Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 11s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

A Review: Wild And Mutant P53 In Cancer Progression And Therapy

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Cite this paper as: Seema Gupta, Biswajit Maity, (2025) A Review: Wild And Mutant P53 In Cancer Progression And Therapy. *Journal of Neonatal Surgery*, 14 (11s), 687-713.

1. INTRODUCTION

Since the discovery of p53 in 1979, extensive studies have been done on p53, which have established the key role of p53 in tumor suppression [1-3]. Loss of wild-type p53 function through mutations of the p53 gene and other mechanisms such as over-expression of negative regulators of p53 (MDM2, MDM4, and PPM1D) has been known as a prerequisite for initiation and/or progression of many human cancers [4-6]. As a transcription factor, p53 executes its tumor-suppressive function mainly through binding to p53 DNA-binding elements in its target genes to regulate their expression. Through transcriptionally regulating these genes, p53 plays critical roles in many important biological processes, including apoptosis, cell cycle arrest, senescence, DNA repair, cell metabolism, and antioxidant defense, which contribute to p53's function in tumor suppression [4-8].

The loss of p53 function is frequently a prerequisite for cancer development. The p53 gene is the most frequently mutated gene in human cancers. p53 mutations occur in >50% of all human cancers and in almost every type of human cancers. Most of p53 mutations in cancers are missense mutations, which produce the full-length mutant p53 (mutp53) protein with only one amino acid difference from wild-type p53 protein [1,8].In addition to loss of the tumor-suppressive function of wild-type p53, many mutp53 proteins acquire new oncogenic activities independently of wild-type p53 to promote cancer progression, termed gain-of-function (GOF). Mutp53 protein often accumulates to very high levels in cancer cells, which is critical for its GOF [1,11].

The p53 phenotype was found in three phages with different functions as delineated bellow:[12-18].

The "null" p53 phenotype: A "null" p53 phenotype indicates a complete absence of functional p53 protein, meaning the gene is completely deleted or mutated to the point where no active protein is produced, leading to a lack of its tumor-suppressing abilities, which often results in increased cancer susceptibility.

*Function: No active p53 protein is present, leading to a complete loss of its tumor-suppressing activity.

*Cellular effect: Cells with a null p53 phenotype are highly susceptible to accumulating DNA damage and uncontrolled cell proliferation, increasing the risk of cancer development.

*Diagnosis: In immunohistochemistry, a null p53 phenotype is often observed as a complete lack of p53 protein staining. [(complete loss of staining) is well-recognized as a "positive or aberrant" result in the diagnostic].

1. The "wild-type" p53 phenotype: A"wild-type" p53 phenotype represents a normal, functional p53 protein that can effectively respond to DNA damage by triggering cell cycle arrest or apoptosis, acting as a tumor suppressor.

*Function: Normal p53 protein functions as a tumor suppressor, responding to DNA damage by inducing cell cycle arrest or apoptosis to prevent the propagation of damaged cells.

*Cellular effect: Maintains genomic stability by preventing the accumulation of mutations and promoting appropriate cellular responses to stress.

*Diagnosis: In immunohistochemistry, wild-type p53 is typically visualized as a moderate level of protein staining with a normal pattern.

2. The "mutant" p53 phenotype: A "mutant" p53 phenotype describes a mutated form of the p53 protein that has lost its normal function, often due to missense mutations, leading to impaired DNA damage response and increased potential for cancer development, sometimes even exhibiting "gain-of-function" properties where the mutant protein disrupts cellular processes beyond its normal role.

*Function: A mutated p53 protein often loses its ability to bind DNA and activate downstream genes, leading to impaired tumor suppression.

*Cellular effect: Mutant p53 can accumulate in cells due to impaired degradation pathways, and depending on the mutation, it may even acquire new, oncogenic functions (gain-of-function) that further promote tumor progression.

