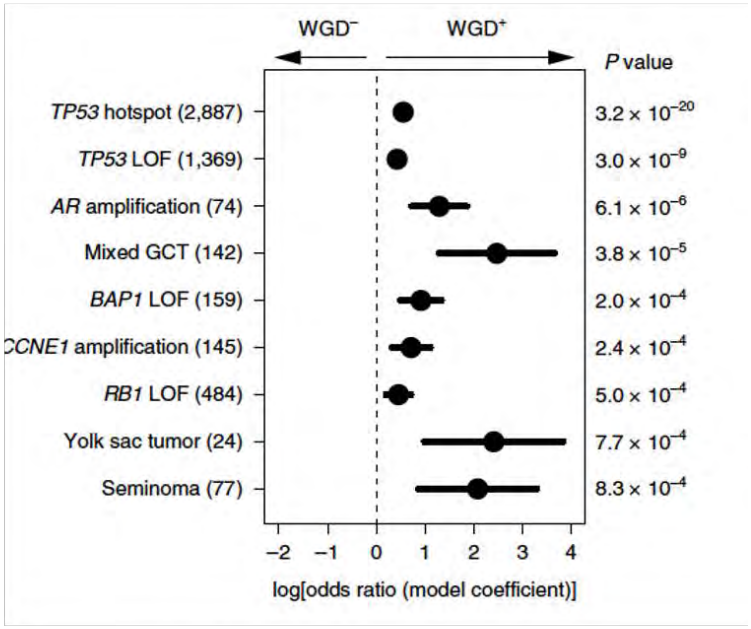
p53 mutations: not just any instability

p53 mutations and CNA



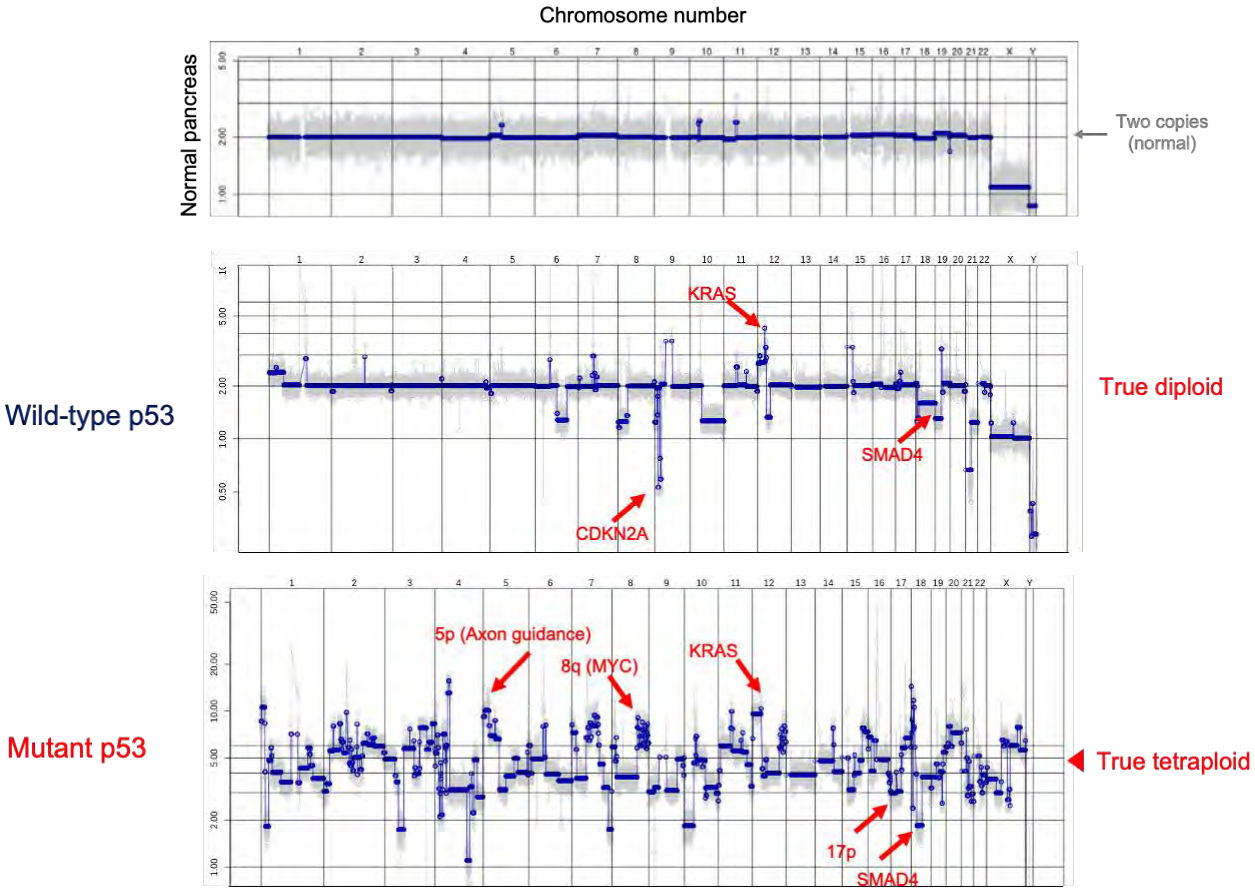
Giovanni Cirello, et al Nature Genet 2013

