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P_{53} and its Applications in Cancer Therapy

Neetu Rajput[1*], Supriya[1], Megha Kadam[1], V. K. Dumka[2] and H. S. Sandhu[2]

[1]Department of Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, Rasalpura - 453446, Mhow, Madhya Pradesh, India; drneeturajput@gmail.com
[2]Department of Pharmacology and Toxicology, GADVASU, Ludhiana - 141012, Punjab, India

Abstract

P53 is a tumour suppressor protein encoded by the TP53 gene, which is one of the most commonly mutated gene in human and animal cancers. Missense Mutation of the p53 gene is the most prevalent genetic abnormalities which gives rise to full length p53 containing single amino acid substitution within the sequence-specific DNA binding domain (|Greenblatt *et al.*, 1994)[14]. p53 plays anti-cancer role, through several mechanisms namely by activating DNA repair, initiating apoptosis and inducing growth arrest. By managing the DNA damage, p53 gene plays an important role in maintaining integrity and stability of the genome and therefore, it has been described as "the guardian of the genome". Restoring p53 function is a major step in the treatment of many cancers by following different strategies like, gene replacement therapy using wild-type p53, reactivation of p53 function, inhibition of p53-Mdm2 interaction etc. Advexin is an Adeno-p53 vector, recognized as an orphan drug by USA, Food and Drug administration in 2003, provides delivery of wild-type p53 to cancer cells and demonstrates anticancer activity following adequate expression of p53 (Nemunaitis and Nemunaitis, 2008)[27]. Similarly, MI-319 restores p53 functions and increases the life span of orally treated follicular lymphoma bearing animals (Mohammad *et al.*, 2009)[25]. These strategies of p53-based cancer therapy differ greatly between themselves both in the mode of action and the degree of success seen in experimental models. Cancer therapy based around p53 proves to be more efficacious, especially in terms of specificity. But the innovation of successful p53-based cancer therapies is only limited by our understanding of p53 biology and the creative use of such knowledge. In future, p53-based cancer therapy can be a valuable option for the treatment of a variety of tumours in human as well as animals.

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1. Introduction

Cancer is a disease resulting from the breakdown of several checkpoints and tumour-suppressing mechanisms[15]. Despite significant advances in cancer diagnostics and therapy there is still little progress in the treatment of advanced disease. Therefore, finding effective ways to treat and cure cancer has become the top priority for the modern society. Global picture revealed in cancer statistics is quite high but, the tumour-suppressing mechanisms are extremely effective against cancer. One of the best known barriers against malignant transformation is a single protein, the tumour suppressor p53.

p53 (also known as protein 53 or tumour protein 53), is a tumour suppressor protein that is encoded by the *TP53* gene. p53 is important in multicellular organisms, where it regulates the cell cycle and, thus, functions as a tumour suppressor that is involved in preventing cancer. As such, p53 has been described as "the guardian of the genome", the "guardian angel gene", and the "master watchman", referring to its role in conserving stability by preventing genome mutation. The name p53 is in reference to its apparent molecular mass of 53-kilodalton (kDa). But, based on calculation from its amino acid residues, p53's mass is actually only 43.7 kDa.

* *Author for correspondence*

2. History

p53 was identified in 1979 by Arnold Levine, David P. Lane and William Old, working at Princeton University, Dundee University (UK) and Sloan-Kettering Memorial Hospital, respectively. p53 was initially presumed to be an oncogene. Its character as a tumour suppressor gene was revealed in 1989 by Bert Vogelstein working at Johns Hopkins School of Medicine. In 1993, p53 was voted molecule of the year by Science magazine[21].

