The p53 Tumor Suppressor

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p53

- Discovered in 1979 (A. Levine, D. Lane) (1988, B. Vogelstein)
- *bona fide* Tumor suppressor
- Transcriptional activator and repressor
- Mutated on over 50% of human tumors
- Cancer therapeutic target (traditional drugs side effects)
Discovery of p53

Studies of SV40-transformed cells show that a (50~55-kDa) protein is coprecipitated with the large-T antigen.

Linzer and Levine found that the 54-kDa protein was overexpressed in a wide variety of SV40 transformed cells, but also in uninfected embryonic carcinoma cells.
Discovery of p53

In 1979, it was found that animals bearing several types of tumors elicited an immune response specific for p53.

Crawford et al. first described antibodies against human p53 protein in 9% of breast cancer patient sera. Caron de Fromentel et al. later found that such antibodies were present in sera of children with a wide variety of cancers. The average frequency was 12%, but the figure was 20% in Burkitt Lymphoma.
Discovery of p53

Early work on p53 suggested that it may be implicated in the promotion of cell proliferation. When the cell was induced to grow by serum stimulation, the level of p53 mRNA and the rate of p53 protein synthesis increased markedly, reaching a peak near the G1/S boundary just prior to initiation of DNA replication (Reich and Levine 1984).

Microinjection of p53 antibody into the nucleus of quiescent Swiss 3T3 mouse cells inhibited the subsequent entry of the cell into the S phase after serum stimulation.
Discovery of p53

Two groups reported that cotransfection of p53 with an activated c-Ha-ras oncogene could transform REF cells in a manner similar to that observed with proto-oncogenes such as myc or E1A (Eliyahu et al. 1984; Parada et al. 1984).

p53 could immortalize normal rat cells leading to cells sensitive to ras transformation (Jenkins et al. 1985; Jenkins et al. 1984).

These observations resulted in the classification of p53 as a nuclear oncogene.
Discovery of p53

- Immunocytochemical and immunohistochemical analysis show that the p53 protein accumulates in the nucleus of transformed or tumor cells. Before 1990, the protein was believed to be wild type.

ACCUMULATION OF p53 IN TUMOR CELLS

p53 (brown) accumulates only in the nucleus of tumor cells. Normal cells (blue nucleus) do not accumulate p53.
p53 is a tumor suppressor

- **p53 in Friend murine erythroleukemia**
- In these tumors induced by the Friend virus, the p53 gene found in the tumor cells is truncated or mutated. In this tumor model, functional inactivation of the p53 gene seems to confer a selective growth advantage to the cells during the development of Friend leukemia in vivo.
p53 is a tumor suppressor

• Wild type p53 has antiproliferative properties and does not cooperate with Ha-ras
• Cotransfection of a plasmid encoding **wild type** p53 reduced the transformation potential of plasmids encoding p53 and an activated Ha-ras gene. Furthermore, wild type p53 was shown to suppress transformation by a mixture of E1A or myc and an activated Ha-ras gene. These transformation experiments indicate that wild type p53 is a suppressor of cell transformation in vitro.
p53 is a tumor suppressor

- p53 gene is mutated in a wide variety of human cancer
- Genetic analysis of colorectal cancer reveals a very high rate of heterozygous loss of the short arm of chromosome 17, which carries the p53 gene (Vogelstein et al. 1988). PCR analysis and sequencing of the remaining p53 allele shows that it often contains a point mutation (Baker et al. 1989). Similar observations have been made in the case of lung cancer. On the heels of these initial observations have come several hundred reports of alterations of the p53 gene in all types of human cancer
p53 mutations in human tumors

Worldwide distribution of cancers and p53 mutations

- Lung: 70%
- Stomach: 45%
- Breast: 20%
- Colon: 60%
- Liver: 20%
- Prostate: 10-30%
- Cervix/Uteri: special
- Head/Neck: 60%
- Esophagus: 40%
- Leukemia: 10%
- Lymphoma: 30%
- Ovary: 60%
- Bladder: 60%
- Non-melanoma skin: 80%

Cases of Cancer per annum (x1000)
p53 is a tumor suppressor

- Germline mutation of the p53 gene are found in Li-Fraumeni patients
- This syndrome presents as a familial association of a broad spectrum of cancers including osteosarcomas, breast cancer, soft tissue sarcoma and leukemia, appearing at a very early age. Statistical analysis predicts that 50% of these individuals will have a tumor before the age of 30, and 90% before the age of 70. Germ-line mutations in the p53 gene have been found in several families with this syndrome.
p53 is a tumor suppressor

- p53 in mouse tumor development
- Mice homozygous for the null allele appear normal but are prone to the spontaneous development of a variety of tumors 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 cancer.