p53 mutations and WGD



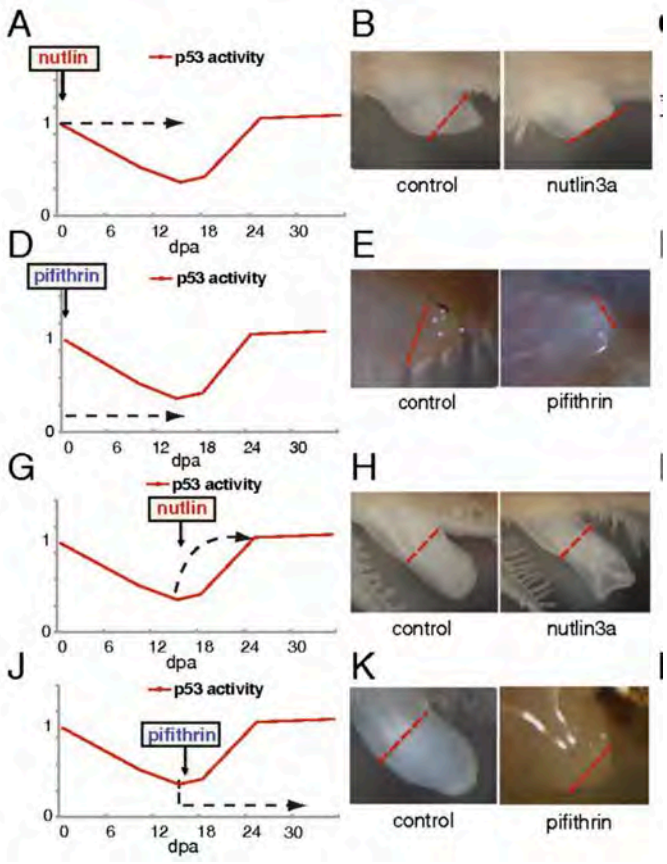
Bielski et al, Nature Genet 2018

CNA, polyploidy, and p53 mutations in pancreas cancer



New roles for p53

- p53 down regulation enables limb regeneration in salamander and promotes lineage plasticity in cancer (Brockes)
- p53 reactivation can revert tumors to premalignant cell fate (Lowe, Finley)
- p53 linked to clonal hematopoiesis (as is p53 target Ppm1d)
- p53 suppresses transposable elements and its inactivation leads to retrotransposition (Gudkov, Abrams)
- p53 can promote survival in some circumstances (Vousden)