3. Structure of p53

P53 is a complex tetrameric structure[20] which is 393 amino acids long and has seven domains:

- N-terminal transcription-activation domain (**TAD**), also known as activation domain 1 (**AD1**), which activates transcription factors: residues 1-42.
- Activation domain 2 (**AD2**) important for apoptotic activity: residues 43-63.
- **Proline rich domain** important for the apoptotic activity of p53: residues 64-92.
- Central DNA-binding core domain (**DBD**). Contains one zinc atom and several arginine amino acids: residues 100-300.
- **Nuclear localization signalling domain**: residues 316-325.
- Homo-oligomerisation domain (**OD**) involved in tetramerization which is essential for the activity of p53 *in vivo*: residues 307-355.
- **C-terminal** involved in down regulation of DNA binding of the central domain: residues 356-393.
    - Wild-type p53 is a labile protein, comprising folded and unstructured regions which function in a synergistic manner.

4. Function of p53

Expression of the mutant protein is high only in tumour tissue, but low in normal tissues, suggesting a promoting role of mutant p53 in cancer progression. Such "gain-of-function" mutants can accelerate tumour progression. Wild-type p53 acts as a tumour suppressor, the mutant form is an inactivated antioncogene, which play active role in the progression of tumour growth[35].

In its anti-cancer role, p53 works through several mechanisms:

- It can activate **DNA repair** proteins when DNA has sustained damage.
- It can initiate **apoptosis**, the programmed cell death, if DNA damage proves to be irreparable.
- It can induce **growth arrest** by holding the cell cycle at the G_1/S regulation point on DNA damage recognition (if it holds the cell here for long enough, the DNA repair proteins will have time to fix the damage and the cell will be allowed to continue the cell cycle).
- It can induce **senescence**.

4.1 DNA Repair

p53 is directly involved in DNA repair. One of its transcriptional target genes, p53R2, encodes ribonucleotide reductase, which is important for both DNA replication and repair. p53 also interacts directly with endonuclease and DNA polymerase which are involved in base excision repair.

4.2 p53-Dependent Apoptosis

One of the most dramatic responses to p53 activation is the induction of apoptosis. Apoptosis occurs through either one of two major pathways described as either the intrinsic mitochondrial or extrinsic death receptor pathway[22].

4.2.1 Intrinsic Mitochondrial Pathway

Nuclear p53 induces Puma expression, which in turn releases cytosolic p53 held inactive in the cytoplasm through binding to Bcl-X_L. Then, cytosolic p53 induces Bax oligomerisation and mitochondrial translocation[1]. In the mitochondrion, p53 induces Bax and Bak oligomerization, physically interacts with protective Bcl-X_L and Bcl-2, antagonizing their antiapoptotic effects, and also forms a complex with cyclophilin D leading to disruption of mitochondrial membrane and subsequent release of both soluble and insoluble apoptogenic factors. p53 has the ability to activate transcription of various proapoptotic genes, including those encoding members of the Bcl-2 family, such as Bax, Noxa, Puma and Fox F_1[36]. The importance of Puma and Noxa to p53-mediated apoptosis became clear when it is reported that certain cell types from puma knockout mice showed almost complete impairment to apoptosis via p53[19]. Cells committed to die *via* p53-dependent apoptosis follow the mitochondrial

pathway, although p53 can also trigger cell death through death receptors.

4.2.2 Extrinsic Death Receptor Pathway

p53 over expression enhances cell surface levels of fas by promoting its trafficking from golgi complex[2]. p53 activates DR5 (death domain-containing receptor) in response to DNA damage and in turn promotes cell death[45]. Genes for proapoptotic proteins that may link apoptotic pathways, such as BID and the p53-induced protein with a death domain (PIDD) were also described as transcriptional targets of p53[26]. p53 is also involved in the activation of the apoptosome *via* induction of APAF-1 expression.

4.3 Cell-Cycle Arrest

Activated p53 binds DNA and activates expression of several genes including WAF1/CIP1 encoding for p21. p21 (WAF1) binds to the G1-S/CDK (CDK2) and S/CDK complexes (molecules important for the G1/S transition in the cell cycle) inhibiting their activity[40]. When p21 (WAF1) is complexed with CDK2 the cell cannot continue to the next stage of cell division i.e. S phase. The progression into the M phase requires Cdc2 which can be inhibited by p21, GADD45 or 14–3–3σ. p53 regulates the expression of these inhibitory proteins to induce growth arrest. Loss of the p21 gene has been shown to result in the failure of the checkpoints that control entry into the S and M phases of the cell cycle after DNA damage[5]. A mutant p53 will no longer bind DNA in an effective way, and, as a consequence, the p21 protein will not be available to act as the "stop signal" for cell division. Thus, cells will divide uncontrollably, and form tumours. Once activated, p53 induce a cell cycle arrest to allow either repair or survival of the cell or apoptosis to discard the damaged cell, but how p53 makes this choice is currently unknown.