*Diagnosis: In immunohistochemistry, mutant p53 can manifest as strong, diffuse staining due to the accumulation of the mutated protein. Advance molecular techniques (SNP, Microarray, RT-PCR, MiRNAs) were developed for early diagnosis. A few studies revealed that the MiRNAs have emerged as a new class of regulators of the expression and function of eukaryotic genomes. Tumor suppressive or oncogenic functions have been attributed to some miRNAs. It was observed that the p53 can alter the transcription of several miRNAs, and in some cases, it can also influence miRNA maturation. Conversely, miRNAs can also modulate the abundance and activity of p53 by direct or indirect mechanisms. Moreover, mutant p53 can actively repress the expression of some miRNAs that are activated by wild-type p53 [6,16-19].

wild-type p53 in cancer:

The loss of wild-type p53 function in tumor suppression, mutp53 often promotes tumor progression through the gain-of-function (GOF) mechanism. The GOF activity of mutp53 was first demonstrated in 1993, when Dittmer et al.(1993) reported that ectopic expression of R175H or R273H mutp53 endowed p53-null cells with an increased ability to form colonies in soft agar and form xenograft tumors in nude mice. Since then, numerous studies, including those using cell culture systems and mouse models and clinical studies, have shown that many missense mutp53 proteins display GOF activities to promote cancer progression, which is independent of wild-type p53 [20-28]. Mutients carrying p53 deletion mutations . GOF mutp53 has also been reported to be associated with poor clinical outcomes in cancer patients [29-34].Various mutp53 GOF activities have been reported so far, including promoting cell proliferation, metastasis, genomic instability, metabolic reprogramming, cell stemness, tumor microenvironment reshaping, immune suppression, and resistance to therapy in cancer [33-37](Figure 1)

In addition to the GOF mechanism, mutp53 has also been reported to inhibit wild-type p53 function through a dominantnegative mechanism in a heterozygous situation, where both wild-type and mutp53 alleles exist [38,39]. Mutp53 was reported to form heterodimer complexes with wild-type p53 to attenuate wild-type p53 function though conformational shifts or inhibiting the DNA-binding activity of wild-type p53 on target genes[21]. Recently, an in vitro mutational scanning of p53 single amino acid mutants in human leukemia cells showed that missense mutants in the DNA-binding domain exert a dominant-negative effect in myeloid malignancies [6,20,40]. Furthermore, analysis of clinical outcomes in patients with acute myeloid leukemia showed no evidence of GOF for p53 missense mutations, suggesting that mutp53 GOF may not play an important role in this type of cancer [41]. Notably, a recent study analyzed p53 mutations in 10225 samples from 32 cancers from The Cancer Genome Atlas (TCGA) and reported that >91% of p53-mutant cancers exhibit loss of the second allele of p53 by mutation, chromosomal deletion, or copy-neutral loss of heterozygosity [42]. This implies that such a heterozygous state of p53 is often transient during cancer progression and there is a selective force driving the inactivation of the remaining wild-type p53 allele in cancers, and also suggests that the dominant-negative effect of mutp53 is not sufficient to completely inactivate the remaining wild-type p53 allele in majority of cancers [43-45]. While mutp53 cannot bind to the p53 DNA-binding elements to transcriptionally regulate target genes of wild-type p53, mutp53 has been reported to exert its GOF activities through different mechanisms to promote tumorigenesis (Figure 1; 6,12,26,46].

Mutp53 interacts with many different proteins other than transcription factors, including tumor suppressors and oncogenic proteins, to affect their functions. Additionally, mutp53 regulates expression of many noncoding RNAs, including microRNAs (miRNAs), circular RNAs (circRNAs), and long noncoding RNAs (lncRNA), to exert its GOF activities [6,19,27,47]. p53 is one of the most intensively studied tumor suppressor proteins, with mutations that lead to loss of wild-type p53 activity frequently detected in many different tumor types. Perturbations in p53 signaling pathways are believed to be required for the development of most cancers, and there is evidence to suggest that restoration or reactivation of p53 function will have significant therapeutic benefit [47-49].

Moreover, the p53 protein is a tumor suppressor encoded by the TP53 gene and consists of 393 amino acids with four main functional domains. This protein responds to various cellular stresses to regulate the expression of target genes, thereby causing DNA repair, cell cycle arrest, apoptosis, metabolic changes, and aging. Mutations in the TP53 gene and the functions of the wild-type p53 protein (wtp53) have been linked to various human cancers. Eight TP53 gene mutations are located in codons, constituting 28% of all p53 mutations. The p53 can be used as a biomarker for tumor progression and an excellent target for designing cancer treatment strategies [50-52].

