p53 and Apoptosis: Master Guardian and Executioner

Chapter 9

Dr Saeb Aliwaini
Strange results!

- Transfection of a p53 cDNA clone into rat embryo fibroblasts revealed that this DNA could collaborate with a co-introduced ras oncogene in the transformation of these rodent cells. Such activity suggested that the p53 gene (which is sometimes termed Trp53 in mice and TP53 in humans) might operate as an oncogene.

- Like myc, the introduced p53 cDNA seemed to contribute certain growth-inducing signals that resulted in cell transformation in the presence of a concomitantly expressed ras oncogene.
• But appearances deceived. As later became apparent, the p53 cDNA had originally been synthesized using as template the mRNA extracted from tumor cells (rather than normal cells).

Frequency of mutant p53 alleles in human tumor cell genomes. Mutant alleles of p53 are found frequently in commonly occurring human tumors.
p53 is discovered to be a tumor suppressor gene

- Deletion of p53 gene copies from the mouse germ line had no significant effect on the development of the great majority of p53−/− embryos.
- Therefore, p53 could not be considered to be a simple negative regulator of cell proliferation during normal development.

Dr Saeb Aliwaini

p53 seemed to be specialized to prevent the appearance of abnormal cells, specifically, those cells that were capable of spawning tumors.

p53−/− resulted in a greatly increased mortality relatively early in life, deriving largely from the development of sarcomas and leukemias.
Mutant versions of p53 interfere with normal p53 function

- Analyses indicated that the great majority of tumor-associated, mutant p53 alleles carry point mutations in their reading frames that create **missense** codons.
- To date, more than 26,000 tumor-associated p53 alleles originating in human tumor cell genomes have been sequenced, 74% of which have been found to carry such missense mutations.
- Consequently, researchers came to the inescapable conclusion that **tumor cells can benefit from the presence of a slightly altered p53 protein rather than from its complete absence.**
How does mutant p53 protein might foster tumor cell formation?

• **1- dominant-interfering or dominant-negative alleles:** Mutation inactivates the normal functioning of the encoded gene product and interferes with or obstructs the ongoing activities of the surviving wild-type copy of this gene in a cell.

• **2- p53 was a nuclear protein that normally exists in the cell as a homotetramer**, that is, an assembly of four identical polypeptide subunits.
As indicated in these pie charts, point-mutated alleles of *p53* leading to amino acid substitutions (*green*) represent the great majority of the mutant *p53* alleles found in human tumors, while other types of mutations are seen relatively infrequently.

But in other tumor suppressor genes (*APC, ATM, BRCA1*) represent reading frame shifts (*yellow*) or nonsense codons (*blue*) in the majority of cases; both of these types of mutation disrupt protein structure, usually by creating truncated versions of proteins that are often degraded rapidly in cells.
Mutations

- **A frameshift mutation** is an insertion or deletion involving a number of base pairs that is not a multiple of three and consequently disrupts the triplet reading frame, usually leading to the creation of a premature termination (stop) codon and resulting in a truncated protein product.

- **Missense mutation** is a point **mutation** in which a single nucleotide change results in a codon that codes for a different amino acid.

- **Nonsense mutations** arise from single bp substitutions and which cause the creation of a premature stop codon.

- **Silent mutations** are base substitutions that result in no change of the amino acid or amino acid functionality when the altered messenger RNA (mRNA) is translated.
How does mutant p53 protein might foster tumor cell formation?

The great majority of p53 mutations (95.1%) affect the DNA-binding domain of the p53 protein.

The transactivation domain enables p53 to interact physically with a number of alternative partners, including the p300/CBP transcriptional co-activator and Mdm2, the p53 antagonist.

Proline rich affects apoptosis!
Mutant allele coexists with a wild-type allele in this cell!

“15 out of the 16 equally possible combinations of mutant and wild-type p53 monomers would contain at least one mutant p53 subunit and might therefore lack some or all of the activity associated with a fully wild-type p53 tetramer.

The presence of only a single mutant p53 protein in a tetramer might well interfere with the functioning of the tetramer as a whole.
How does mutant p53 protein might foster tumor cell formation?

• Mutant p53 has a highly active promoter that is far more active than the gene promoter controlling expression of the native p53 gene copies...!!!

• Furthermore, in the great majority of human tumor cells that are mutant at the p53 locus, the p53 locus is found to have undergone a loss of heterozygosity, yielding a cell with two mutant p53 alleles.
Why is elimination of the surviving wild-type $p53$ allele even necessary?

