ejpmr, 2017,4(2), 740-751

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

P53 REACTIVATING PEPTIDES AS A NOVEL FINDING FOR TREATMENT OF CANCER

***Manju Kumari[1] and Ramandeep Kaur[2]**

[1]Department of Pharmaceutical Chemistry, Himachal institute of Pharmaceutical Education and Research, Bela, Nadaun.

[2]Adesh Institute of Pharmacy and Biomedical Seciences, Adesh University, Bathinda.

Corresponding Author: Manju Kumari

Department of pharmaceutical chemistry, Himachal institute of pharmaceutical education and research,Bela, Nadaun.

Article Received on 21/12/2016 **Article Revised on 11/01/2017** **Article Accepted on 31/01/2017**

ABSTRACT

The p53 is a transcription factor is also known as tumour suppressor gene TP53, involved in regulating the cell responses to DNA damage to support genomic stability. p53 was first identified in 1979 as a cellular protein that bound to the simian virus (SV40) large antigen and a 53-kilodalton phosphoprotein, the product of a 20-kilobase gene on the short arm of human chromosome 17. p53 is usually activated by disruption of its interaction with Mdm2 and it has been shown that cocompartmentalization of both proteins is essential for p53 degradation.

INTRODUCTION

The p53 is a transcription factor is also known as tumour suppressor gene TP53, involved in regulating the cell responses to DNA damage to support genomic stability.[1] p53 was first identified in 1979 as a cellular protein that bound to the simian virus (SV40) large antigen.[2] and a 53-kilodalton phosphoprotein, the product of a 20-kilobase gene on the short arm of human chromosome 17.[3] The tumour suppressor protein is a central factor in the prevention of cancer in human. It has been famously called "the guardian of the genome" due to its ability to respond to genotoxic stress, such as DNA damage and other stress signals, and to protect the genome by inducing a variety of biological responses including DNA repair, cell cycle arrest, and apoptosis.[4]. The cell cycle arrest reconciled by p53 depends on its ability to act as a sequence-specific DNA-binding transcription factor.[5].p53 is a tumour suppressor i.e. maintained at low levels in a normal cells, but rapidly accumulates in the nucleus in response to stress, such as DNA damage, hyper proliferation, chemotherapeutic agents, ultraviolet light, and hypoxia. The half life of p53 is 6-20 min in healthy cells. However, the concentratiorn of p53 is increased three to four fold, and the half-life is improved to hours in response to stress.[6]. p53 instability is primarily controlled by its negative regulator MDM2.[7] and binds to p53 and prevent p53 functioning effectively as a transcriptional activator and target it for proteasomal degradation.[8] p53 is usually activated by disruption of its interaction with Mdm2 and it has been shown that cocompartmentalization of both proteins is essential for p53 degradation.[9]. p53 is activated as a transcription factor in response to oncogene activation, hypoxia, nitric oxide, mitotic spindle damage, ribonucleotide depletion and specially DNA, damage resulting in growth arrest or apoptosis or by repressing the expression of antiapoptotic proteins.[10]. p53 regulated apoptosis by interactions between BCL2 family proteins, including the antiapoptotic proteins:-

1) BCL2,
2) BCL2- related myeloid cell leukemia sequence 1(MCL2)
3) BCL2- associated X protein (BAX)
4) BCL-2 antagonist/killer1 (BAK1).

By protein like BH3, PMAIP1, BCL2L11, play key roles in coupling the specific death stimuli to the core apoptotic machinery.

The displaced BCL2L11 then interacts with BAX or BAK1, resulting in change in their conformation and insertion into the outer mitochondrial membrane. The subsequent release of cytochrome c, somatic (CYCS) from the mitochondria activates the CASPs, which are required for the initiation of apoptosis). It has been shown that TP53 translocates to the mitochondria within 1 h after γ-irradiation in various cancer cell lines. This translocation promotes changes in the mitochondrial membrane potential and subsequent release of CYCS.[11]

The activity of p53 is regulated primarily through post-translational mechanism, including stabilization of the protein by phosphorylation, increased nuclear localization, and changes in conformation leading to enhanced DNA binding.

Some of the genes induced by p53 include:-

a) $p21^{waf7}$, which contributes to growth arrest;
b) *Bax*, which induces apoptosis;
c) GADD45, which functions in DNA repair;[12] and
d) Cyclin G, which modulate apotosis.[13].

Mutations in p53 have been implicated in many cancer types. It is inactive in normal, unstressed cells, but becomes active when DNA is damaged.[14] In most tumour mutations, missense base substitutions occur in the p53 coding sequence that change a single amino acid in the core domain, which governs conformation and specific interactions with DNA. Mutations are occurred at points where the proteins is in close proximity to DNA or makes direct contact when the tetramer combines to its recognition structure.[15] Mutations within introns affect gene expression w13x. Mutations in the p53 gene have been recorded to increase resistance to ionizing radiation in transgenic mouse cells[3]. Inactivation of p53 through mutation is generally found in a wide range of occurring at irregular interval cancers.[16] The regularity of p53 gene mutations is high in cancers of the colon, breast, lung, overy, brain. As well as in leukemias and osteosarcomas. The most common abnormalities obtained are missense point mutations that are collection between exons 5-8. Insertions occurred at 1-14 nucleotides in length and these duplicate the sequences of the neighbouring region. Deletions were observed more generally and occurred from 1-37 nucleotides.[17]

An essential mediator of cellular mediator to oncogene is mutation of the tumour suppressor p53, is the most frequent genetic alteration in human cancer. About 30% of the mutations that inactivate p53 simply lower the melting temperature (Tm) of DNA binding domain of p53, so that it denatures fastly in cells. Tm of a mutant, and its activity, can be reserved by molecules that attach to its native structure and not its denatured states. Temperature sensitive mutants of p53 are transcriptionally active at low temperature(< 37˚ C) demonstrated that the activity of the such mutant can be rescued in a biological context.

The principle of p53 reactivation by small molecules was provided using a small peptides, CDB3, which binds reversibly to the DNA- binding domain of p53. The peptide was shown to stabilize wild-type and mutant p53 in vitro and elevated the activity of mutant p53 in cancer cell lines.A group of molecules like PRIMA-1 and MIRA-1 or their respective hydrolysis products may react covalently with p53 via modification of cysteine residues, suggesting a possible mechanism for targeting p53 in cells. It is also possible that molecule bind to cysteine residues in p53 may also regulate p53 activity through modification of the proteins redox state. Given the ramifications toward the development of novel anticancer drugs, there is a fundamental need to discover small molecules that directly stabilize temperature sensitive p53 mutants.[18]

The use of peptides in cancer therapeutics has recently famous because of their potency, specificity, low toxicity and limitations of viral vector gene therapy approaches. One necessary requirement of these peptide-therapy is the ability of these molecules to be efficiently cross the cancer cell membranes.[19] In general, cellular plasma membranes are largely impermeable to proteins and peptides. But certain short peptie sequence, composed mostly of basic, positively charged aminoacids (eg Arg, Lys and His), have the capability not only to transport themselves across cell membranes, but also to carry binded molecules (proteins, DNA, or even large metallic beads) into cells . These basic sequences, mostly derived from DNA binding proteins are now commonly known as protein transduction domains(PTD) and have been successfully employed to transport cargo proteins.[20] across a varity of cell membrane. The principle of p53 reactivation by small molecules was first explained using a small peptides, CDB3, which binds reversibly to the DNA- binding domain of p53.[21]

Structure of p53 protein

The p53 gene encodes for p53 protein of 393 amino acids with a molecular weight of 53 kDa.[22] p53 protein form a homotetramer, or dimmer of dimmers with each subunit containing

1) N- terminal transactivation domain,
2) DNA – binding core domain (p53 DBD) i.e. the site of the vast majority of tumor derived substitution mutations,
3) Tetramerization domain structured as a dimer of dimmers and
4) C-terminal regulatory domain.[23]