In wild-type p53-carrying cancers, abnormal signaling of the p53 pathway usually occurs due to other unusual settings, such

as high MDM2 expression. These differences between cancer cell p53 and normal cells have made p53 one of the most important targets for cancer treatment [53-58].

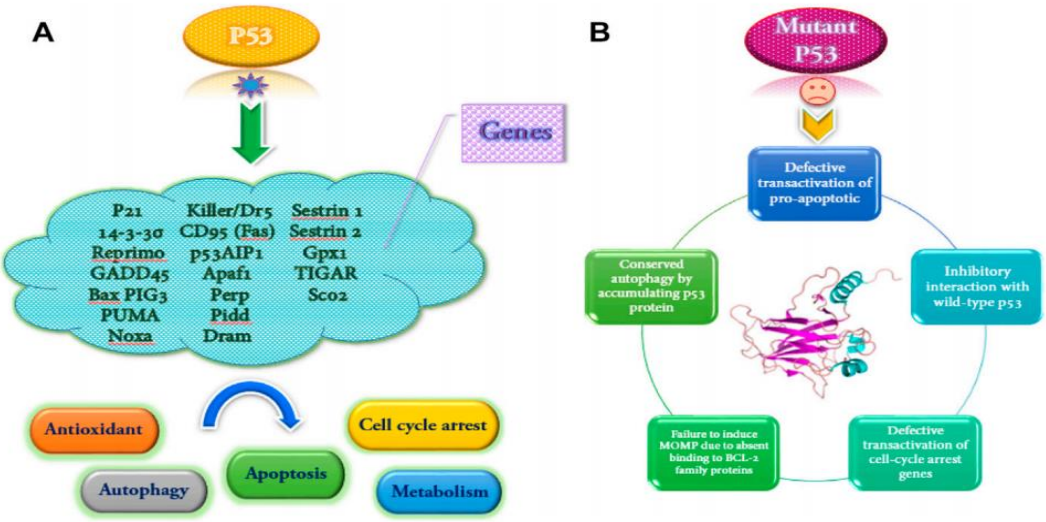
Structure of wild-type p53 and mutations leading to cancer:

The p53 protein consists of 393 amino acids, with four main functional domains: transcription, DNA binding, tetramerization, and regulatory . Also, this protein includes five protected regions under the headings (I, II, III, IV, and V) and the loop-helix structure (L, S, and H). Highly protected domains overlap with loop domains and are part of the protein's three-dimensional structure. In addition, there is a strong association between mutations and p53 three-dimensional structural domains [59]. Typically, wild-type p53 loses its function with a single-point mutation. This mutation causes a change in the structure of the core DNA binding domain of the protein (conformational mutation) or a change in the DNA binding capacity (contact mutation). Recent findings suggest that p53 mutants and their fragments can form protein masses both in vitro and in vivo [57-61]. Accumulation of p53 in both contact and conformational mutations in samples taken from patients' tumor tissue has been observed in several cancer cell lines; this indicates an association between mutated p53 accumulation and tumor growth [62]. In most human cancers, p53 hot spot mutations (both conformational and structural) are observed at the amino acid sites 175, 245, 248, 249, 273, and 282 [60]. Meanwhile, R248Q, R248W, and R175H mutations showed p53 protein accumulation in different tumor samples, while p53 protein accumulation was not reported in tumor samples containing R273H and R249S hot spot mutations [63,64].

The loss of wild-type p53 function in oncogenic processes and cancer development:

In cancer biology, the vital point about p53 is that; the p53 mutant protein is found in 50% or more of 50% of human cancers. In addition to losing function, the mutant p53 can have a dominant-negative effect on the remaining wild-type p53 allele and subsequently inactivate it by losing heterozygosity (LOH). Also, some p53 mutations have additive functions, which will cause the tumor to grow. In cancers in which wild-type p53 is conserved, it is usually in regulatory genes that encode the up or down pathways of p53; changes are observed[35,36]. Among p53 mutants, missense mutations not only cause the mutated p53 protein to lose its wild-type (LOF) function and gain dominant-negative activity but also increase the function of the mutated p53, leading to the tumor's more aggressive behavior and drug resistance [12,37,38, 65-68].