- The answer seems to lie in the residual one-sixteenth of fully normal $p53$ gene function; even this little bit seems to be more than most tumor cells care to live with. So, being most opportunistic, they jettison the remaining wild-type $p53$ allele in order to proliferate even better.
p53 protein molecules usually have short life times

• The nuclear localization of this protein in many normal and neoplastic cells suggested that it might function as a transcription factor (TF).

• *Regulation of the activity of p53 transcription factor.*

• 1- Concentrations of the transcription factor in the nucleus are modulated.

• Cycloheximide treatment of cells with wild-type *p53* alleles, the p53 protein disappeared with a half-life of only 20 minutes.

• Similar behaviors have been associated with other cellular proteins such as Myc!
p53 protein molecules usually have short life times

- Why should a cell invest substantial energy and synthetic capacity in making a protein molecule, only to destroy it almost as soon as it has been created?
- The cell can double the concentration of p53 protein in 20 minutes simply by blocking its degradation.
- The net result of this is a very low “steady-state” level of the protein within this cell.

- In response to certain physiologic signals, however, the degradation of p53 is blocked, resulting in a rapid increase of p53 levels in the cell.
A variety of signals cause p53 induction

- X-rays, ultraviolet (UV) radiation, certain chemotherapeutic drugs that damage DNA, inhibitors of DNA synthesis, and agents that disrupt the microtubule components of the cytoskeleton.
- low oxygen tension (hypoxia).
- Introduction of either the adenovirus E1A or myc oncogene
- E2F1 transcription factor

- **2- By post-translational stabilization of the normally labile p53 protein.**
p53-activating signals and p53’s downstream effects

- lack of nucleotides
- UV radiation
- ionizing radiation
- oncogene signaling
- hypoxia
- blockage of transcription

- cell cycle arrest
- DNA repair
- block of angiogenesis
- apoptosis

- OR

- senescence
- return to proliferation

Dr Saeb Aliwaini
• X-rays, evoked an increase in cellular p53 levels, the levels of the p21Cip1 protein increased subsequently; this induction was absent in cells expressing mutant p53 protein.

In the same time, normal cells must also avoid excessive p53 activity, since it threatens to end their lives and thereby cause depletion of the cells needed to maintain normal bodily functions.

Dr Saeb Aliwaini
DNA damage and deregulated growth signals cause p53 stabilization

- The sensors of dsDNA breaks transfer signals to the ATM kinase to chk2, phosphorylate p53.

- ssDNA sensors activate ATR kinase, which acts via the Chk1 kinase, to phosphorylate p53, again protecting it from degradation.

- Loss of the DNA repair and genome-stabilizing functions promoted by p53 will make descendants of a p53−/− cell more likely to acquire further mutations and advance more rapidly down the road of malignancy.
Mdm2 destroys its own creator

- [link](https://www.youtube.com/watch?v=hvNJ3yWZQbE)
- MDM2 recognizes p53 as a target that should be ubiquitylated shortly after its synthesis and therefore marked for rapid destruction.
- In many human lung tumors, Mdm2 (as we will call it) is overexpressed through mechanisms that remain unclear.

Dr Saeb Aliwaini
Mdm2 destroys its own creator

• Mdm2 binding to p53 immediately blocks the ability of p53 to function in this role.
• Mdm2 directs the attachment of a ubiquitin moiety to p53 and the export of p53 from the nucleus.
• Protection of p53 is often achieved by phosphorylation of p53, which blocks the ability of Mdm2 to bind p53 and trigger its ubiquitylation.
• At the same time, the DNA damage–activated ATM kinase can phosphorylate Mdm2 in a way that causes its functional inactivation and destabilization
Yet another mechanism that affects Mdm2 has been revealed through the discovery of an Mdm2 antagonist, which is termed p19^{ARF} in mouse cells and p14^{ARF} in human cells. Astute sequence analysis led to the discovery of ARF, as we will call it hereafter. Its encoding gene was originally uncovered in mouse cells as a gene whose sequences are intertwined with those specifying p16^{INK4A}, the important inhibitor of the CDK4 and CDK6 kinases that initiate pRb phosphorylation (see Section 8.4).

Dr Saeb Aliwaini
Mdm2 destroys its own creator

- The signaling pathway that favors cell survival through activation of the PI3 kinase (PI3K) pathway leads, via the Akt/PKB kinase, to Mdm2 phosphorylation (at a site different from that altered by the ATM kinase described above) and to the resulting translocation of Mdm2 from the cytoplasm to the nucleus, where it is poised to attack p53.