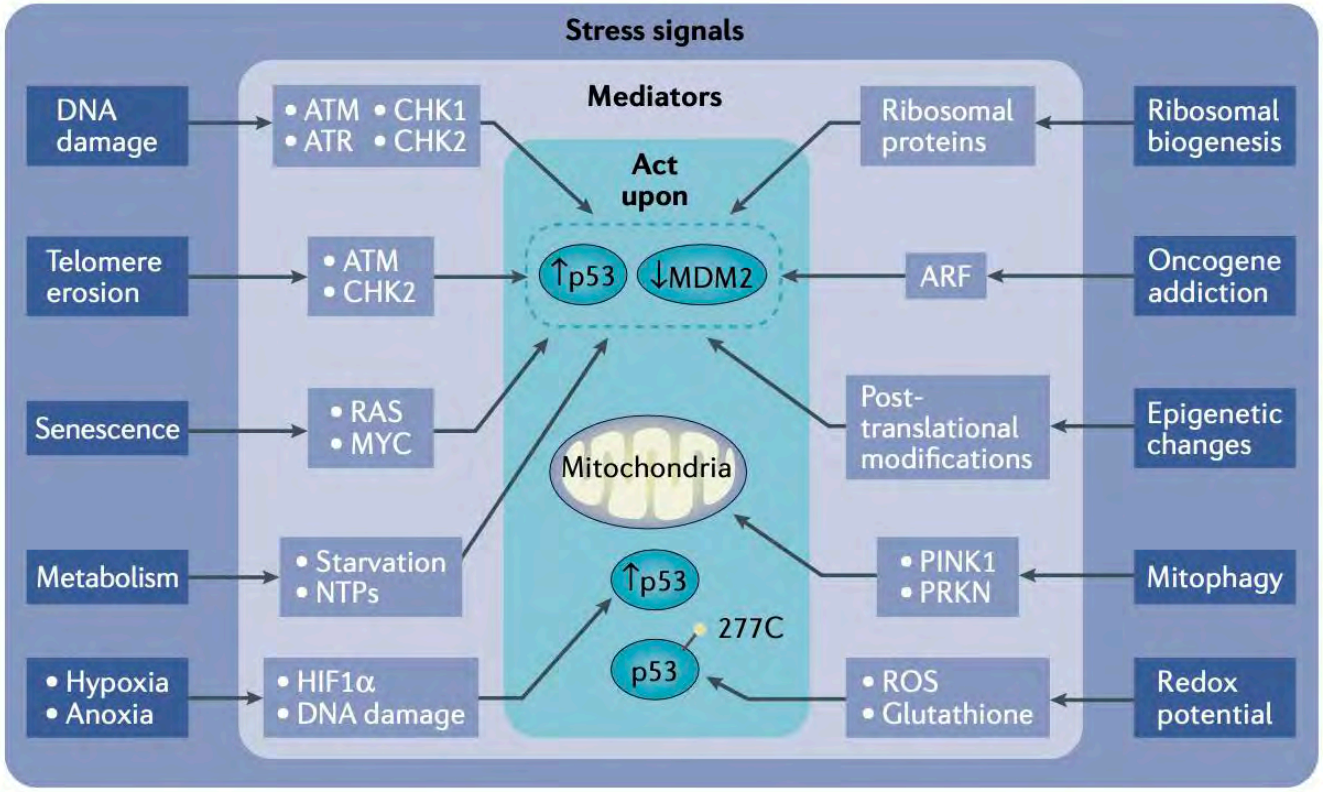


Yun et al, PNAS 2013

Decade 5

Unanswered questions and therapeutic opportunities

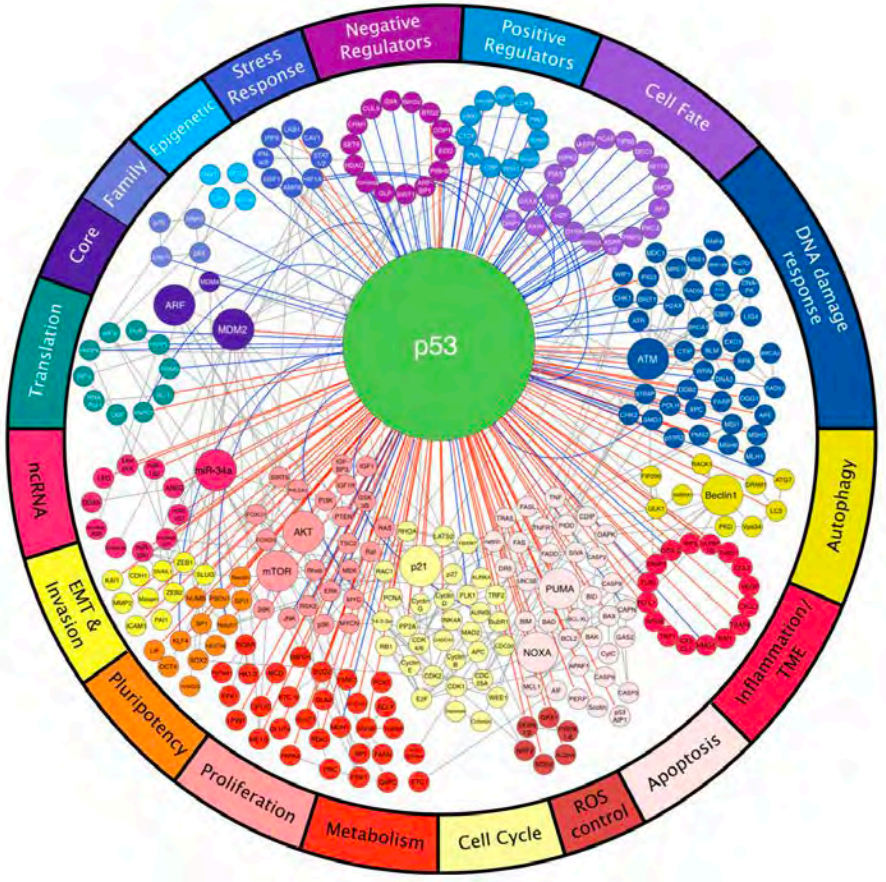
p53 can be activated by a wide variety of triggers