N-terminal domain responsible for transactivation (TAD) domain (residue 1-40) possesses a highly conserved region (15-29) called Box1. This region is mostly unstructured in a solution, however, residues 19-25 form an alpha helix when bound to the hydrophobic pocket of the p53 regulatiry protein MdM2. p53 contains a number of conserved serine, threonine and lysine residues which are the accepted sites for phosphokinase involved in the regulatory pathways.[24]. The central or core domain of p53, which is comprised of residue 94-312, is responsible for specific DNA interactions, but it has also been describrd to be involved in nonspecific DNA binding.[25] The p53 TAD can be divided into two subdomains, TAD1 (residues 1–40) and TAD2 (residues 40–83), and both subdomains display independent transcriptional activation functions. TAD1 specifically recognizes MDM2, the TATA binding protein (TBP), and CBP/p300, while TAD2 interact with the replication protein A (RPA70), among others (26). ArhGAP11A as an important neurite inducer that physically binds with p53 in embryonic oligodendrocytes, and RhoGAP domain binds to the p53 tetramerization domain (TD). The tetrameric conformation of the p53 TD is crucial for the interaction with RhoGAP, and multiple hydrogen bonds are involved in the RhoGAP-p53 TD interaction. Upon DNA damage stress, ArhGAP11A is upregulated and translocated to the nucleus, where it binds to p53 and induces cell-cycle arrest and apoptosis.[26]

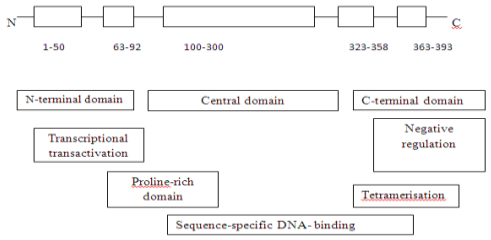

Figure1. p53 protein structure.[27]

In terms of three-dimensional structure, the DNA-binding domain is made of a frame of beta-sheets that supports flexible loops and helixes, which are in direct contact with DNA. The most common mutations that occur in cancer alter this structure either by abrogating protein–DNA contacts or by disrupting protein folding.[28]

FUNCTIONS OF p53

1) Human malignancies can be caused by the inactivation of tumor suppressor p53. Restoration of p53 function causes death of tumor cells and is potentially suitable for gene therapy of cancer.[29]

2) Its unique G1 cell cycle arresting mechanism that is maintained by p21WAF1, there are signals transduced by p53 to multiple apoptotic effectors perhaps due to the importance of apoptosis in suppressing tumors.[30]

3) p53 is the central component of the system that prevents the generation of genetically altered cells in the body.[31]

4) p53 is used in the management of esophageal adenocarcinoma which is useful in surveillance and multiple non targeted biopsies.[32]

5) p53 prevents cancer by Cell cycle arrest in G1, allowing time for the repair of DNA damage, thereby eliminating cells with damaged genomes.[33]

6) The cyclin dependent kinase inhibitor p21Waf1/Cip1 is a direct p53 target and deletion of this gene significantly reduces the cell cycle arrest response to p53.[34]

7) p53 can bind and inactivate proliferating cell nuclear antigen (PCNA), which plays an essential role in DNA replication. It also activates RRM2B/P53R2(p53-inducible ribonucleotide reductase) and DDB2 (damage – specific DNA binding protein 2) genes that have distinct roles in DNA repair. Individuals who inherit only one functional copy of p53 gene have the possibility of developing Li Fraumeni's syndrome.[35]

8) Tax-mediated p53 inactivation for ATL (adult T-cell leukemia) genesis process in which Tax can immortalize the virus-harboring T-cells of the HTLV-1-infected individuals and destabilize their genome, so that these cells may progress towards the ultimate leukemic state by a stepwise accumulation of oncogenic mutations.[36]

Classification of p53 reactivating peptides

1) p53-MDM2 interaction inhibitors: The MDM2 (murine double minute 2) protein (also known in humans as HDM2) was first identified as the product of a gene developed over 50-fold on acentromeric extrachromosomal bodies (called "double minutes") found in a 3T3DM spontaneously transformed mouse cell line and able to interact to the transactivation domain of p53 through a "p53-interacting domain" on the MDM2 N-terminus.[37] and stimulates transport of p53 from nucleus to cytoplasm.[38] The direct cooperation between the two proteins has been bounded to a relatively small (amino acids 25–109) hydrophobic pocket domain at the NH_2 terminus of MDM2 and a 15-amino acid amphipathic peptide at the NH_2 terminus of p53.[39]. (Site-directed mutagenesis has shown the importance of p53 residues Leu14, Phe19, Leu22, Trp23, and Leu26, of which Phe19, Trp23, and Leu26 are the most important.[39,40]

The mutations of MdM2 at residues Gly58, Glu68, Val75, or Cys77 result in lack of p53 binding [41] The interacting domains show a tight key-lock configuration of the p53-MDM2 interface. The hydrophobic side of the amphipathic p53 a-helix, which is formed by amino acid 19–26 (with Phe19, Trp23, and Leu26 making contact), fits deeply into the hydrophobic cleft of MDM2. Thr18 is very important for the stability of the p53 a-helix.[42]. The activation of the p53 function by the inhibition of the protein-protein interaction of p53-MDM2 is regarded as an effective approach in cancer therapy[43]. MDM2, negatively regulates p53 function by a variety of mechanisms, including-

a) Ubiquitin independent proteosomal associated degradation of p53.
b) Ubiquitylation.
c) phosphorylation
d) transportation of p53 from nucleolus to cytoplasm
e) p53 regulations by metal ions.

a) Ubiquitin independent proteosomal associated degradation of p53: p53 is the most generally mutated tumor suppressor gene in various types of cancers. DNA damage produces p53 accumulation in an Ataxia Telangiectsia Mutated (ATM)-dependent manner. p53 interacts with mouse double minute2 (MDM2) protein and undergoes ubiquitination and 26S proteasomal degradation. ATM controls p53 stability by phosphorylation of of MdM2 and E3 ligase processivity. Not only 26S proteasomal degradation of p53, 20S proteasomal degradation pathway was also reported. NQO1 and NQO2 interact with p53 and protect p53 against 20S proteasomal degradation. NQO1 knock-out mouse showed reduced p53 induction and increased susceptibility to chemically-induced tumors.[44] NADH quinone oxidoreductase 1 (NQO1). NQO1, or DT-diaphorase, is a flavin-containing quinine reductase with a large substrate specificity. NQO1 activates the reduction in various quinones through a two-electron reduction mechanism using either NADH or NADPH as a reducing cofactor, and it is inhibited by the competitive inhibitor dicoumarol.[45]

The proteasome is a large, multi-catalytic protease that degrades proteins to small peptides. The 26S proteasome

is made up of a core 20S catalytic chamber, capped at both ends with 19S regulatory units. The 19S regulatory particles are important for recognizing poly ubiquitinated proteins, unfolding them, and opening an orifice into the 20S core catalytic chamber.[46] Free 20S core particles establish a major portion of the total amount of proteasomes and are present both in the nucleus and cytoplasm of the cell.[47] NQO1 co-fractionates with the 20S core particle but not the 26S proteasome.[48] NQO1 inhibits p53 degradation by defect, this provided the information that the 20S but not the 26S proteasome regulates this process. The 20S core of the 26S proteasome digests uncoiled protein substrates, and the uncoiling step is finished by the 19S regulatory particle in a process that is ATP dependent.[49]] By reversing chaperon activity (a guide or companion whose purpose is to ensure propriety or restrict activity) of the 19S that is important in denaturing the substrate to fit the 20S chamber, it was initially rather baffling that the ubiquitinin dependent process does not require the 19S particle. A number of in vitro studies showed that certain proteins that are naturally unfolded, such as proteins that are fully or regionally intrinsically unstructured, undergo degradation by the 20S proteasome. Recently, it was suggested that as much as 20% of all cellular proteins can be degraded or cleaved by the 20S proteasome specifically at unstructured domains.[50].The susceptibility to the 20S proteasome may be used as an operational definition approach to determine whether a given protein is unstructured.[51] Consistently, p53 that is unstructured at both N- and C-termini28.[52] undergoes 20S proteasomal degradation in vitro. NQO1 associates with the 20S proteasome, and it prevents the degradation of proteins with unstructured regions, such as p53, p73,[53] NQO1 plays the role of 'gatekeeper' of the 20S proteasome . NADH regulates the organisation of NQO1 with the potential 20S proteasome substrates, but does not control NQO1 organisation with the 20S proteasome. At high levels of NADH the substrates are protected and do not enter the 20S catalytic chamber. At low levels of NADH the substrates are not effectively protected and are degraded by the proteasome. This model explains how certain small drugs that compete with NADH, such as dicoumarol, sensitize p53 to degradation. Interestingly, a similar molecular mechanism was recently described in yeast in the context of the transcription factor Yap4 protein.[54] Lot6 is the NQO1 ortholog in yeast31 and binds to the 20S proteasome. It was recommended that like p53 in mammalian cells Yap4 in yeast becomes associated with the Lot6–proteasome complex in the presence of NADH. NADH is needed because Lot6 must be reduced to bind Yap4. Remarkably, the binding of Yap4 to the Lot6–proteasome complex protects it from the ubiquitin-independent proteasomal degradation.[55]