![Graph showing survival rates of mice deficient for p53 compared to wild type mice.](image-url)
p53 protein

- Human p53 protein can be divided into five domains, each corresponding to specific functions:
  - I) The amino-terminus part 1-42 contains the acidic transactivation domain and the mdm2 protein binding site. It also contains the Highly Conserved Domain I (HCD I).
  - II) Region 40-92 contains series repeated proline residues that are conserved in the majority of p53. It also contains a second transactivation domain.
  - III) The central region (101-306) contains the DNA binding domain. It is the target of 90% of p53 mutations found in human cancers. It contains HCD II to V.
  - IV) The oligomerization domain (307-355, 4D) consists of a beta-strand, followed by an alpha-helix necessary for dimerization, as p53 is composed of a dimer of two dimers. A nuclear export signal (NES) is localized in this oligomerization domain.
  - V) The C-terminus of p53 (356-393) contains 3 nuclear localization signals (NLS) and a non-specific DNA binding domain that binds to damaged DNA. This region is also involved in downregulation of DNA binding of the central domain.
p53 protein

• The amino-terminus of p53
• AD1: activation domain 1
  AD2: activation domain 2
• PRD: proline rich domain
• NES: nuclear exclusion domain
• HCD I: Highly Conserved Domain I
p53 protein

- The carboxy-terminus of p53
- Tetra (4D): oligomerization domain
- NEG: negative regulation domain
- NES: nuclear exclusion domain
- NLS: nuclear localization domain
p53 is a DNA-binding protein

The core domain structure consists of a beta sandwich that serves as a scaffold for two large loops and a loop-sheet-helix motif.

The loops and the loop-sheet-helix motif consist of the conserved regions of the core domain and contain the majority of the p53 mutations identified in tumors.

The structure supports the hypothesis that DNA binding is critical for the biological activity of p53, and provides a framework for understanding how mutations inactivate it.

p53 structure

p53 tumor suppressor binds to DNA using all four of its arms.
p53 structure

p53 tumor suppressor is a flexible molecule composed of four identical protein chains. Flexible molecules are difficult to study by x-ray crystallography because they do not form orderly crystals, and if they do crystallize, the experimental images are often blurry. So, p53 has been studied in parts, by removing the flexible regions and solving structures of the pieces that form stable structures.

Three of these compact, globular portions, termed "domains", have been studied.

At the center of p53 is a tetramerization domain) that ties the four chains together

A long flexible region in each chain then connects to the second stable domain: a large DNA-binding domain that is rich in arginine residues that interact with DNA. This domain recognizes specific regulatory sites on the DNA.

The third stable domain studied thus far is the transactivation domain, found near the end of each arm, that activates the DNA-reading machinery.
• **P53/DNA structure**

• p53 tumor suppressor binds to DNA using all four of its arms.

• The typical binding site for the whole molecule is composed of three parts: a specific binding site for two p53 domains, a variable stretch of 0 to 13 base pairs, and a second specific binding site for the other two p53 domains.

• The tetramerization domain is behind the helix, tying all four chains together, and the four transactivation domains extend along the DNA helix, ready to activate neighboring proteins involved in reading the DNA.

• The flexible chains that connect all four arms together allow p53 to bind to many different variants of this binding site, allowing it to regulate transcription at many places in the genome.
p53 Family Proteins

In 1997 and 1998, p63 and p73 have been cloned as two p53 related genes. p63 and p73 have “p53-like” function in vitro but are not frequently mutated in human tumors.
Mdm2 is a repressor of p53

- MDM2 (murine double minute 2) was first identified as the gene responsible for the spontaneous transformation of an immortalized murine cell line, BALB/c 3T3.
- Mdm2 interacts with p53 and acts as a E3 ubiquitin ligase for p53
- Mdm2 is a repressor for p53-mediated transcriptional activation.
- The critical role of Mdm2 in regulating p53 is best illustrated by studies carried out in mice where inactivation of p53 was shown to completely rescue the embryonic lethality caused by the loss of Mdm2 function.
Mdm2 is a repressor of p53

- MDM2 is a RING-finger E3 ubiquitin ligase that specifically interacts with p53 through its N-terminal p53-binding domain.
- MDM2 is also a negative regulator of p53-mediated transcriptional activity.
- The MDM2 gene is a transcriptional target of p53. The existence of this auto-regulatory feedback loop between MDM2 and p53 adds a complex feature to the p53–MDM2 pathway and makes MDM2 one of the most important regulators of p53 activity.
Mdm2/Mdmx (Mdm4)

- MDM4 is a RING-finger protein that specifically interacts with p53 through its N-terminal p53-binding domain.