4.4 Senescence

By forcing cells with damaged DNA to stop dividing and become senescent or even to die by apoptosis, it protects the organism from the threat of those cells becoming cancerous but at the expense of reducing cell renewal (e.g., by decreasing the size of the pools of stem cells). Mice that are forced to produce higher-than-normal levels of the p53 protein show many signs of premature aging[11]. It is important to note that just a slight constitutive hyper activation of p53 induces an alarming premature aging phenotype in mice[24].

5. Regulation of p53

p53 signaling pathway occurs in response to a broad range of stressors, intrinsic and extrinsic to the cell, which stabilize and affect p53 by a series of post-translational modifications[46]. Phosphorylation is classically regarded as the first crucial step of p53 stabilization. p53 is regulated through control of protein stability, a mechanism mediated by Mdm2[42]. Mdm2 is itself a transcriptional target of p53, therefore, generating an autoregulatory negative feedback loop in which p53 activates the expression of its own inhibitor Mdm2[48]. It has been demonstrated that the Mdm2 protein can inhibit p53 by regulating its stability, cellular localization, and transactivation. Mdm2 functions as an E3 ubiquitin ligase targeting p53, and Mdm2 itself, for proteasome degradation. The ubiquitin ligase activity of Mdm2 also contributes to the efficient nuclear export of p53. Some oncogenes can also stimulate the transcription of proteins which bind to Mdm2 and inhibit its activity. Deacetylase enzymes, such as Sirt1 and Sirt7, can deacetylate p53, leading to an inhibition of apoptosis[39]. There are multiple layers of p53 regulation by Mdm2 in the following ways:

- Expression of Mdm2 is activated by p53.
- Binding of p53 by Mdm2 can trigger the degradation of p53 via the ubiquitin system.
- Phosphorylation of p53 at Ser15, Thr18 or Ser20 will disrupt its binding with Mdm2. In normal cells, these three residues are not phosphorylated, and p53 is maintained at low level by Mdm2.
- DNA damage may activate protein kinases (such as ATM) to phosphorylate p53 at one of these three residues, thereby increasing p53 level. Since Mdm2 expression is activated by p53, the increase of p53 also increases Mdm2, but they have no effect while p53 is phosphorylated. After the DNA damage is repaired, the ATM kinase is no longer active. p53 will be quickly dephosphorylated and destroyed by the accumulated Mdm2.

Ubiquitylation and acetylation of lysine residues are mutually exclusive modifications. Purified acetylated p53 cannot be ubiquitinated by its specific repressor mouse double minute-2 (Mdm-2) oncoprotein and ubiquitylation is significantly reduced upon induction

of acetylation. The ability of p53 to integrate each kind of post-translational modification allows it to adequately respond by modulating the expression of different subsets of target genes.

6. Nature of p53 Dysfunction in Cancer

6.1 Mutation

p53 status has considerable potential as a clinical marker in malignancy. The most prevalent genetic abnormalities of the p53 gene are missense mutations which give rise to full-length protein products containing single amino acid substitutions within the sequence-specific DNA binding domain (Greenblatt *et al.,* 1994). It has been estimated that more than 50% of all human malignancies have detectable p53 mutations. Mutant p53 often don't induce Mdm2, and are thus able to accumulate at very high concentrations. Mutant p53 protein itself can inhibit normal p53. Mutation of p53 may affect its ability to suppress tumourigenesis through one, or a combination, of the following modes:

- Loss of wild-type p53 function
- Dominant negative effect of mutant p53 over wild-type p53
- Gain of additional oncogenic properties that are not dependent upon inhibition of wild-type p53.