Figure-1:Some genes are transactivated by wild-type p53, and several functional consequences of p53 activation (A). Functional implications of mutant p53 (B).



p53 null phenotype:

The concept of a "p53 null phenotype" (complete loss of staining) is well-recognized in the gynecologic pathology literature, implicitly reflecting that this staining pattern represents a TP53 mutation. However, in the genitourinary pathology literature, a p53 null phenotype has only been addressed regarding the prognosis of invasive urothelial carcinoma, and not as a diagnostic biomarker for urothelial carcinoma in situ (CIS). Herein, 25 cases of urothelial carcinoma in situ [diagnoses made on hematoxylin and eosin (H&E) stained sections] showing null pattern p53 staining were retrieved from 22 different patients (16 males and 6 females, age range 52–85 years; average 69.6 years), most commonly showing large cell pleomorphic pattern morphology. One representative tissue block per case was selected for next-generation DNA sequencing (NGS). All 21 cases (100%) passing quality control for NGS showed at least 1 TP53 mutation (majority nonsense or frameshift mutations), including 3 cases with 2 mutations and 3 cases with 3 mutations. Three patients with multiple available samples harbored 1

or more shared TP53 mutations at 2 different time points, indicating clonality of the temporally distinct lesions. Additionally, 2 patients had an additional unique TP53 mutation at a later time point, suggesting intratumoral heterogeneity and/or temporal clonal evolution. While urothelial CIS remains an H&E diagnosis in most cases, a p53 immunostain may be useful in a subset of challenging cases. This study demonstrates that a p53 null phenotype represents an aberrant result in urothelial CIS with supportive molecular analysis showing a previously unknown level of complexity for TP53 mutations among these noninvasive lesions. Adequate recognition of the p53 null phenotype as a "biologically supportive result", similar to strong and diffuse staining with p53, is important and may warrant a formal consensus statement for recommended p53 reporting (i.e., "wild type" versus "aberrant or mutant"). [69]

Figure-2 a &b: Schematic structure of TP53 and its different domains:

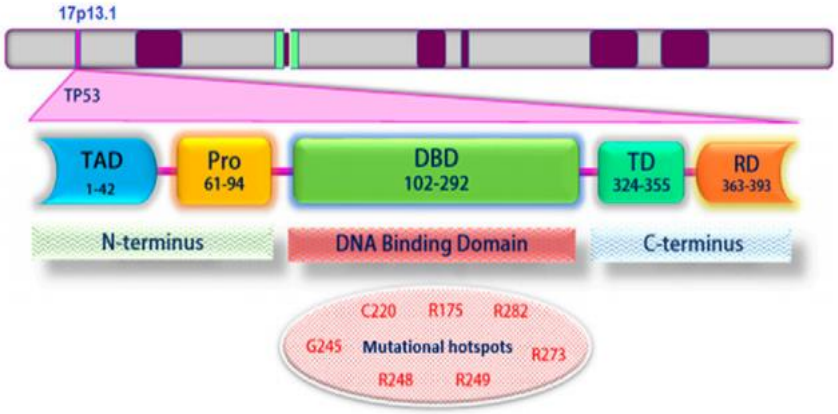


Figure-2a, illustrating that the mutations frequently occur within the DNA-binding domain. Mutant codons are shown in red: Transcriptional activation domain (TAD); Proline-rich domain (PRD); DNA binding domain (DBD); Tetramerization domain (TD); Regulatory domain (RD).