☞ Despite its high sequence homology with Mdm2 and the presence of a RING domain, Mdmx does not have intrinsic E3-ligase activity for p53 but was instead shown to inhibit p53-induced transcription via their interactions.

☞ Mdmx knockout mice die even in the presence of Mdm2 and this lethality is also rescued by inactivation of p53.
P53 is a sensor of stress

- p53 pathway in stress response
  - i) The stress signals that activate the pathway
  - ii) The upstream mediators that detect and interpret the upstream signals.
  - iii) The core regulation of p53 through its interaction with several proteins that modulate its stability
A Classic model of p53 stabilization upon DNA damage

p53 stabilization: upstream signaling after DNA damage (gamma irradiation). Phosphorylation of p53 and mdm2 disrupt the interaction between the two proteins.
A model of p53 activation by ARF

p53 activation by ARF

ARF (known as p14 in humans and p19 in mice) was originally identified as an alternative transcript of the Ink4a/ARF tumor suppressor locus, a gene that encodes the Ink4a/p16 inhibitor of cyclin-dependent kinases. ARF suppresses aberrant cell growth in response to oncogenic stress mainly by activating the p53 pathway.

MULE = ARF-BP1
Multiple functions of p53

p53 pathways: downstream pathway
• 1) the core regulation of p53 through its interaction with several proteins that modulate its stability and activity
  2) the downstream events, act mainly through transcriptional activation
  3) The final outcome, growth arrest, apoptosis, DNA repair and etc
A model of p53-mediated cell cycle arrest.

P53-mediated growth arrest:

downstream signaling, G1 arrest via p21 transcription. The CDKI p21 will prevent Rb phosphorylation via inhibition of the CDK4 and CDK2 kinases.
p53 and Cell Cycle Arrest

- p53
- p21
- GADD45
- 14-3-3σ
- cdk2
- cdc2

G1 arrest
G2 arrest
p53 and Apoptosis

Other p53 apoptotic targets: Noxa, Fas, DR5, Apaf-1, p53AIP1, PIDD and etc
Transcription-dependent and independent p53 apoptotic pathways. Nuclear p53 induces expression of PUMA and Bax. In the mitochondria, p53 induces Bax and Bak oligomerization, antagonizes the Bcl-2 and Bcl-Xₐ antiapoptotic effect, which result in marked disruption of mitochondrial membranes and subsequent apoptosis.
Several transcriptional targets of p53 can promote autophagy, a response that has a tumour-suppressive role. However, basal levels of p53 function directly in the cytoplasm to inhibit autophagy, an activity that is shared by cytoplasmic tumour-derived p53 mutants (mt-p53). BAX, BCL2-associated X protein; DRAM, damage-regulated autophagy modulator; PUMA, p53-upregulated modulator of apoptosis; SESN2, sestrin 2.
p53 and Metabolic regulation.
Key targets: SCO2, Hexokinase, GLUT1,3,4, TIGAR and PGM
p53 in Tumorigenesis

- Telomere shortening
- Chemical exposure
- DSBs (γ-IR)
- Oncogenic stress
- Metabolic stress

- Telomere shortening
- Chemical exposure
- DSBs (γ-IR)
- Oncogenic stress
- Metabolic stress

- HAUSP
- Mdmx
- Mdm2
- Sirt1
- HDAC1/2

- p300
- PCAF
- CBP
- Tip60
- hMOF
- p300
- PCAF
- CBP
- Tip60
- hMOF

- DNA repair autophagy
- Apoptosis
- Transient cell cycle arrest
- Senescence

- Metabolism Or others?

- Tumorigenesis
Regulation of p53

Protein modifications

- **Acetylation**
- **Ubiquitination /Phosphorylation**
- Others: (SUMO, NEDD8, Methylation)

Quick response: Modification!