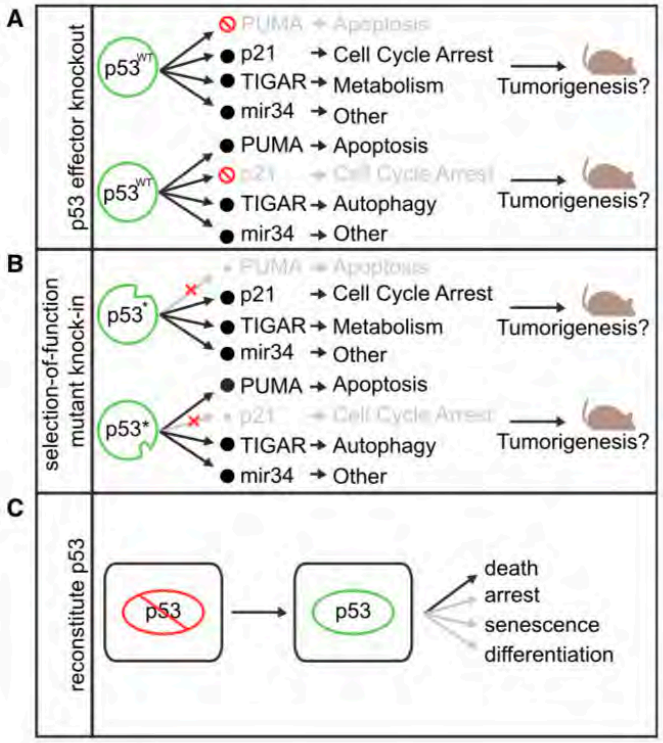
Levine, AJ, Nature Reviews Cancer 2020

The p53 transcriptional network

- It is now clear that p53 controls many genes that are linked to a wide range of biological processes
- Some are cell intrinsic and others cell extrinsic'
- p53 likely acts through coordination of multiple anti-proliferative programs
- Did this evolve to suppress cancer or other processes?

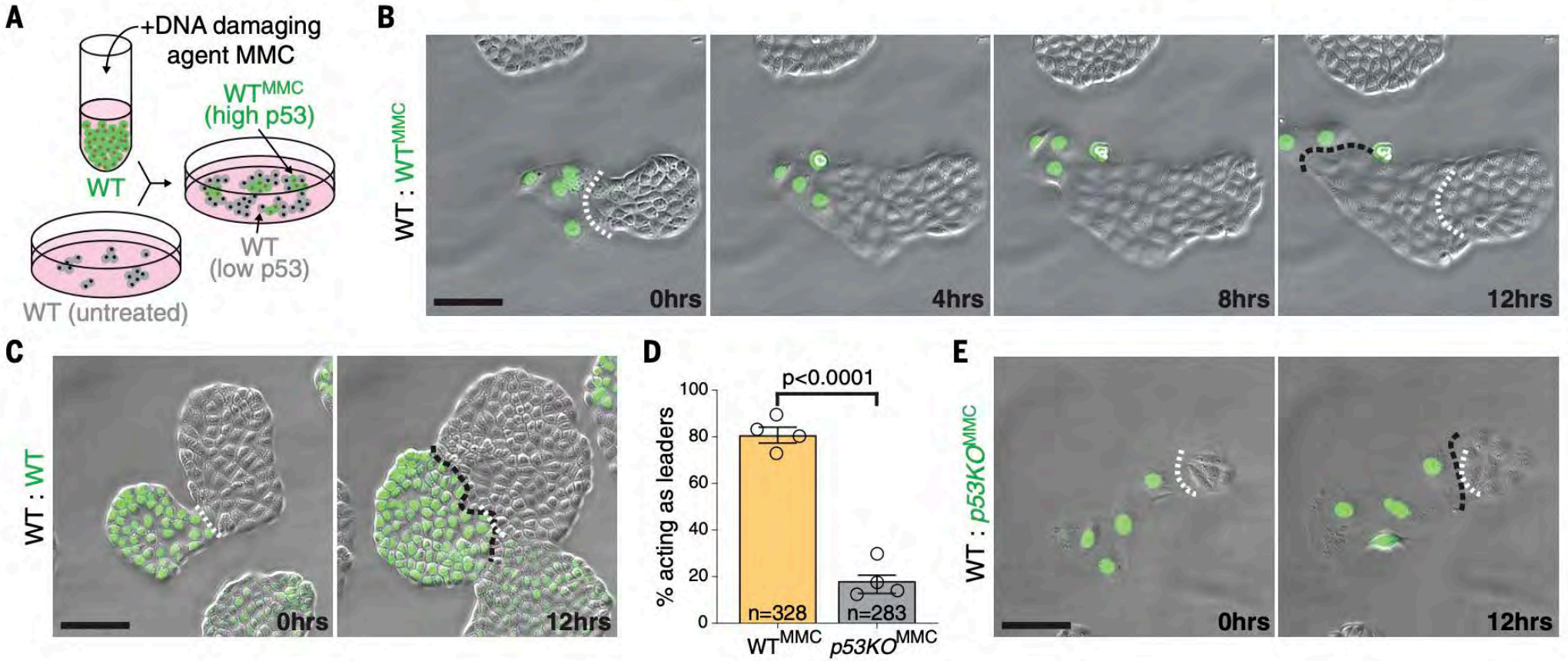


Context, context, context



- p53 likely coordinates a tumor suppressive program through multiple effectors
- Some may be more or less dependent on context
- Contextual factors include tissue type, activating stimulus and the presence of other oncogenic events
- What dictates context in p53 output?
- Can contextual functions of p53 be exploited?