b) Ubiquitylation: Ubiquitin is an abundant and essential cellular 9-kd protein.[56]. Ubiquitin is composed of 76 amino acid residues and its primary arrangement is highly conserved from yeast to mammals, thus resulting in essentially identical tertiary structure.[57] Ubiquitination is the covalent connection of molecules of the small 76 amino-acids protein ubiquitin to a target protein which is then marked for proteasome destruction or endocytosis or participation in a range of processes. Ubiquitination along with other post-translational modifications of proteins such as phosphorylation, hydroxylation and acetylation is a regulated process for the execution of which a multitude of regulators exist.[58]

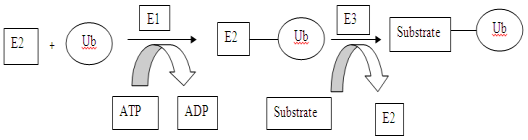

Figure 2. The ubiquitination cascade. E1 denotes ubiquitin-activating enzyme, E2: ubiquitin conjugating enzyme and E3 ubiquitin ligase. Ub: ubiquitin.

Ubiquitination requires binding of the target protein to the appropriate E3 ubiquitin ligase. E3 ubiquitin ligases are recognised by the N-end rule, based on the finding that the in vivo half-life of a protein is related to the properties of its amino-terminal residue. Short-lived proteins commonly have basic or bulky hydrophobic residues at their N-terminus, and more stable proteins have one of the amino acids of cysteine, alanine, serine, threonine, glycine, valine, or methionine at the N terminus.[59] The E3 ubiquitin ligase E3/Ubr1 is engaged for targeting N-end rule substrates. However, most proteins are targeted for ubiquitination by more complex mechanisms than acceptance of an N-terminal amino acid. For example, post-translational modifications, such as phosphorylation, are common signals for ubiquitination. A number of important transcription factors are affected by phosphorylation-dependent ubiquitination. Nuclear factor-kappa B is activated after its inhibitory chaperone IB is phosphorylated and consequently ubiquitinated.[60] Catenin, which is regulated by ubiquitination and targeted for ubiquitin ligation by phosphorylation at N-terminal serine residues is part of the T-cell factor/lymphoid enhancer factor (TCF/LEF) heterodimeric transcription complex,[61]. Some short-lived proteins contain a PEST sequence, which is a phosphorylation site improved in the four amino acids of proline, glutamic acid, serine, and threonine that regulates ubiquitination.[62] Ubiquitination can also be regulated by activation of some E3 ligases, which themselves may be synthesized as inactive enzymes and undergo post-translational modification as the activation step. The anaphase-promoting complex/ cyclosome E3 ligase is phosphorylated late in mitosis to initiate degradation of cyclin B and progression of the cell cycle.[63, 64, 65, 66]. Cyclins are cell cycle–regulatory proteins that are rapidly activated and degraded to control progression through the different phases of the cell cycle.[67, 68]. These critical cell cycle control processes are susceptible to interference early during viral infection. Because of the critical role that ubiquitin ligation has in regulating the cell cycle, viruses have

evolved mechanisms to sustain cell division after infection and thus assure viral replication.

c) Phosphorylation: Almost 20% of the amino acids on the MDM2 protein are either serine or threonine residues, and the MDM2 protein is phosphorylated. Two arrays of phosphorylation sites are located at the NH_2 terminal (amino acids 1–193) and central (amino acids 194–293) domains of murine MDM2, respectively. Mapping of these arrays fits well with more recent studies (carried out for the most part with human MdM2) which have identified a number of sites that are phosphorylated in a cellular context, including (with the modifying enzymes, where known, given in parentheses) ser166 (Akt), ser186 (Akt), thr219 (cyclin A-CDK1/2), ser229, ser232, ser240, ser242, ser246, ser253, ser256, ser260, ser262, ser269 (CK2), tyr294 (c-Abl), and ser295 (ATM [''ataxia telangiecta siamutated'' protein kinase]). In addition to these well-characterized modifications, two other phosphorylation sites in this region, threonine 168 and serine 189 (murineMdm2).[69]

Phosphorylation happens on multiple sites in MdM2, and the phosphorylated residues array into 2 functional domains:
(1) N-terminal domain that interacts with p53 and inhibits p53 transactivity and
(2) highly disordered acidic domain in the central part of MDM2 that serves as the docking site for many binding partners.[70]

Two protein kinases have been involved directly in p53 modifications induced by ionizing radiation (IR) and radiomimetic chemicals: ATM, required for the initial phase of p53 aggregation in response to this damage, and ATR, involved in the later phase of this process. In response to IR and radiomimetic treatment of cells, ATM is activated and mediates rapid phosphorylation of p53 on Ser15, whereas ATR seems to be involved in the subsequent maintenance of this phosphorylation. Importantly, both of these kinases phosphorylate p53 in vitro on Ser15. A third enzyme involved in cellular responses to DNA damage, the DNA-dependent protein kinase (DNA-PK), is capable of phosphorylating p53 in vitro on Ser15 and Ser37 , but is probably not necessary for damage-induced p53 activation and accumulation.
ATM, ATR, and DNA-PK belong to a family of protein kinases with carboxyl-terminal domains showing similarity to phosphoinositide 3 kinases. Members of this family are involved in controlling genome stability, cell cycle progression, and responses to DNA damage in various organisms. Lack of ATM in humans causes the genetic disorder ataxia-telangiectasia (A-T), characterized by neurodegeneration, immunodeficiency, genome instability, cancer predisposition, sensitivity to IR, and defective activation of cell cycle checkpoints by DNA damage . DNA-PK deficiency in mice leads to severe combined immunodeficiency (scid), which shares many features with A-T.[71]

d) Transportation of p53 from nucleolus to cytoplasm: p53 is a very unstable protein that is typically nuclear and present in very amount. p53 transports between nucleus and cytoplasm during the cell cycle.[72, 73, 74, 75] and its nuclear entry and exit are mediated by specific import and export machinery as it exceeds the 40-50kDa limit for passive nuclear transport[76] p53 accomodates nuclear localization and nuclear export signals, and its subcellular localization reflects a balance between the rates of import and export.[77,78,79,80,81] p53 inactivates in the cytoplasm by some tumors[82, 83, 84]. p53 has two nuclear export signals (NES), a C-terminal one within the tetramerization domain.[79] and a second that overlaps the N-terminal transactivation domain.[81] Because treatment of cells with leptomycin B (LMB), which inhibits the nuclear export receptor CRM1.[85, 86] results in p53 nuclear localization, either or both are potential CRM1 targets.[79]. The C-terminal NES has the potential to link p53 structure with subcellular localization and nuclear functions. The crystal structure of the tetramerization domain indicates that the NES it contains should be covered in the tetramer, but exposed in monomers or dimers. The priority of the C-terminal NES for controlling p53 subcellular localization is indicated by the p53 nuclear restriction caused by C-terminal NES mutations.[79]The positioning of an NES in the tetramerization domain allows for factors that affect p53 tetramerization and dissociation to be linked to subcellular localization and binding of p53 to its response elements. For example, phosphorylation of serine 392 (human p53) in the C-terminus might stabilize p53 tetramers, while phosphorylation of serines 315 and 392 might destabilize tetramers.[86,87,88] and MDM2-mediated ubiquitination of p53 may expose the p53 C-terminal NES to enable p53 export to the cytoplasm.[89, 90,91]

p53 nuclear export can be induce by N-terminal NES in unstressed cells, but is inactivated by DNA damage to allow for rapid nuclear accumulation.[79] However, this supposed NES lies within the transactivation domain, and overlaps the sequences known to bind MDM2,[92] While DNA damage induced N-terminal phosphorylations inactivate the N-terminal NES[79] these modifications occur in regions that could affect MDM2-p53 combination and [93,94,95]. Also, DNA damage also induces modifications on MDM2.[96] that can reduce MDM2 stability [97] which also impedes MDM2-p53 interaction. Furthermore, mutations in the N-terminal NES limit interaction with the export receptor were made in residues that prevent association with MdM2[98,99].Consequently, these mutations stabilize p53, leading to its tetramerization, and constitutive nuclear localization.