Senescence
Cell growth arrest
Apoptosis
Autophagy
Metabolism
Aging
The p53-ubiquitination pathway

p53 ubiquitination:
1. **Mdm2** Mono vs. Poly
   (Ub—Nuclear export)
2. Deubiquitination: HAUSP
3. Mdm2-independent ubiquitination
   ARF-BP1, COP1, PIRH2 and etc.
The difference between Mono- and Poly-ubiquitination

Mono-Ubiquitination

Multi Mono-Ubiquitination

Poly-Ubiquitination
Mdm2 can induce both mono- and polyubiquitination of p53

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MW (Kd)

- p53 (poly-Ub)
- p53 (mono-Ub)
- p53
Monoubiquitination of p53 induces nuclear export

B

GFP-p53

+ Mdm2

GFP-p53 +

+ Mdm2

+ LLNL&MG132
Polyubiquitination of p53 induces nuclear degradation

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<tr>
<td>GFP-p53 + Mdm2 + LLNL&amp;MG132</td>
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Mdm2

PARC/Cul-7

Ub

Nucleus

Cytoplasm

26S

Mdm2 dependent

??

Ub

p53

Ub

Ub

Ub

Ub

Ub

Ub
P53 is degraded by both Mdm2-dependent and – independent ubiquitination

- Mdm2 is the major E3 ligase
- Other E3 ligases such as ARF-BP1/MULE, Pirh2, COP1 are also involved in p53 degradation
Oncogene Activation (c-Myc, Ras)

ARF

ARF-BP1

unknown substrates

p53-independent cell growth inhibition

Mdm2

p53-dependent cell growth arrest, apoptosis, senescence
Inactivation of ARF-BP1, but not Mdm2, induces cell growth repression in p53-null cells.
p53 activation in vivo in ARF-BP1-null e13.75 embryos

Kon and Gu unpublished
HAUSP/USP7

UBP: ubiquitin-specific process protease

- Stabilize p53, Mdm2 and Mdmx
- A substrate-specific deubiquitinase

- Li et al., Nature, 416, 648, 2002
- Hu et al., Cell, 111, 1041, 2002
- Li et al., Mol Cell, 2004
The HAUSP Consensus Binding Site on p53 and Mdm2
HAUSP reduction/ablation has a profound effect on p53/Mdm2

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HCT116 HAUSP wt

HCT116 HAUSP -/-
Analysis of e11.5 embryos from Hausp conditional knockout

Hausp^{+/cl}
Embryo

Hausp^{ko/cl, ERT2}
Embryo
Oncogenic stress

HAUSP \rightarrow Mdm2 \rightarrow ARF

ARF-BP1/Mule, COP1, PIRH2 and etc.

HAUSP

p53

p53
Regulation of p53 by acetylation/Deacetylation
(general mechanism for non-histone proteins)
~1400 substrates

Gu et al., Cell 1997; Nature 1997.
Luo et al., Cell, 2001; Tang et al., 2006
p53 is acetylated at Lysine 120 by Tip60/hMOF
(Tang et al., 2006; Sykes et al., 2006; Li et al., 2009)

A. N-terminal domain  DNA-binding domain  C-terminal domain

1-97  98-299  300-393

B. Tip60

K120

C. p53_H. sapiens  110 RLGFLH.SGTAKSV...TCTYPALN 131
p53_M. musculus  104 HLGFLQ.SGTAKSV...MCTYSPPLN 125
p53_G. gallus  95 RVGFVE.AGTAKSV...TCTYSPVLN 116
p53_X. laevis  84 QLDFOQ.NGTAKSV...TCTYSPELN 105
p53_D. melanogaster  93 FSMVLI..DEPPKSL....WMYSIPLN 112
p53_C. elegans  229 DVLK...QKVAKSSDMAFAISSEHEK 251

Actinomycin D (hr)

MG132  0  2  4  8  12

p53

Tip60 siRNA  -  +  -  +
Control siRNA  +  -  +  -
Actinomycin D  -  -  +  +

C. crude extracts
DNA damage-mediated p53 activation in the K117R mutant mouse

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<th>Fold change in mRNA level</th>
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DNA damage-mediated p53 activation in the K117R mutant mouse
No treat | 12.5Gy IR
---|---
**Cleaved Caspase 3 (testis)**

WT | K117R

**Cleaved Caspase 3 (intestine)**

WT | K117R

**Cleaved Caspase 3 (spleen)**

WT | K117R

**Cleaved Caspase 3 (thymus)**

WT | K117R
Is p53-mediated apoptosis dispensable for tumor suppression?

\[ p53^{K117R/K117R} \]

**Percent survival**

- p53\(^{-/-}\) (n=19)
- p53\(^{K117R/K117R}\) (n=14)