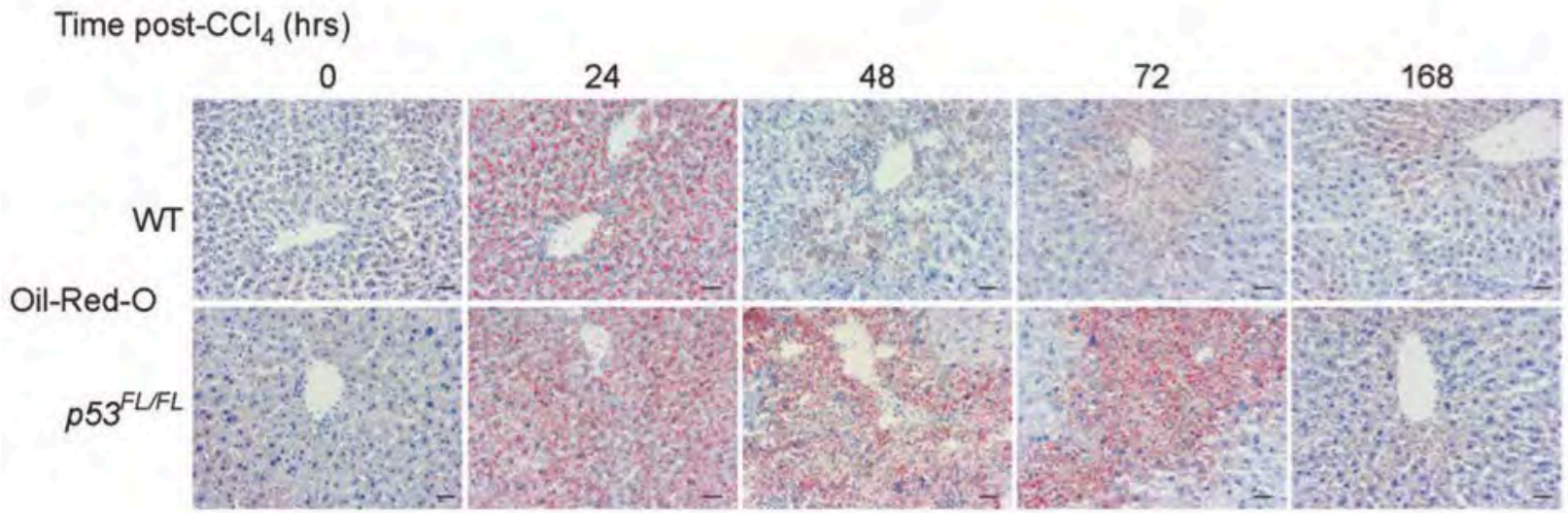
A role for p53 in wound healing responses?



Kozyrska. Science 2022

A role for p53 in wound healing responses?

Oil Red O staining of livers post-damage



Humpton Cell Death Diff 2022

p53 action in would healing is intriguing


SEMINARS IN MEDICINE

OF THE

BETH ISRAEL HOSPITAL, BOSTON

JEFFREY S. FLIER, M.D., *Editor*

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TUMORS: WOUNDS THAT DO NOT HEAL

Similarities between Tumor Stroma Generation and Wound Healing

HAROLD F. DVORAK, M.D.

Cancer: A wound that does not heal

HISTOLOGICAL SIMILARITIES

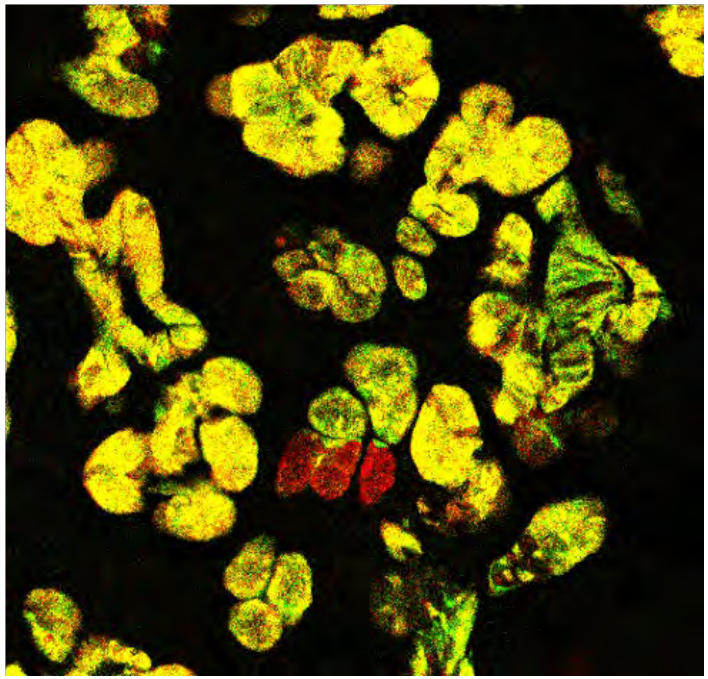
- Cancers often arise at sites of wounds
- Both involve inflammation, blood clotting factors, blood vessel formation, cell plasticity
- Both require tissue remodeling and cell proliferation

CELL AND MOLECULAR SIMILARITIES

- Overlapping changes in cell composition and cell state in the tissue
- Critical role for stem cells and regenerative programs
- Molecular pathways important in would healing are mutated in cancer