Figure-2b:The transcriptional model of mutp53

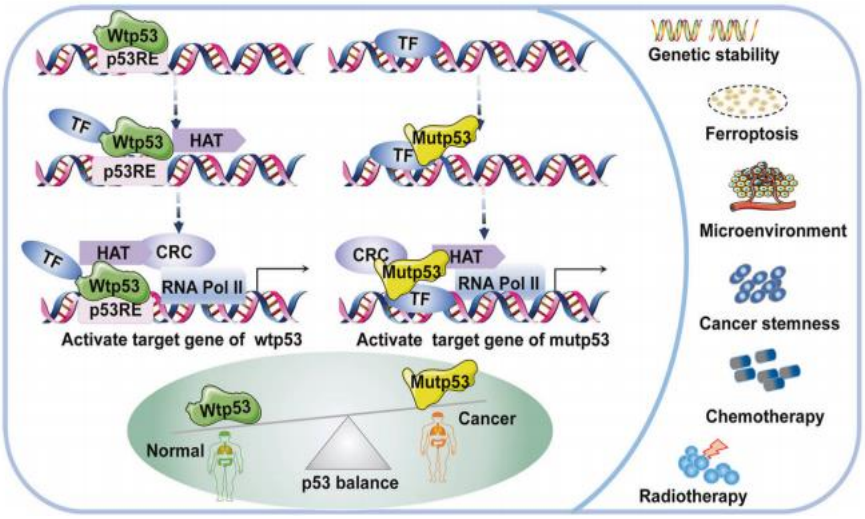


Figure-2b: The transcriptional model of mutp53 and its function in tumors: In contrast to wtp53, mutp53 cannot bind directly to DNA RE and it exerts function through interactions with TFs.

Figure-3:Prototypic histomorphology and immuno profile of study cases:

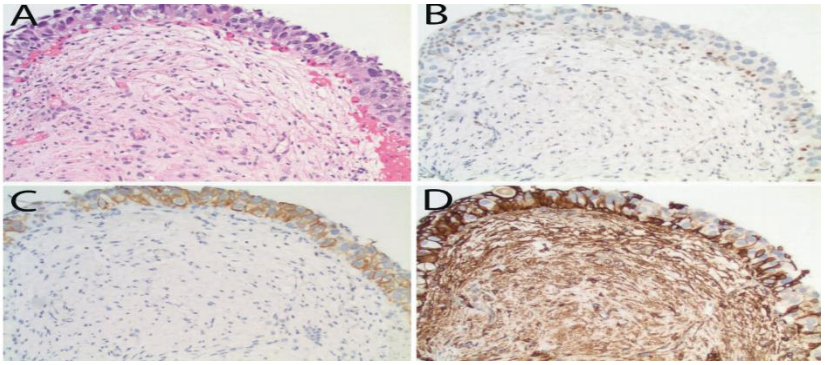


Figure-3, illustrating that theH&E of large cell pleomorphic pattern urothelial carcinoma in situ (A, 200×). Paired p53 immunostains showing null phenotype pattern (complete loss of staining in lesional cells) (B, 200×). Paired positive keratin 20 staining in lesional cells (C, 200×) and negative CD44 staining in lesional cells (D, 200×).

In this review, It was summarized the recent advances in studies on Wild type / Mutent (mutp53 GOF) and Null in cancer and mutp53-targeted cancer therapies. it has also been have dealt with various issues, such as the relative contribution of wild-type p53 loss of function, including transactivationdependent and transactivation-independent activities in oncogenic processes and their role in cancer development. We also discuss the role of p53 in the process of ferroptosis and its targeting in cancer treatment. Finally, it was focused on p53-related drug delivery systems and investigate the challenges and solutions [70-77].

Mutp53 GOF activities and mechanisms:

Cell proliferation:

p53 plays a critical role in suppression of cancer cell proliferation through different mechanisms, such as cell cycle arrest, senescence, and apoptosis [70-74]. In contrast, GOF mutp53 promotes cancer cell proliferation. Mutp53 forms a complex with the transcription factor NF-Y and co-factor p300 and transcriptionally activates NF-Y target genes, such as cyclin A, cyclin B1, CDK1, and CDC25C, to promote cell cycle progression [45]. Mutp53 binds to the promoter of MAP2K3, an upstream activator of the p38 MAPK, and recruits NF-Y and NF-jB to the MAP2K3 promoter, inducing MAP2K3 expression to promote cell proliferation [46]. Mutp53 binds to the transcription factor YAP to induce the transcription of cyclin A, cyclin B, and CDK1 to promote cellproliferation [77]. Mutp53 promotes colorectal tumor growth through interacting with the transcription factor STAT3 to activate STAT3 transcription program [48]. In addition, R249S mutp53 interacts with Pin1 after being phosphorylated by CDK4/cyclin D1 at the S249 residue and then is imported into the nucleus to stabilize c-Myc protein, resulting in the transcriptional activation of Myc target genes to promote proliferation of hepatocellular carcinoma cells [79,80].