e) p53 regulations by metal ions: Chromium[Cr(VI)] compounds are used widely in industry and are found in the environment.cancers of respiratory system.[100] Exposure to Cr(VI)- containing compounds is known to

induce lung toxicity and increased chances of cancers of respiratory system.[101, 102, 103,104] The activation of p53 is at the protein level instead of the transcriptional level. The degradation of p53 was dramatically decreased upon stimulation by Cr(VI). In addition, Cr(VI) treatment decreased the interaction of p53 with mdm2 proto-oncoprotein, which blocks the transactivation ability of p53 and promotes the degradation of p53 protein, In response to Cr(VI) treatment, p53 protein became phosphorylated and acetylated at Ser15 and Lys 382, respectively. The phosphor -rylation levels at either Ser20 or Ser392 did not show any significant alterations. Ser15 instead of Ser20 may play a kay role in the dissociation of mdm2 in response to Cr(VI).Erk, a member of mitogen-activated protein kinase for the phosphorylation of the p53 Ser15 site.[105].MDM2 to interact, even though more weakly, with the C-terminal and core domains of p53. The disruption of any of these regulatory functions by MDM2 is a viable strategy to reactivate p53, especially through inhibition of the p53/MDM2 binding interaction. The p53/ MDM2 interaction is largely hydrophobic, and the binding interface of the two proteins is also quite small, making small, peptidic or non-peptidic molecular mimics of the p53 binding site good candidates for inhibitors of p53/MDM2 interaction. This hydrophobic binding domain has been efforted to rationally design synthetic p53/MDM2 inhibitors with excellent affinity. Three major classes of synthetic p53/MDM2 inhibitors, the nutlins, spirooxindoles, and benzodiazepinediones, have taken advantage of the Phe19-Trp23-Leu26 binding core to displace p53 and bind MDM2 with much greater affinity and in vitro potency. Approximately half of all amino acids utilized in cyanobacterial natural products are modified with the most frequent modifications including N-methylation, N'-N'-dimethylation, ketide extension, and halogenation to a lesser extent. Many features of marine cyanobacterial compounds, including their molecular weight distribution (median 604 Da), lipophilicity, and chemical diversity, lend themselves to favorable bioactivity and medicinal chemistry profiles, particularly as it pertains to p53/MDM2 inhibition.

Hoiamide D, a peptide- derived p53/MDM2 inhibitor, isolated in both its acid and carboxylate forms, from two separate collections of the Papua New Guinea cyanobacterium Symploca species.[106]

An in vitro screen for agents that blocked doxorubicininduced, p53-dependent, lacZ-encoded h-Gal expression led to the identification of a small-molecule inhibitor of p53, is pifithrin-α[PFT-a,2-(2-imino-4,5,6,7-tetrahydrobenzothiazol-3-yl)-1-(4-methylphenyl)ethanone]. This compound inhibited a number of p53- dependent processes including UV-induced, p53- dependent h-gal expression, UV-induced cyclin G, p21, and MDM-2 protein expression.[107]

The side chains of hydrophobic residues F19', W23' and L26' are responsible for the interaction of p53 with MDM2. The binding of p53 to MDM2 is directly disrupted by these residues and it may be an attractive pathway of targeted anticancer therapy. Many drug candidates, such as small-molecule inhibitors, peptides, and peptide-analogue are designed to target the interaction between p53 and MDM2. And the design novel potent inhibitors have become the current goal for cancer therapy development. Recently, two peptide inhibitors pDI (LTFEHYWAQLTS) and pMI (TSFAEYWNLLSP) was identified identified using phage display. Using pDI and pMI for comparison, a quadruple mutant peptide (pDIQ) was reported as the most potent inhibitor against MDM2[10].

2) p53 mitochondria reactivating peptides:- The mitochondrion plays a critical role in apoptosis. Tumor protein p53 (TP53) controls apoptosis, via positive or negative transcriptional regulation of various BCL2 family proteins such as BCL2, BBC3, BAX and PMAIP1 (phorbol-12-myristate-13-acetate-induced protein[10]. A trademark of apoptosis is the activation of a caspase cascade, resulting in cleavage of many structural and signaling proteins. This can be achieved by two major cellular signaling pathways, known as the intrinsic (or mitochondrial) pathway and the extrinsic pathway also known as death receptor pathway. The intrinsic pathway is eliminated in a mitochondria-dependent fashion and is controlled by the pro- and anti-apoptotic members of the Bcl-2 family of proteins. In counter to an apoptotic signal, such as oxidative stress or DNA damage caused by many anticancer therapeutics, the pro-apoptotic Bcl-2 members Bax or Bak are activated at the mitochondria, thereby triggering a cascade of signaling events including cytochrome c release into the cytoplasm and the activation of the initiator caspase-9. The administrator caspase-3 is subsequently activated, leading to cleavage of numerous cellular proteins and that basically ends with the death of the cell. Anti-apoptotic Bcl-2 family proteins, including Bcl-2, Bcl-XL, Bcl-w, Mcl-1 and A1, prevent cell death by binding and sequestering pro-apoptotic proteins. The activity of caspases is also tightly regulated by cytosolic inhibitor of apoptosis (IAP) proteins (XIAP, cIAP1, cIAP2, survivin), which can directly bind to and block caspase activation.[108]

Mitochondria are central death regulators in response binds to BclXL via its DNA binding domain. DNA damage, growth factor withdrawal, hypoxia, and oncogene deregulation and are critical for p53-dependent death. When mitochondria receive a death signal, the outer mitochondrial membrane (OMM) endures immobilization which causes the release of potent death factors from the intermembraneous space into the cytosol. These apoptogenic factors activate caspase-9 (cytochrome c), inhibit cytosolic IAPs (Smac, Htra2), induce chromatin condensation (AIF), or degrade DNA (Endonuclease G). OMM immobilization is regulated by the opposing actions of pro- and antiapoptotic Bcl2 proteins. The antiapoptotic members, described by Bcl2

and BclXL, fundamently reside at the OMM and moderate an analytical pro-survival function by stabilizing the OMM and preventing the release of moderate apoptosis by transcriptional activation of pro-death factors. Overexpressed Bcl2 and BclXL abolish p53-dependent and independent cell death. The pro-apoptotic members consist of the BH3-only class, which reconciles the protective Bcl2/XL proteins, and the multidomain BH123 class. The type II BH3-only proteins Noxa, Puma, Bik, Bim, and Bad couple death signals to mitochondria and in healthy cells are sequestered to cytosolic sites other than the OMM. Upon appreciated death stimuli, BH3-only proteins undergo post translational modifications and mitochondrial translocation. Translocated BH3-only proteins then bind to Bcl2/XL via their BH3 domain, thereby inactivating their protective function. In resting cells, BH123 proteins exist as inactive monomers in the cytosol (Bax) or at mitochondria (Bak) and can be induced to homooligomerize and insert into the OMM by tBid after death stimuli, leading to cytochrome c release. BH3-only proteins are upstream of BH123 proteins since Bax/Bak double null cells are resistant to Bim- and Bad-induced apoptosis.[109] As a cytosolic protein, p53 acts in association with 79 several members of the Bcl-2 family proteins, facilitating the mitochondrial release of cytochrome c (Cyt c); this triggers a cascade of 81 events leading to caspase-3 activation and cell death.[110]

3) p53-oxidative stress reactivating peptides: By definition, oxidative stress is a condition under which free radicals in excess of the antioxidant defences are present[111]. Oxidative stress as an imbalance between the production of ROS (prooxidants) and antioxidants defense system in an organ or the organism as a whole, in favor of the first and brings about cellular disruption. This imbalance occurs due to two reasons; either by the overproduction of ROS such as the superoxide radical or hydroxyl radical (OH), or by the decrease in the elimination of ROS by oxidant defense mechanisms. The most important sources of ROS generation include the mitochondrial electron transport chain (one of the important sites involves in the production of significant amounts of H_2O_2), per -oxisomes and the cytochrome P450 system. Furthermore, production of ROS can be accelerated by the action of various enzymes such as cyclooxygenases, xanthine oxidase, uncoupled NOS and NADPH oxidases. Different drugs, such as doxorubicin, cisplatin, acetaminophen and nimesulide, toxicants such as heavy metals (As, Pb, Cd, Hg, etc.), acrolein, chloroform, and carbon tetrachloride, tertiary butyl hydroperoxide, xenobiotics, ultraviolet (UV) irradiation, environmental pollutants (oxides of nitrogen, SO_2, CO_2, etc.), and other factors enhance the process of ROS production.