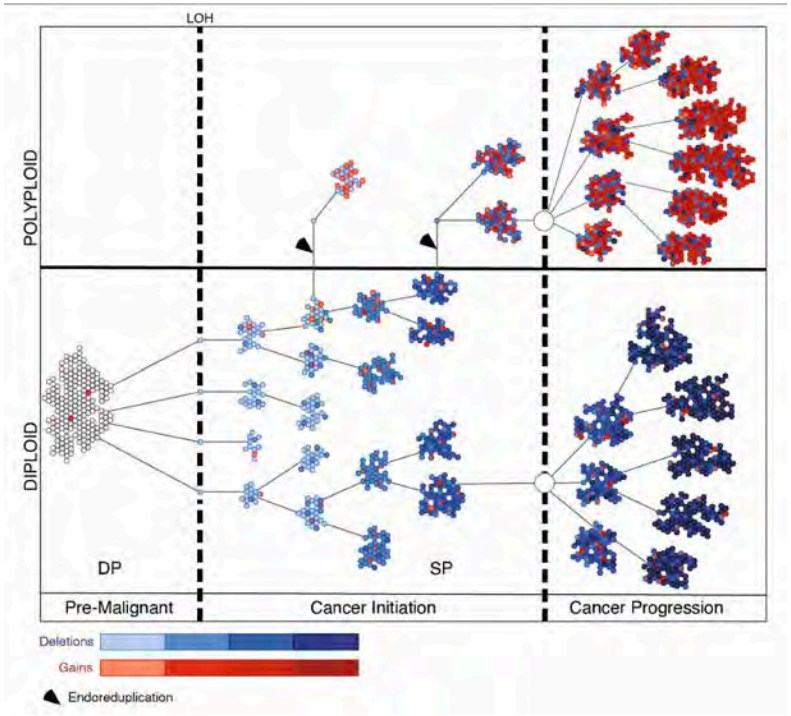
p53 as ‘guardian of the genome’

Fluorescence reporter to identify cells that sporadically inactivate p53



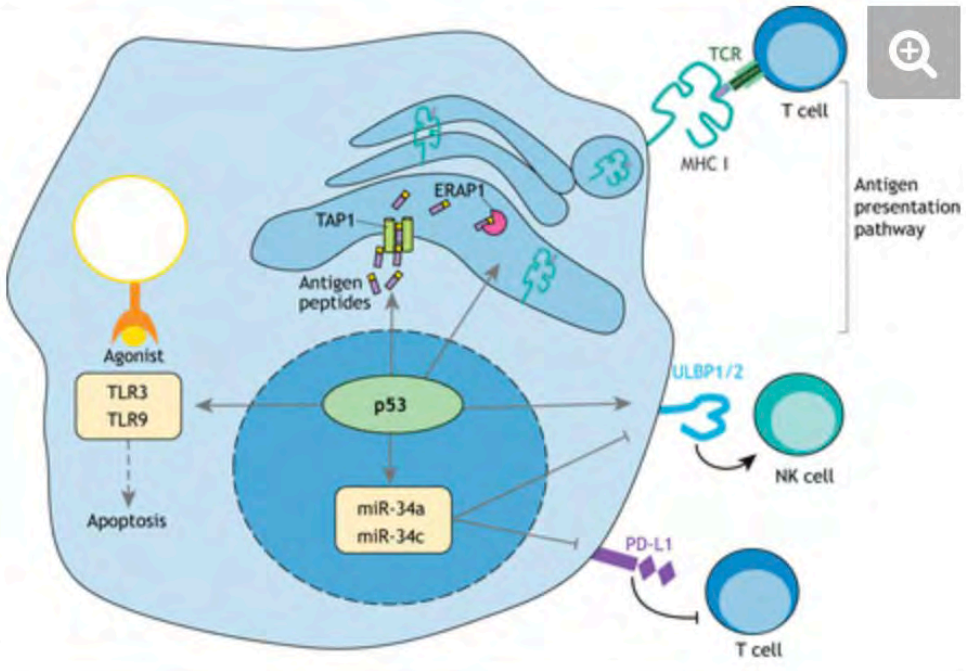
Baslan, Morris, Zhao, Nature 2023

Schematic of copy number evolution following p53 inactivation



p53 and immune modulation

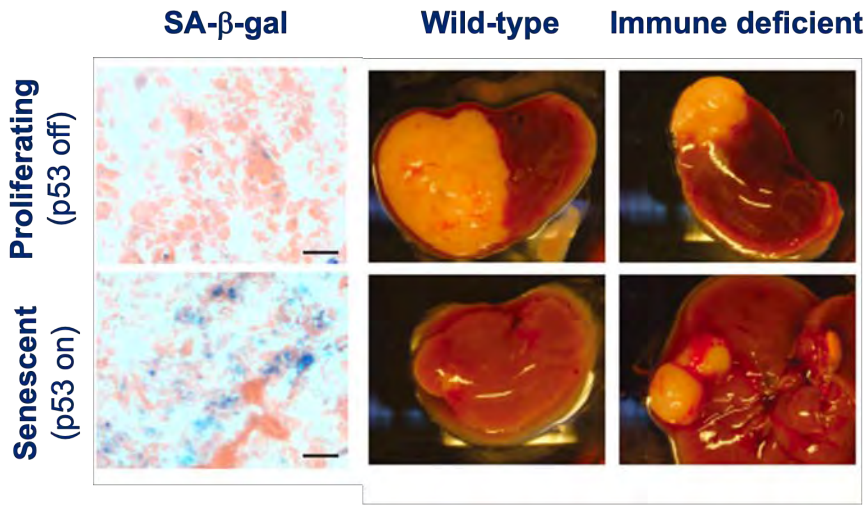
- Emerging evidences suggests p53 directly controls immune surveillance functions
- p53 directly regulates factors that are important for NK and T cell surveillance
- Consistent with a role for p53 in limiting viral responses