**Age (weeks)**
Making mouse p53-3KR (K117R, K161R and K162R) knockin mouse

A

Human p53-8KR

Transactivation domain

Proline-rich domain

DNA binding domain

N-terminal

Central core

C-terminal

1-42

63-97

98-292

324-355

363-393

K120

K164

p300/CBP

NLS

NES

KKKKKKK

370/372/373/381/382/386

B

Mouse p53-3KR

N-terminal

Central core

C-terminal

Transactivation domain

Proline-rich domain

DNA-binding domain

Tetramerization domain

C-terminal regulatory domain

K117R

K161R

K162R
Loss of p53-mediated activation of PUMA and p21 in p53-3KR MEFs
Loss of p53-mediated senescence in p53-3KR MEFs
Are p53-mediated apoptosis and senescence absolutely required for tumor suppression?
If cell-cycle arrest, apoptosis and senescence are not absolutely required, what else could be important for tumor suppression?
Rethink the mechanisms of p53-mediated tumor suppression

- Apoptosis
- Cell growth arrest
- Senescence
- Cell Metabolism (new targets)

Additional targets?
3KR mouse model retains regulation on metabolic targets

Li et al, Cell (2012)
Functions of TIGAR

Tigar function:
- Shunts glycolytic intermediates into pentose phosphate shunt
  - \(\downarrow\) fructose-2,6-bisphosphate (positive allosteric regulation on glycolysis)
  - \(\downarrow\) glycolytic rate
- Increases GSH:GSSG ratio
- Lowers intracellular ROS

Bensaad et al, Cell (2006)
TIGAR promotes tumor survival in mouse model

TIGAR Is Required for Efficient Intestinal Regeneration and Tumorigenesis

Eric C. Cheung,1 Dimitris Athineos,1 Pearl Lee,1 Rachel A. Ridgway,1 Wendy Lambie,1 Colin Nixon,1 Douglas Strathdee,1 Karen Blyth,1 Owen J. Sansom,1 and Karen H. Vousden1,*
1CR-UK Beatson Institute, Switchback Road, Glasgow G61 1BD, UK
*Correspondence: k.yousden@beatson.gla.ac.uk
http://dx.doi.org/10.1016/j.devcel.2013.05.001

B
C
D
M

Total # of tumors
Total tumor burden
Average tumor size

↑ ROS scavenger (glutathione)
↑ Nucleoside synthesis (allow for cell proliferation)

Cheung et al., Dev Cell (2013)
SLC7A11 is regulated by p53-3KR

A

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1 2 3 4
SLC7A11:

(1) A key component of the cystine/glutamate antiporter (system $x^-$), critical for cellular cystine uptake
(2) Located @ chromosome 4q28-31, overexpressed in human cancers
(3) Involved in regulating ROS levels
(4) Non-apoptotic cell death
Regulation of Cystine Update by p53-wt and p53^{3KR}

A

Slc7a11

Hprt

1 2 3

Slc7a11 Relative mRNA level

p53^{+/+} p53^{-/-} p53^{3KR/3KR}

B

Cystine uptake level

C

Tet - +

Cystine uptake level

p53^{+/+} p53^{-/-} p53^{3KR/3KR}
Ferroptosis: An Iron-Dependent Form of Nonapoptotic Cell Death

Scott J. Dixon, Kathryn M. Lemberg, Michael R. Lamprechts, Rachid Skouta, Eleina M. Zaitsev, Caroline E. Gleason, Darpan N. Patel, Andras J. Bauer, Alexandra M. Cantley, Wan Seok Yang, Barclay Morrison III, and Brent R. Stockwell

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Apoptosis, necrosis, autophagy

Erastin → System L → Amino acid uptake
Erastin → System x^- → Cys

Sulfasalazine
β-ME → sh-CS, sh-ACSF2

H2O2 → Lipid and soluble ROS
Gin → Ferroptosis
Regulation of Ferroptosis by p53-wt and p53^{3KR}
SLC7A11 expression abrogates p53-3KR mediated tumor suppression

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<tbody>
<tr>
<td>p53-3KR</td>
<td>-  -  +  +</td>
<td>p&lt;0.05</td>
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<tr>
<td>SLC7A11</td>
<td>-  +  -  +</td>
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![Tumor weight (mg)](image)

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<tr>
<td>SLC7A11</td>
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<tr>
<td>SLC7A11</td>
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<td>p53(3KR)</td>
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<td>VINCULIN</td>
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1 2 3 4
p53-mediated tumor suppression

Apoptosis

Cell growth arrest Senescence

p21 PML PAI-1

K120 K164 K163

SLC7A11 and Others Metabolic targets

Metabolism ferroptosis

PUMA NOXA BAX