6.2 Cellular and Viral Proteins

Mutation of the p53 gene is not the only mechanism by which the p53 pathway can be inactivated. A large number of cellular and viral proteins have been shown to cause p53 dysfunction through either sequestration or degradation of the wild-type p53 protein. A prime example is the protein product of the Mdm2 cellular proto-oncogene which is over expressed in a variety of tumours and can inhibit p53 function through direct binding and covering of the transcriptional activation domain of p53[8].

Mdm2 protein may also target wild type p53 protein for ubiquitin-dependent degradation. One or more p53-interacting cytoplasmic proteins are responsible for preventing p53 from reaching its nuclear site of action in these cells. Viral proteins which have the ability to bind to and disrupt wild-type p53 function include the human papilloma virus (HPV) E6 and the hepatitis B virus X proteins. For example, the oncogenic HPV E6 protein is believed to inhibit p53 function either by blocking the sequence-specific DNA binding activity of p53 or by exposing newly synthesised p53 for ubiquitin mediated degradation[38].

6.3 Defects in the Upstream Signalling Pathway

Inactivation of the p53 pathway may also result from defects in the upstream signalling pathway which normally leads to activation of p53 in response to stress. Evidence is there to indicate that any alterations in the pattern of phosphorylation and acetylation occur within p53 subsequent to stress leads to inactivation of the p53 pathway[31]. However, the signalling intermediates leading from stress to such modifications within p53 are still unclear. Some potential candidates include protein kinases such as the ATM gene product and the histone acetyltransferases.

In certain tumours, defect may be present in critical downstream components of the p53 pathway, thus switching p53 activation into an important event. In particular, p53 dysfunction correlates with more aggressive tumours, early metastasis and decreased survival rates.

7. p53-based Cancer Therapies

Following strategies in cancer therapeutics have been developed to target p53:

- Gene replacement therapy using wild-type p53
- Reactivators of p53 function
- Inhibitors of p53-Mdm2 interaction
- Ribozyme strategy
- Selective chemotherapy
- Immunotherapy
- Attenuated adenovirus therapy

8. Gene Replacement Therapy using Wild-Type p53

Restoring p53 function is a major step in curing many cancers[41]. Both cell transformation and neoplastic cell growth can be suppressed by re-introduction of the wild-type p53 gene into tumour cells with lost or dysfunctional p53. The effectiveness of many conventional therapeutic agents is determined, at least in part, by a p53-dependent mechanism. Therefore, restoration of p53 function might also lead to sensitisation of resistant tumour cells to such

agents. Effort has been made to design clinically effective gene replacement strategies using wild-type p53 for the treatment of cancer. The primary requirement of such cancer gene therapy is the necessity for highly efficient delivery of the wild-type p53 gene into tumour cells *in vivo*, sufficient expression of functional p53 protein to mediate tumour suppression either through a direct mechanism involving cell death/growth arrest or by increasing sensitivity to conventional anti-tumour agents and low level of toxicity towards normal cells and the absence of a detrimental host immune response against the gene delivery system used.

Two types of delivery system can be used in p53-based gene replacement therapy-

- Viral delivery system
- Non-viral delivery system

8.1 Viral Delivery Systems

Number of viruses is being used to deliver p53 gene into tumour cells-

8.1.1 Retrovirus

Retroviruses are important for use in cancer gene therapy applications. These viral vectors integrate stably into the genome of infected cells and require cell division for transduction. Jack Roth and his colleagues had been demonstrated that retrovirus mediated gene transfer of the wild-type p53 gene into both human lung tumour cell line and xenograft models could lead to inhibition of tumour cell growth (Roth *et al.,* 1997). The adverse effect on cell growth is limited to tumour cells which contained a defective p53 pathway (they were either null for p53 or had inactivating missense mutations within the p53 gene).