Blagih et al, J Cell Sci 2020

p53 and immune modulation

- Emerging evidences suggests p53 directly controls immune surveillance functions
- p53 directly regulates factors that are important for NK and T cell surveillance
- Consistent with a role for p53 in limiting viral responses
- Reactivation of endogenous p53 in tumors can provoke immune mediated cancer regressions
- p53 can coordinate cell intrinsic and extrinsic tumor suppression

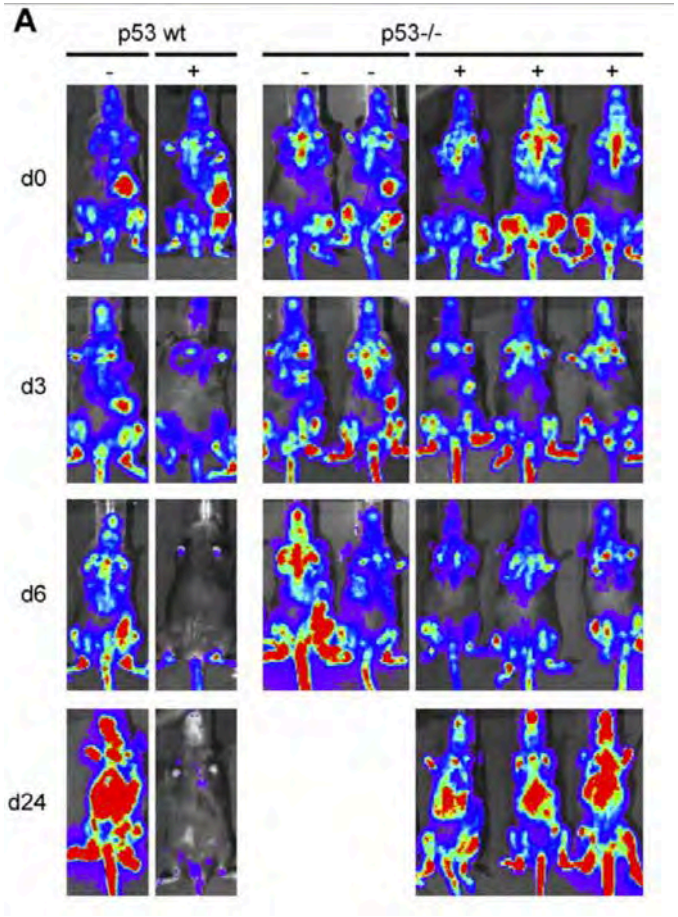


Chen, Cancer Discovery 2022

Therapeutic opportunities for targeting p53

(p53 wild-type tumors)

- p53 activation in tumors by chemotherapy can be potently tumor suppressive
- Agents in clinical trials that disrupt the Mdm2-p53 (or mdmx-p53) interactions - will they be too toxic?
- Can p53 *inhibitors* be useful for certain indications (ischemic injury, neurodegeneration)?