A number of metabolic disorders, such as insulin resistance, familial amyotrophic lateral sclerosis, obesity, and diabetes mellitus all assist the formation of ROS in the biological system.[112]

Paraquat (PQ) causes toxicity mainly due to generation of superoxide anions in the mitochondria and cytosol of mammalian cells, which leads to the formation of several reactive oxygen species (ROS). Because the toxicity mechanism of PQ is mainly due to a sustained redox-cycling effect resulting in oxidative stress-related. Upon exposure of human neural progenitor cells(hNPCs) to find out the detection of ROS production and on the change of caspase-3 activity, intracellular calcium level, p21, p53 mRNA transcripts and NF-κB activity[113].

Perfluorooctanoic acid (PFOA) caused oxidative stress and mitochondrial dysfunction in HepG2 cells, which was firmly linked to cell cycle arrest and induction of apoptosis. The induction of cell apoptosis by PFOA is also done by ROS and caspase pathway.[114]

The neurotoxic effect of Aβ[25–35] peptide has been mediate by an increasing production of reactive oxygen species. The redox-active iron (Fe) has been determined toassemble in amyloid-β deposits in Alzheimer's disease (AD) brains. A hydroxyl radical is stoichiometrically generated by oxidation of Fe^{2+} by H_2O_2 to Fe^{3+}, which is called the Fenton reaction. These outcomes that Aβ-generated H_2O_2 in combination with Fe^{2+} may contribute to the progression and/or pathogenesis of AD. H_2O_2 can trigger by itself the activation of multiple signalling pathways that influence the cytotoxicity in affected cells including caspase-3 activation, the phosphorylation cascades leading to the activation of mitogen-activated protein kinases (MAPKs) and nuclear factor κB (NF-κB). Indeed, the MAPK c-Jun N terminal kinase/stress activated protein kinase (JNK/SAPK) is responsible for the phosphorylation of a variety of proteins including downstr- eam kinases and transcription factors such as c-Jun which, in turn, activates transcription genes involved in apoptosis. Although, both NF-κB and c-Jun have been postulated as antiapoptotic factors, they have also been linked to the onset of apoptosis.

NF-κB has been shown to activate tumour suppressor p53 which is a transcriptional factor involved in regulating apoptosis . p53 has been discribed to be a direct transcriptional activator of bax gene (proapoptotic gene belonging to a large bcl-2 gene family).[115]

4) Direct involvement of the tumor suppressor p53 in nucleotide excision repair: The NER mechanism involves several biochemical steps which include damage recognition, damage site unwinding, dual incision and gap-filling DNA synthesis. The damage recognition step may involve RNA polymerase II or XPC-hHR23B if the damage is or is not in the transcribing region, respectively. Once the damage has been identified and verified, the damage site, including its 3' and 5'-end adjacent nucleotides, is free by helicases (XPB and XPD of the TFIIH complex) before the damaged DNA is excised by two endonucleases (XPG and ERCC1-XPF). The oligonucleotide excision is followed by repair synthesis which is mediated by DNA

polymerases δ or ε, PCNA, RPA and replication factor C. The remaining gap is then sealed by ligase I.[116]

REFERENCES

1. Mohideen, A. K. S.; Sheriff, M. A. Sequence analysis of the Tumour suppressor protein p53 and its implications. *J. Biochem. Tech*. 2012; 3: 306-308.
2. Hofseth, L. J.; Hussain, S. P.; Harris, C. C. p53: 25 years after its discovery. *Trends Pharmacol. Sci*. 2004; 25: 177–181.
3. Sidransky, D.; Hollstein, M. Clinical implications of the p53 gene. *Annu. Rev. Med*. 1996, 47, 285–301.
4. Teodoro, J. G.; Evans, S. K.; Green, M. R. Inhibition of tumor angiogenesis by p53: a new role for the guardian of the genome. *J. Mol. Med*. 2007; 85: 1175–1186.
5. Peng, Y- C.; Kuo, F.; Breiding, D. E.; Wang, Y-F.; Mansur, C. P.; Androphy, E. J. AMF1 (GPS2) Modulates p53 Transactivation. *Mol. Cell. Biol*. 2001; 21: 5913–5924.
6. Davis, J. R.; Mossalam, M.; Lim, C. S. Controlled Access of p53 to the nucleus regulates its proteasomal degradation by MDM2. *Mol. Pharm*. 2013; 10: 1340−1349.
7. Wang, Z.; Sun, Y. Targeting P53 for novel anticancer therapy. *Transl. oncol*. 2010: 3: 1–12.
8. Poyurovsky, M. V.; Prives, C. Unleashing the power of p53: lessons from mice and men. *Gene Dev*. 2006; 20: 125-131.
9. Nayak, S. K.; Panesar, P. S.; Kumar, H. p53-Induced Apoptosis and Inhibitors of p53. *Curr. Med. Chem*. 2009; *16:* 2627-2640.
10. Kuo, K. K.; Chen, Y. L.; Chen, L. R.; Li, C. F.; Lan, Y. H.; Chang, F. R.; Wu, Y. C.; Shiue, Y. L. Involvement of phorbol-12-myristate-13-acetate-induced protein 1 in goniothalamin-induced TP53-dependent and independent apoptosis in hepatocellular carcinoma-derived cells. *Toxicol. Appl. Pharm*. 2011; 256: 8–23.
11. Rubbi, C. R.; Milner, J. Disruption of the nucleolus mediates stabilization of p53 in response to DNA damage and other stresses. *EMBO J*, 2003; 22: 6068-6077.
12. Turpin, E.; Luke, K.; Jones, J.; Tumpey, T.; Konan, K.; Schultz-Cherry, S. Influenza Virus Infection Increases p53 Activity: Role of p53 in Cell Death and Viral Replication. *J. virol*. 2005; 79: 8802-8811.
13. Freedman, D. A.; Levine, J. A. Regulation of the p53 Protein by the MDM2, Oncoprotein. *Cancer Res*. 1999; 59: 1-7.
14. Okamoto, K.; Prives, C. A role of cyclin G in the process of apoptosis. *Oncogene*. 1999; 18: 4606-4615.
15. Okorokov, A. L.; Sherman, B.; Plisson, C.; Grinkevich, V.; Sigmundsson, K.; Selivanova, G.; Milner, J.; Orlova, E. L. The structure of p53 tumour suppressor protein reveals the basis for its functional plasticity. *EMBO J*. 2006; 25: 5191-55200.
16. DeWoody, A. J. Nucleotide variation in the *p53* tumor-suppressor gene of voles from Chernobyl, Ukraine. *Mutat. Res*. 1999; 439: 25–36.
17. Hardcastle, I. R.; Ahmed, S. U.; Atkins, H.; Calvert, A. H.; Curtin, N. J.; Farnie, G.; Golding, B. T.; Griffin, R. J.; Guyenne, S.; Hutton, C.; Kallblad, P. K.; Kemp, S. J.; Kitching, M. S.; Newell, D. R.; Norbedo, S.; Northen, J. S.; Reid, R. J.; Saravanan, K.; Willems, H. M. G.; Lunec, J. Isoindolinone-based inhibitors of the MDM2–p53 protein–protein interaction. *Bioorg. Med. Chem Lett.* 2005; 15: 1515–1520.
18. Basse, N.; Kaar, J. L.; Settanni, G.; Joerger, A. C.; Rutherford, T. J.; Fersht, A. R. Toward the Rational Design of p53-Stabilizing Drugs: Probing the Surface of the Oncogenic Y220C Mutant. *Chem. Biol*. 2010; 17: 46–56.
19. Rosal, R.; Brandt-Rauf, P.; Pincus, M. R.; Wang, H.; Mao ,Y.; Li, Y.; Fine, R. L. The role of alpha-helical structure in p53 peptides as a determinant for their mechanism of cell death: necrosis versus apoptosis. *Adv. Drug Delivery Rev*. 2005; 57: 653–660.
20. http://www.answers.com/topic/cargo-protein.
21. Keshava, R.; Jothi, M.; Gope, M. L.; Gope, R. Functional modulation of the p53 gene and its gene and its protein in human tumors. *Ann. Neurosci*. 2008; 15: 75-86.
22. Ho, W. C.; Fitzgerald, M. X.; Marmorstein, R. Structure of the p53 Core Domain Dimer Bound to DNA. *J.Biol.Chem*. 2006; 281: 20494–20502.
23. Zawacka-Pankau J.; Krachulec J.; Grulkowski I.; Bielawski K.P.; Selivanova G. The p53-mediated cytotoxicity of photodynamic therapy of cancer: recent advances. *Toxicol. Appl. Pharm*. 2008; 232: 487–497.
24. Ishimaru, D.; Bom, A. P. D. A.; Lima, L. M. T. R.; Quesado, P. A.; Oyama, M. F.C.; Moura Gallo, C. V. D.; Cordeiro, Y.; Silva, J. L. Cognate DNA Stabilizes the Tumor Suppressor p53 and Prevents misfolding and aggregation. *Biochemistry*, 2009; 48: 6126–6135.
25. Lello, P. D.; Jenkins, L. M. M.; Jones, T. N.; Nguyen, B. D.; Hara, T.; Yamaguchi, H.; Dikeakos, J. D.; Appella, E.; Legault, P.; Omichinski, J. G. Structure of the Tfb1/p53 complex: Insights into the Interaction between the p62/Tfb1 Subunitof TFIIH and the activation domain of p53. *Molecular Cell*. 2006; 22: 731–740.
26. Xu, J.; Zhou, X.; Wang, J.; Li, Z.; Kong, X.; Qian, J.; Hu,Y.; Fang, J-Y. RhoGAPs attenuate cell proliferation by direct interaction with p53 tetramerization domain. *Cell Rep*. 2013; 3: 1526–1538.
27. http://www.expertreviews.org/
28. Bouchet, B. P.; Fromentel, C. C. D.; Puisieux, A.; Galmarini, C. M. p53 as a target for anticancer drug development. *Oncology/Hematology(O/H)*, 2006; 58: 190–207.