The growth rate of non-infected lung cancer cells are to be reduced by cells which had been transduced with the wild-type p53 gene, therefore suggesting the possibility of a '**bystander**' effect [7]. The exact molecular basis of this 'bystander' effect is unclear at present, but, it may involve:

- Exudation of cell death signal molecules from transduced, dying cells to neighbouring untransduced cells.
- Phagocytotic uptake of apoptotic vesicles by untransduced cells which in turn induce apoptosis.
- Activation of an immune response against the tumour.

Advantage of such a 'bystander' killing process is that less than 100% of cells within a tumour need to be transduced with the wild-type p53 gene for a complete therapeutic response to occur. Only a small proportion (less than 10%) of transduced cells are necessary for successful killing of tumour cells.

8.1.2 Adenovirus

Currently the viral vector for gene replacement therapy using wild-type p53 is based on the double stranded DNA adenovirus serotype 5. Adenoviruses are capable of high efficiency transduction of a wide range of cell types and are not limited to actively proliferating cells. As adenoviruses do not integrate into the genome of the host cell, they are transient expression vectors. This is considered as a disadvantage if sustained expression of p53 is required to mediate tumour suppression through a reversible growth arrest mechanism, but not in the case of those cell types where p53 activation leads to either apoptosis or irreversible growth arrest. Use of adenoviral vectors for gene therapy can lead to induction of an adverse immune response within the treated patient, which in turn limit the repeated administration of therapeutic virus. Despite this, many studies have indicated enhancing results with respect to tumour cell growth suppression when using adenovirus-mediated transfer of the wild-type p53 gene[28].

In general, gene delivery by p53-adenovirus cause specific inhibition of tumour cell growth only in those cells which contain mutated p53 or p53 null. Both normal cells and tumour cells retaining p53 function are not adversely affected to any significant degree by the expression of exogenous wild-type p53, at doses of p53-adenovirus required to inhibit the growth of p53 deficient cells. Combination of p53 adenovirus treatment with other traditional cancer therapies, for example radiation and cisplatin, is more effective than either treatment alone.

One of such Adeno-p53 vectors has been commercialized by Introgen Therapeutics under the name **Advexin** and received the status of an orphan drug from the USA Food and Drug Administration in 2003. Advexin provides delivery of wild-type p53 to cancer cells and demonstrates anticancer activity following adequate expression of p53 (Nemunaitis and Nemunaitis, 2008). Advexin has been used in patients with several different types of cancer including head and neck cancer, nonmetastatic non-small cell lung cancer and bronchioalveolar lung cancer.

8.1.3 Other Viral Vectors

Other viral vectors may play an important role as delivery vehicles for gene replacement therapy using wild-type p53 in certain clinical situations, for example the utilisation of **herpes simplex virus (HSV)** for gene therapy of brain tumours[16]. A recent study indicated that the HSV protein VP22 alone is sufficient to mediate intercellular delivery of functional p53 protein.

8.2 Non-Viral Delivery Systems

Several different non-viral delivery system are studied for their ability to deliver wild-type p53 expression plasmids *in vivo* and to mediate effective antitumor responses[13]. These delivery system can be subdivided into two categories-

- Direct DNA Injection
- Cationic lipid-mediated gene transfer (Liposomes)

Although non-viral delivery system is less efficient than the viral systems described above in actual delivery of the wild-type p53 to target cells, but the advantages of such non-viral approaches include an apparent tolerance by the immune system.

8.2.1 Direct DNA Injection (Naked DNA)

Following direct injection *in vivo* of "naked" (i.e., without viral or synthetic vector) plasmid DNA, cells can spontaneously take the foreign DNA and permit expression of the enclosed transgene[44]. At present, four out of the eight hepatocellular carcinoma patients injected with the wild-type p53 expression plasmid have demonstrated significant reductions in both the levels of a fetoprotein, a serum marker for malignancy, and in tumour volume (more than 50%). The naked DNA injection do not directly cause mortality or morbidity, but the complications like transient fever, hypotension and hypertension can be observed.