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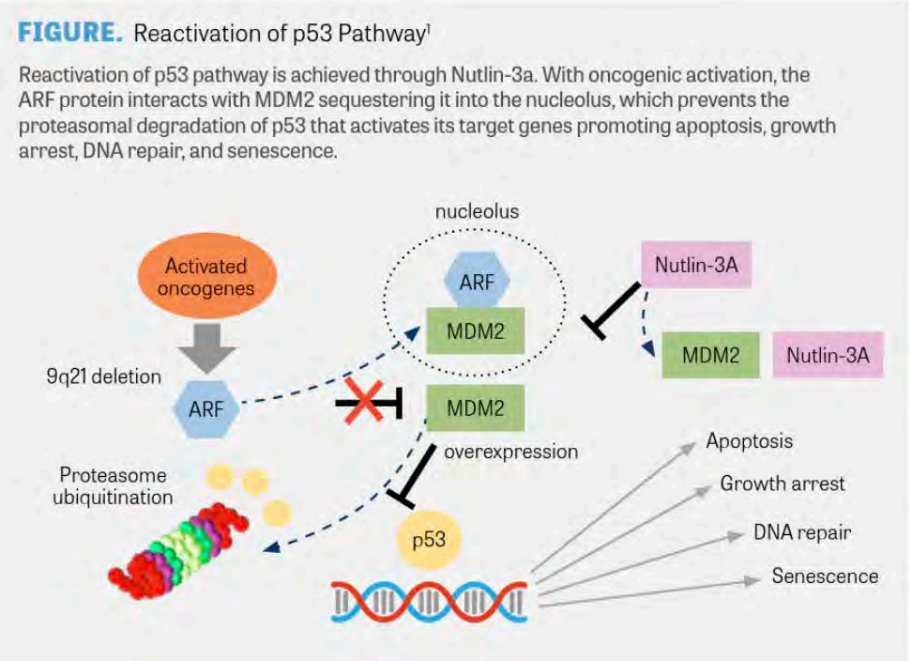
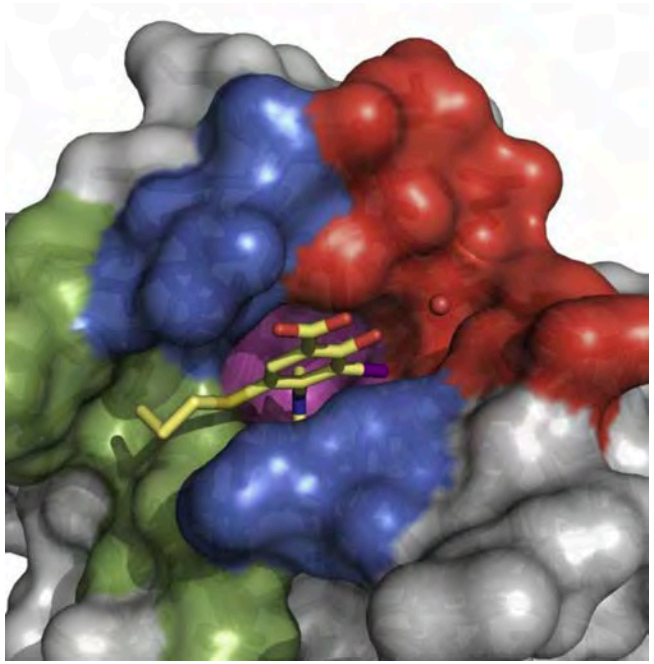


Negrao, Targeted Therapies in Oncology 2022

- Small molecule inhibitors of Mdm2 and/or MdmX (e.g. Nutlin) can effectively reengage p53
- Drugs do not work in p53 null cells (i.e. specific)
- Many clinical trials have been performed showing limited efficacy and toxicities
- Drugs can cause thrombocytopenia, gastrointestinal issues, and clonal expansion of p53 mutant cells

Therapeutic opportunities for targeting p53
(p53 mutant tumors)

- Studies suggest that p53 mutants can be refolded to produce wild type p53 protein
- R220C is a chemically tractable p53 mutant
- One purported p53 refolding drug failed in clinical trials (though many doubt it is on target)
- Synthetic lethal approaches tried and failed to date



Alan Fersht

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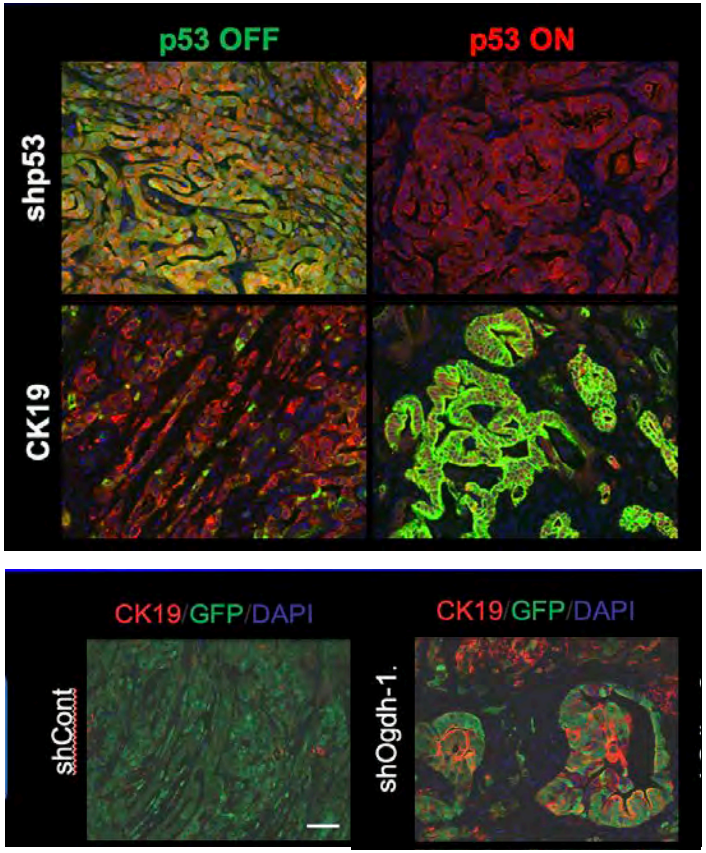
- Mimic p53 reactivation
- NEED TO SOLVE : ~5 million deaths/year due to cancers with p53 mutations



Morris et al Nature 2019

‘All models are wrong, but some are useful’

–George Box

‘All models are wrong, but some are useful’

–George Box

‘All models are wrong, it just a matter of how wrong’

–p53 researcher