29. Kochetkov D. V. ; Iiyinskaya G. V. ; Komarov, P. G.; Strom, E.; Agapova, L. S.; Ivanov, A.V.; Budanov, A. V.; Frolova, E. I.; Chumakov, P. M. Transcriptional inhibition of the human Papilloma virus reactivates tumor suppressor p53 in cervical carcinoma cells. *Mol Biol (Mosk*). 2007; 41: 515–523.
30. El-Deiry, W.S. Insights into cancer therapeutic design based on p53 and trail receptor signaling. *Cell Death Diff*. 2001; 8: 1066 -1075.
31. Almazov, V. P.; Kochetkov, D. V.; Chumakov, P. M. Use of p53 for therapy of human cancer. *Mol Biol (Mosk)*. 2007; 41: 947–963.
32. Keswani, R. N.; Noffsinger, A.; Waxman, I.; Bissonnette, M. Clinical use of p53 in Barrett's esophagus. *Cancer Epidem. Biomar*. 2006; 15*:* 1243.
33. Chao, C.; Saito, S. I.; Kang, J.; Anderson, C. W.; Appella, E.; Xu, Y. p53 transcriptional activity is essential for p53 dependent apoptosis following DNA damage. *EMBO*. 2000; 19: 4967-4975.
34. Ryan, K. M.; Phillips, A. C.; Vousden, K. H.. Regulation and function of the p53 tumor suppressor protein. *Curr. Opin. Cell Biol.* 2001; 13: 332–337.
35. Tabakin-Fix, Y.; Azran, I.; Schavinky-Khrapunsky, Y.; Levy, O.; Aboud, M. Functional inactivation of p53 by human T-cell leukemia virus type 1 Tax protein: mechanisms and clinical implications. *Carcinogenesis*. 2006; 27: 673–681.
36. Shadfan, M.; Lopez-Pajares, V.; Yuan, Z-M. MDM2 and MDMX: alone and together in regulation of p53. *Transl. Cancer Res.* 2012; 1: 88-99.
37. Miyazaki, M.; Kawato, H.; Naito, H.; Ikeda, M.; Miyazaki, M.; Kitagawa, M.; Seki, T.; Fukutake, S.; Aonuma, M.; Soga, T. Discovery of novel dihydroimidazothiazole derivatives as p53–MDM2 protein–protein interaction inhibitors: Synthesis, biological evaluation and structure activity relationships. *Bioorg. Med. Chem. Lett.* 2012; 22: 6338–6342.
38. Chen, J., Marechal, V., and Levine, A. J. Mapping of the p53 and mdm-2 interaction domains. *Mol. Cell. Biol*. 1993; 13: 4107–4114.
39. Bottger, A., Bottger, V., Garcia-Echeverria, C., Chene, P., Hochkeppel, H. K., Sampson, W., Ang, K., Howard, S. F., Picksley, S. M., and Lane, D. P. Molecular characterization of the hdm2-p53 interaction. *J. Mol. Biol*. 1997; 269: 744 – 756.
40. Freedman, D. A., Epstein, C. B., Roth, J. C., and Levine, A. J. A genetic approach to mapping the p53 binding site in the MDM2 protein. *Mol. Med*. 1997; 3: 248 – 259.
41. Mihara, M.; Erster, S.; Zaika, A.; Petrenko, O.; Chittenden, T.; Pancoska, P.; Ute M. Moll, U. M. p53 has a direct apoptogenic role at the mitochondria. *Mol. Cell*. 2003; 11: 577–590.
42. Zhang,S.; Kuang, G.; Zhao, G.; Wua, X.; Zhang, C.; Lei, R.; Chen,T. X. J.; Wanga, Z.; Maa, R.; Li, B.; Yang, L.; Aiguo Wanga, A. Involvement of the mitochondrial p53 pathway in PBDE-47-induced 4 SH-SY5Y cells apoptosis and its underlying activation mechanism. *Food Chem. Toxicol*. 2013(in press).(DOI.org/10.1016/j.fct.2013.10.008)
43. Sato, M.; Takagi, M.; Mizutani, S. Irradiation-induced p53 expression is attenuated in cells with NQO1 C465T polymorphism. *J Med Dent Sci*. 2010; 57: 139－145.
44. Hollander, P. M.; Bartfai, T.; Gatt, S. Studies on the reaction mechanism of DT diaphorase. Intermediary plateau and trough regions in the initial velocity vs substrate concentration curves. *Arch Biochem Biophys*. 1975; 169: 568–576.
45. Hershko, A.; Ciechanover, A. The ubiquitin system. *Annu Rev Biochem*. 1998; 67: 425–479.
46. Glickman, M. H.; Ciechanover, A. The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. *Physiol. Rev*. 2002; 82: 373-428.
47. Brooks, P.; Fuertes, G.; Murray, R. Z.; Bose, S.; Knecht, E.; Rechsteiner, M. C.. Subcellular localization of proteasomes and their regulatory complexes in mammalian cells. *Biochem. J*. 2000; 346: 155–161.
48. Asher, G.; Tsvetkov, P.; Kahana, C.; Shaul, Y. A mechanism of ubiquitin-independent proteasomal degradation of the tumor suppressors p53 and p73. *Genes Dev*. 2005; 19: 316–321.
49. Zwickl, P.; Voges, D.; Baumeister, W. The proteasome: A macromolecular assembly designed for controlled proteolysis. *Philos Trans R Soc Lond B Biol Sci*. 1999; 354: 1501–1511.
50. Baugh, J. M.; Viktorova, E. G.; Pilipenko, E. V. Proteasomes can degrade a significant proportion of cellular proteins independent of ubiquitination. *J. Mol. Biol*. 2009; 386: 814–827.
51. Tsvetkov, P.; Asher, G.; Paz, A.; Reuven, N.; Sussman, J. L.; Silman, I. Operational definition of intrinsically unstructured protein sequences based on susceptibility to the 20S proteasome. *Proteins*. 2008; 70: 1357–1366.
52. Bell, S.; Klein, C.; Muller, L.; Hansen, S.; Buchner, J. p53 contains large unstructured regions in its native state. *J. Mol. Biol*. 2002; 322: 917–927.
53. Al-Assaf, A. H.; Alqahtani, A. M.; Alshatwi, A. A.; Syed, N. A.; Shafi, G.; Hasan, T. N. Mechanism of cadmium induced apoptosis inhuman peripheral blood lymphocytes: The role of p53, Fas and Caspase-3. *Environ. Toxicol. Pharmaco.* 2013; 36: 1033–1039.
54. Sollner, S.; Schober, M.; Wagner, A.; Prem, A.; Lorkova, L.; Palfey, B. A.. Quinone reductase acts as a redox switch of the 20S yeast proteasome. *EMBO Rep*. 2009; 10: 65–70.
55. Sollner, S.; Nebauer, R.; Ehammer, H.; Prem, A.; Deller, S.; Palfey, B. Al. Lot6p from Saccharomyces cerevisiae is a FMN-dependent reductase with a potential role in quinine detoxification. *FEBS J*. 2007; 274: 1328–1339.