8.2.2 Cationic Lipid-Mediated Gene Transfer (Liposomes)

An important non-viral delivery approach is the utilisation of cationic liposomes, complexed with plasmid DNA carrying the transgene of interest, which can be efficiently endocytosed by mammalian cells[12]. The main advantages of lipid-mediated gene transfer are that the process is relatively non-toxic and non-immunogenic. Hence, liposomes may permit multiple administrations of the wild-type p53 gene, which is necessary for the successful treatment of metastatic tumours. In a similar fashion to adenoviruses, liposomes are transient expression vectors.

A number of pre-clinical studies have indicated that this means of gene delivery can be highly effective in terms of tumour suppression. Direct injection of liposome-p53 complexes into breast tumours grown as implants within nude mice caused not only regression of the primary tumour but also prevention of both tumour relapse and metastasis[23]. No evidence of toxicity within the mouse was seen with dosages of liposome-p53 complex that was sufficient to markedly inhibit growth of the breast tumours. The addition of transferrin to liposome-p53 complexes dramatically increase the transfection efficiency of this vector system and may provide some degree of selective targeting, as transferrin receptors have been found to be elevated in various types of cancer cells. Transferrin-liposome-mediated transfer of the wild-type p53 gene into a radio resistant squamous cell carcinoma cell line results in sensitisation of these cells to ionising radiation treatment[47]. Advantage with liposome-based p53 delivery is the ease of regional administration through the airways to sites within the lung bearing cancerous lesions.

9. Reactivators of p53 Function

Various strategies have been proposed to restore p53 function in cancer cells. Several compounds with capability to restore p53 function have been identified by random screening and by drug design. Although such chemical compounds differ in structure and in the pattern of susceptible mutants, they form new group of potential drugs - reactivators of p53. Various reactivators of p53 are-

9.1 CP-31398

CP-31398 is the first small molecule identified with the ability to restore the wild-type conformation to mutant p53[43]. Cp-31398 is a styrylquinazoline, a small molecule compound which is able to interfere with mutant p53 and restore wild type function[37]. Cp-31398 induce mitochondrial translocation of p53, leading to changes in mitochondrial membrane permeability pore transition (MPT) and consequent cytochrome c release leading to apoptosis in cells with mutant p53. CP-31398 cause stabilization of newly synthesized p53 *via* interaction with p53 tetramers, results in inhibition of ubiquitination of p53 and hence it's degradative pathway.

9.2 PRIMA-1

PRIMA-1 represents a model compound from reactivators of p53 group. PRIMA-1 induces apoptosis in tumour cells with different p53 mutations. It activate transcription of several p53 target genes, including p21, Mdm2 and puma only in cells with mutant p53 and shown antitumor activity in mouse models, with no apparent toxicity[6]. PRIMA-1 does not exert any effect on wild-type p53. Whether PRIMA-1 is able or not to induce apoptosis in the cells without p53 is not clear. Therefore detailed studies are necessary before further application of PRIMA-1.

9.3 CDB3

CDB3 is a nine-amino-acid peptide designed rationally by analysis of the interaction and stabilization of p53 by p53-binding protein 2 (53BP2)[18]. It interacts with the DNA-binding domain and corrects some misfolded mutants. This has been shown to induce transcription of several target genes like Mdm2, GADD45 and p21 accompanied by p53-dependent restoration of apoptosis. CDB3 did not activate apoptosis in cells with wild-type p53. Although, CDB3 did not elicit a full biological response, it bind to and rescue p53 in cells and so can serve as a lead for the development of novel drugs for anticancer therapy.

9.4 PAb421 or Synthetic Basic Peptides

Reactivation of the transcriptional activation function of mutant p53 may also be accomplished through exposure to certain anti-p53 monoclonal antibodies such as PAb421 or synthetic basic peptides derived from the C-terminal domain of p53. Such agents are believed to be act by alleviating the constitutive activation by the C-terminus on sequence-specific DNA binding activity[33]. Mutant p53 can be activated by this mechanism.

9.5 CTS-1

Another approach to reactivate cells with mutated p53 employs a chimeric adaptor protein. Such a construct consists of p73 DNA-binding and tetramerization domains fused to p53 oligomerisation domain and alone do not exert transcriptional activity because it lacks a transactivation domain. CTS-1 has several interesting properties such as enhanced transcriptional activity, resistance to inactivation by either Mdm2 overexpression or oncogenic p53 mutants, reduced sensitivity to E6-induced degradation and enhanced apoptotic activity[10].

In the presence of mutant p53, CTS-1 serves as a link between mutant p53 and target sequences in DNA and activates transcription of target genes. Delivery of CTS-1 with adenoviral vectors into cells with mutant form of p53 cause activation of p53 target genes and apoptosis (Roth *et al.*, 1997).

10. Inhibitors of the p53-Mdm2 Interaction

More recently, the design and development of small-molecular inhibitors of the p53-Mdm2 interaction has emerged as a promising and novel cancer therapeutic strategy. Various inhibitors of p53-Mdm2 interaction are-

10.1 Nutlin

The *cis*-imidazoline analogs, named nutlins, bind to the p53-binding site on the Mdm2 molecule, displace p53 from its complexes with Mdm2 and prevent Mdm2 from targeting p53 to degradation (Shangary and Wang, 2009). This leads to accumulation of p53 and activation of p53 target genes. Antitumor effects of nutlins were observed in cells with wild-type p53, but not in cells expressing mutant p53 or without p53. Induction of wild type p53 may also serve to protect normal cells from the detrimental effects of chemotherapy or radiotherapy.

10.2 MI-319 and MI-219

MI-319 restores p53 functions and increases the life span of orally treated follicular lymphoma bearing animals (Mohammad *et al.,* 2009). MI-319 is designed to stabilize p53 protein in cells by blocking the p53-Mdm2 interaction. Although many genes in addition to p53 are usually altered in tumours, recent studies suggest that restoring p53 function alone is sufficient to cause regression of established sarcomas, lymphomas and liver tumours in mice. MI-319 is a very close analogue of MI-219.

Nutlin and MI-219 are already in preclinical development or early phase clinical trials. Both display a high binding affinity and specificity to Mdm-2, a strong activity in cancer cells with wild-type p53, and a highly desirable pharmacokinetic profile[32].

A growing number of recent reports suggest that small molecule inhibitors targeting the p53-Mdm2 interaction may represent very promising, specific and novel therapeutics against various types of cancers. Using small molecules to block the p53-Mdm2 interaction is an attractive approach to stabilize functional p53 protein and restore its anti-tumour activity in tumours with wild-

type p53 gene. Unlike in many solid tumours, alterations of the p53 gene are far less common in haematological malignancies. Therefore, small-molecule inhibitors that interrupt the p53-Mdm2 interaction might represent a new therapeutic strategy for the treatment of most patients with this kind of disease.

10.3 Super TIP

A synthetic Mdm2 binding mini protein has been recently designed which specifically targets the p53-binding site[4]. An optimised Mdm2-binding peptide, was cloned into the active site loop of the thioredoxin protein, the resulting fusion being called super TIP (thioredoxin insert protein). Thioredoxin was chosen as the protein scaffold due to its small size, ease of expression in both eukaryotic and prokaryotic cells, stability and inert quality. The potential of super TIP to displace Mdm2 from an inhibitory complex with p53 was shown to be around fifty times greater than a TIP containing the natural Mdm2-binding site within p53.

Displacement of Mdm2 from p53 by super TIP activates p53-dependent transcription, lead to nuclear accumulation of p53 and stimulate cell cycle arrest. Release of active p53 by inhibitors like super TIP is in a reciprocal relationship with the Mdm2 content of the cell. Accordingly, cells with amplified Mdm2 levels are less responsive than cells with normal expression of Mdm2.