56. Mani, A.; Gelmann, E. P. The Ubiquitin-Proteasome pathway and Its Role in Cancer. *J. clin. Oncol.* 2005; 23: 4776- 4789.
57. Vijay-Kumar, S.; Bugg, C. E.; Wilkinson, K. D.; Vierstra, R. D.; Hatfield, P. M.; Cook, W. J. Comparison of the three-dimensional structures of human, yeast, and oat ubiquitin. *J. Biol. Chem*. 1987; 262: 6396–6399.
58. Voutsadakis, I. A. The ubiquitin–proteasome system and signal transduction pathways regulating Epithelial Mesenchymal transition of cancer. *J. Biomed. Sci*. 2012; 19: 67.
59. Bachmair, A.; Finley, D.; Varshavsky, A. In vivo half-life of a protein is a function of its amino-terminal residue. *Science*. 1986; 234: 179-186.
60. Chen, Z.; Hagler, J.; Palombella, V. J.; Signal-induced site-specific phosphorylation targets I kappa B alpha to the ubiquitin-proteasome pathway. *Genes Dev*. 1995; 9: 1586-1597.
61. Orford, K.; Crockett, C.; Jensen, J. P. Serine phosphorylation-regulated ubiquitination and degradation of beta-catenin. *J. Biol. Chem*. 1997; 272: 24735-24738.
62. Yaglom, J.; Linskens, M. H.; Sadis, S. P34Cdc28-mediated control of Cln3 cyclin degradation. *Mol. Cell Biol*. 1995; 15: 731-741.
63. Lahav-Baratz, S.; Sudakin, V.; Ruderman, J.V. Reversible phosphorylation controls the activity of cyclosome-associated cyclin-ubiquitin ligase. *Biochem.* 1995; 92: 9303-9307.
64. Shteinberg, M.; Protopopov, Y.; Listovsky, T. Phosphorylation of the cyclosome is required for its stimulation by Fizzy/cdc20. *Biochem. Biophys. Res. Commun.* 1999; 260: 193-198.
65. Rudner, A. D.; Murray, A. W. Phosphorylation by Cdc28 activates the Cdc20-dependent activity of the anaphase-promoting complex. *J. Cell Biol*. 2000; 149: 1377-1390.
66. Kramer, E. R.; Scheuringer, N.; Podtelejnikov, A. V. Mitotic regulation of the APC activator proteins CDC20 and CDH1. *Mol. Biol. Cell.* 2000; 11: 1555-1569.
67. Hershko A, Ganoth D, Sudakin V, et al: Components of a system that ligates cyclin to ubiquitin and their regulation by the protein kinase cdc2. *J. Biol. Chem.* 1994; 269: 4940-4946.
68. Sudakin, V.; Ganoth, D.; Dahan, A. The cyclosome, a large complex containing cyclin selective ubiquitin ligase activity, targets cyclins for destruction at the end of mitosis. *Mol. Biol. Cell*. 1995; 6: 185-197.
69. Meek, D. W.; Knippschild, U. Posttranslational Modification of MDM2. *Mol. Cancer Res.* 2003; 1: 1017–1026.
70. Shi, D.; Gu, W. Dual Roles of MDM2 in the Regulation of p53: Ubiquitination Dependent and Ubiquitination Independent Mechanisms of MDM2 Repression of p53 Activity. *Genes & Cancer*. 2012; 3: 240– 248.
71. Khosravi, R.; Maya, R.; Gottlieb, T.; Oren, M.; Shiloh, Y.; Shkedy, D. Rapid ATM-dependent phosphorylation of MDM2 precedes p53 accumulation in response to DNA damage. *PNAS*. 1999; 96: 14973–14977.
72. David-Pfeuty, T.; Chakrani, F.; Ory, K.; Nouvian-Dooghe, Y. Cell cycle-dependent regulation of nuclear p53 traffic occurs in one subclass of human tumor cells and inuntransformed cells. *Cell Growth Differ*. 1996; 7: 1211-1225.
73. Moll, U. M.; Laquaglia, M.; Benard, J.; Riou, G. Wild-type p53 protein undergoes cytoplasmic sequestration in undifferentiated neuroblastomas but not in differentiated tumors. *Proc Natl Acad Sci USA*. 1995; 92: 4407-4411.(84)
74. Ostermeyer, A. G.; Runko, E.; Winkfield, B.; Ahn, B.; Moll, U. M. Cytoplasmically sequestered wild-type p53 protein in neuroblastoma is relocated to the nucleus by a C-terminal peptide. *Proc Natl Acad Sci USA*. 1996*;* 93: 15190-15194.
75. Shaulsky, G.; Goldfinger, N.; Tosky, M. S.; Levine, A. J.; Rotter, V. Nuclear localization is essential for the activity of p53 protein. *ONC*. 1991; 16: 2055-2065.
76. Gorlich, D.; Kutay, U. Transport between the cell nucleus and the cytoplasm. *Annu. Rev. Cell Dev. Biol*. 1999; 15: 607-660.
77. Henderson, B. R.; Eleftheriou, A. A comparison of the activity, sequence specificity, and CRM1-dependence of different nuclear export signals. *Exp. Cell Res*. 2000; 256: 213- 224.
78. Shaulsky, G.; Goldfinger, N.; Ben-Ze'ev, A.; Rotter, V. Nuclear accumulation of p53 protein is mediated by several nuclear localization signals and plays a role in tumorigenesis. *Mol. Cell Biol*. 1990; 10: 6565-6577.
79. Stommel, J. M.; Marchenko, N. D.; Jimenez, G. S.; Moll, U. M.; Hope, T. J.; Wahl, G. M. A leucine-rich nuclear export signal in the p53 tetramerization domain: regulation of subcellular localization and p53 activity by NES masking. *EMBO J*. 1999; 18: 1660-1672.(87, 91, 92)
80. Stommel, J. M.; Wahl, G. M. Accelerated MDM2 auto-degradation induced by DNA-damage kinases is required for p53 activation. *EMBO J*. 2004; *23*: 1547-1556.
81. Zhang, Y.; Xiong, Y. A p53 amino-terminal nuclear export signal inhibited by DNA damage- induced phosphorylation. *Science*. 2001; *292*: 1910-1915.(84)
82. Moll, U. M.; Riou, G.; Levine, A. J. Two distinct mechanisms alter p53 in breast cancer: mutation and nuclear exclusion. *Proc Natl Acad Sci USA*. 1992; 89: 7262-7266.
83. Sun, X. F.; Carstensen, J. M.; Zhang, H.; Stal, O.; Wingren, S.; Hatschek, T.; Nordenskjold, B. Prognostic significance of cytoplasmic p53 oncoprotein in colorectal adenocarcinoma. *BJC*. 1992; 340: 1369-1373.
84. Kudo, N.; Wolff, B.; Sekimoto, T.; Schreiner, E. P.; Yoneda, Y.; Yanagida, M.; Horinouchi, S.; Yoshida, M. Leptomycin B inhibition of signal-