11. Ribozyme Strategy

Expression of p53 may be targeted in a number of ways and one of the important way is by ribozyme. An anti-p53 ribozyme, which is designed to cleave p53 pre-mRNA, efficiently reduce the level of mutant p53 mRNA[17]. The ribozyme recognize the substrate RNA by base pairing to it through the internal guide sequence (IGS) of the ribozyme. Target RNAs can be recognized by the ribozyme at any accessible uridine residue found upstream of the mutation in the target transcript that is to be corrected. The ribozyme then cleaves the target RNA, releases the downstream RNA sequence containing the mutation, and replaces the sequence with a 3′ exon that encodes the correct sequence for the wild-type p53 transcript[34].

12. Selective Chemotherapy

Most of the currently used chemotherapeutic agents like, DNA cross linkers, anti-metabolites and topoisomerase inhibitors are generally less effective in cells that contain a defective p53 pathway[29]. Therefore the main aim of selective chemotherapy is to use such anti-tumour agents which work independently of the p53 pathway and thereby act preferentially in p53 defective cells. Anti-mitotic anticancer chemotherapeutic agent like taxol and vincristine, work independently of p53 status of the cells and can be used selectively for the treatment of cancer.

13. Immunotherapy

p53-based immunotherapy include triggering of a cytotoxic T-lymphocyte (CTL) response against tumour cells expressing mutant p53[9]. Mutant p53-specific epitopes presented on the cell surface could serve as selective targets. CTLs demonstrate the ability to differentiate between mutant and wild-type p53 proteins. There appears to be an immunoselection process against those tumours which express immunopositive mutant p53-specific epitopes. Alternatively, since overexpression of mutant p53 is a frequently occurring phenomenon in tumour cells, so by triggering cytotoxic T-lymphocyte response causes ablation of tumour. The advantage of triggering a CTL response against mutated p53-specific epitopes is clear when one considers the vast number of possible missense mutation sites within p53. Mutated p53-based immunotherapy has shown some efficacy with regard to tumour eradication in mouse models[3].

14. Attenuated Adenovirus Therapy

Genetically modified adenovirus ONYX-015 has been engineered. ONYX-015 does not express E1B and is not able to interfere with wild-type p53. Such a virus induces wild-type p53 response in normal cells, which halts viral replication and allows the cell to survive. On the other hand, it replicates freely in cells expressing mutant p53, causing cell death. The attenuated adenovirus is able to replicate in, and ultimately lyses cells with dysfunctional p53 while retaining an inability to progress within normal cells. The most attractive feature of this approach is the potential for virus spread which may permit improvement upon the low efficiency of gene transfer in gene therapy for cancer[30].

15. Conclusion

Tremendous progress has been made in understanding the various functions of p53. The important role that p53 plays in response to stresses such as DNA damage, clearly indicates a central biological pathway within the cell, while all of the upstream and downstream components of the p53 pathway have yet to be deciphered and wired together. A large number of investigators have tried to exploit known aspects of p53 in the design of p53-based cancer therapies. The strategies which have been developed differ greatly between themselves both in the mode of action and the degree of success seen in experimental models. Rapid progress is expected to be made regarding the identification of conventional pharmaceutical agents which either work in a p53-independent manner or act preferentially in p53 defective cells. Gene replacement therapy with wild-type p53 also holds considerable potential for obtaining clinically relevant results quickly. Cancer therapy based around p53 proves to be more efficacious in the long run, especially in terms of specificity. As with many other fields, the innovation of successful p53-based cancer therapies is only limited by our understanding of p53 biology and the creative use of such knowledge. In future p53-based cancer therapy can be a valuable approach for the treatment of a variety of tumours in human as well as in animals.

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Nomenclature

p53 is also known as:

- Cellular tumour antigen p53
- Antigen NY-CO-13
- Phosphoprotein p53
- Transformation-related protein 53 (TRP53)
- Tumour suppressor p53

LOCATION OF *TP53* GENE

For these mammals, the *TP53* gene is located on different chromosomes:

Chimp and Orang-Utan : Chromosome 17
Cow : Chromosome 19
Dog : Chromosome 5
Horse : Chromosome 11
Human : Chromosome 17
Macaque : Chromosome 16
Mouse : Chromosome 11
Opossum : Chromosome 2
Pig : Chromosome 12
Rat : Chromosome 10