mediated nuclear export by direct binding to CRM1. *Exp. Cell Res.* 1998; 242: 540-547.
85. Wolff, B.; Sanglier, J. J.; Wang, Y. Leptomycin B is an inhibitor of nuclear export: inhibition of nucleo cytoplasmic translocation of the human immunodeficiency virus type 1 (HIV-1) Rev protein and Rev-dependent mRNA. *Chem. Biol*. 1997; 4: 139-147.
86. Sakaguchi, K.; Sakamoto, H.; Lewis, M. S.; Anderson, C. W.; Erickson, J. W.; Appella, E.; Xie, D. Phosphorylation of serine 392 stabilizes the tetramer formation of tumorsuppressor protein p53. *Biochem*. 1997; 36: 10117-10124.
87. Jimenez, G. S.; Khan, S. H.; Stommel, J. M.; Wahl, G. M. p53 regulation by posttranslational modification and nuclear retention in response to diverse stresses. *ONC*. 1999; 18: 7656-7665.
88. Liang, S. H.; Clarke, M. F. Regulation of p53 localization. *Eur. J. Biochem*. 2001; 268: 2779-2783.
89. Boyd, S. D.; Tsai, K. Y.; Jacks, T. An intact HDM2 RING-finger domain is required for nuclear exclusion of p53. *Nat. Cell Biol*. 2000; 2: 563-568.
90. Geyer, R. K.; Yu, Z. K.; Maki, C. G. The MDM2 RING-finger domain is required to promote p53 nuclear export. *Nat. Cell Biol*. 2000; 2: 569-573.
91. Lohrum, M. A.; Woods, D. B.; Ludwig, R. L.; Balint, E.; Vousden, K. H. C-terminal ubiquitination of p53 contributes to nuclear export. *Mol. Cell Biol*. 2001; 21: 8521-8532.
92. Michael, D.; Oren, M. (2003). The p53-Mdm2 module and the ubiquitin system. *Semin Cancer Biol*. 2003; 13: 49-58.
93. Dumaz, N.; Milne, D. M.; Jardine, L. J.; Meek, D. W. Critical roles for the serine 20, but not the serine 15, phosphorylation site and for the polyproline domain in regulating p53 turnover. *Biochem. J*. 2001; 359: 459-464.
94. Shieh, S. Y.; Ikeda, M.; Taya, Y.; Prives, C. DNA damage-induced phosphorylation of p53 alleviates inhibition by MDM2. *Cell*. 1997; 91: 325-334.
95. Siliciano, J. D., Canman, C. E., Taya, Y., Sakaguchi, K., Appella, E., Kastan, M. B. DNA damage induces phosphorylation of the amino terminus of p53. *Genes Dev*. 1997; 11: 3471- 3481.
96. Unger, T.; Sionov, R. V.; Moallem, E.; Yee, C. L.; Howley, P. M.; Oren, M.; and Haupt, Y. Mutations in serines 15 and 20 of human p53 impair its apoptotic activity. *Oncogene*. 1999b; 18: 3205-3212.
97. Maya, R.; Balass, M.; Kim, S. T.; Shkedy, D.; Leal, J. F.; Shifman, O.; Moas, M.; Buschmann, T.; Ronai, Z.; Shiloh, Y. ATM-dependent phosphorylation of Mdm2 on serine 395: role in p53 activation by DNA damage. *Genes Dev*. 2001; *15*: 1067-1077.
98. Kussie, P. H.; Gorina, S.; Marechal, V.; Elenbaas, B.; Moreau, J.; Levine, A. J.; Pavletich, N. P. Structure of the MDM2 oncoprotein bound to the p53 tumor suppressor transactivation domain. *Science*. 1996; 274: 948-953.
99. Lin, J.; Chen, J.; Elenbaas, B.; Levine, A. J. Several hydrophobic amino acids in the p53amino-terminal domain are required for transcriptional activation, binding to mdm-2 and the adenovirus 5 E1B 55-kD protein. *Genes Dev*. 1994; 8: 1235-1246.
100. Singh, J.; Carlish, D.L.; Patierno, S. Chromium induced genotoxicity and apoptosis: Relationship to chromium carcinogenesis. *Oncol. REP*. 1998; 5: 1307-1318.
101. De Flora, S.; Bagnasco, M.; Serra, D.; Zanacchi, P. Genotoxicity of chromium compounds; a review. *Mutat. Res*. 1990; 238: 99-172.
102. Freeman, N.; Lioy, P.J. Exposure to chromium dust from homes in a Chromium Surveillance Project. *Arch. Environ. Health*, 1997; 52: 213-226.
103. Hayes, R. B. Review of occupational epideminology of chromium chemicals and respiratory cancer. *Sci. Total environ*. 1988; 71: 331-339.
104. Langard, S. One hundred years of chromium and cancer: a review of epidemiological evidence and selected case reports. *Am. J. Ind. Med*. 1990; 17; 189-215.
105. Wang, S.; Shi, X. Mechanisms of Cr (VI)-induced p53 activation : the role of Phosphorylation, mdm2 and ERK, *Carcinogensis*. 2001; 22: 757-762.
106. Malloy, K. L.; Choi, H.; Fiorilla, C.; Valeriote, F. A.; Matainaho, T.; Gerwick, W. H. Hoiamide D, a marine cyanobacteria-derived inhibitor of p53/MDM2 interaction. *Bioorg. Med. Chem. Lett*. 2012; 22: 683–688.
107. Walton, M. I.; Wilson, S. C.; Ian R. Hardcastle, I. R.; Mirza, A. R.; Workman, P. An evaluation of the ability of pifithrin-A and -B to inhibit p53 function in two wild-type p53 human tumor cell lines. *Mol Cancer Ther*. 2005; 4: 1369-1377.
108. Niu, R-J.; Zheng, Q-C.; Zhang, J-L.; Zhang, H-X. Molecular dynamics simulations studies and free energy analysis on inhibitors of MDM2-p53 interaction. *J*. Mol. *Graphics* Modell. 2013; 46: 1-30.
109. Brunelle, J. K.; Zhang, B. Apoptosis assays for quantifying the bioactivity of anticancer drug products. *Drug Resist. Updates*. 2010; 13: 72–179.
110. Mihara, M.; Erster, S.; Zaika, A.; Petrenko, O.; Chittenden, T.; Pancoska, P.; Moll, U. M. p53 has a direct apoptogenic role at the mitochondria. *Mol. Cell*. 2003; 11: 577–590.
111. Velez-Pardo, C.; Ospina, G. G.; Rio, M. J. D. Aβ[25–35] Peptide and Iron Promote Apoptosis in Lymphocytes by an Oxidative Stress Mechanism: Involvement of H_2O_2, Caspase-3, NF-κB, p53 and c-Jun. *NeuroToxicol*. 2002; 23: 351–365.
112. Rashid, K.; Sinha, K.; Sil, P. C. An update on oxidative stress-mediated organ pathophysiology. *Food Chem. Toxicol*. 2013; 62: 584–600.
113. Chang, X.; Lu, W.; Dou, T.; Wang, X.; Lou, D.; Sun, X.; Zhou, Z. Paraquat inhibits cell viability via enhanced oxidative stress and apoptosis in human neural progenitor cells. *Chem-Biol Inter*. 2013; 206: 248–255.

114. Huang, Q.; Zhang, J.; Martin, F. L.; Peng, S.; Tian, M.; Mu, X.; Shen, H. Perfluorooctanoic acid induces apoptosis through the p53-dependent mitochondrial pathway in human hepatic cells: A proteomic study. *Toxicol Lett*. 2013; 223: 211– 220.
115. Kim, E-J.; Kho, J-H.; Kang, M-R.; Um, S-J. Active regulator of SIRT1 cooperates with SIRT1 and facilitates suppression of p53 activity. *Molecular Cell*. 2007; 28: 277–290.
116. Chang, Y- C.; Janb, K-Y.; Cheng, C-A.; Liao, C-B.; Liu, Y-C.; Direct of the tumor suppressor p53 in nucleotide excision repair. *DNA repair*. 2008; 7: 751– 761.