Figure-4: Mutp53 GOF in cancer.

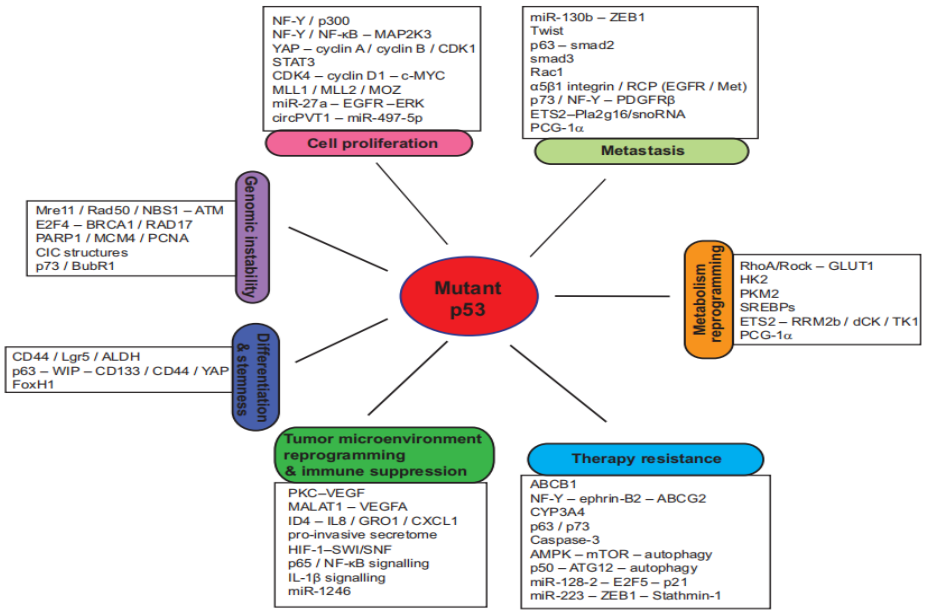


Figure-4, illustrating that the Mutp53 regulates cell proliferation, metastasis, genomic instability, differentiation and stemness, metabolic reprogramming, tumor microenvironment, immune response, and cancer therapy resistance to exert its GOF in tumorigenesis.

Metastasis:

p53 plays a key role in suppression of migration, invasion, and metastasis of cancer cells [51-53]. In contrast, promoting cancer metastasis is a well-known GOF activity of mutp53. R172H and R273H mutp53 knock-in mice develop more metastatic tumors than p53 / mice, providing clear evidence of mutp53 in promoting tumor metastasis in vivo [12,18,21]. Mutp53 has been reported to promote metastasis through different mechanisms. One important mechanism is through promoting epithelial–mesenchymal transition (EMT). Mutp53 transcriptionally represses miR-130b to upregulate ZEB1, a key EMT-related transcription factor, to promote EMT and cancer cell invasion [80]. Mutp53 also promotes EMT and metastasis by upregulating the EMT-related transcription factor Twist1 [81]. and interacting with p53 family member p63 to form a complex with Smad2 to activate the TGF-b signaling, which is important for EMT [56]. In addition to EMT, other mechanisms include modulating cell motility and extracellular matrix.

Genomic instability:

Genome instability is a hallmark of cancer. While p53, as a guardian of genome, plays a critical role in maintaining genomic stability, GOF mutp53 promotes genomic instability, such as chromosomal and amplification instability [6,57].The proper DNA damage response and DNA repair function are crucial for maintaining genomic stability in cells. Mutp53 can induce genomic instability through impairing DNA damage response and DNA repair. R248W and R273H mutp53 can bind to the nuclease Mre11 and prevent the association of the Mre11–Rad50–NBS1 (MRN) complex to DNA double-stranded breaks (DSBs), which in turn impairs ATM activation and DNA damage response (Song et al., 2007). Mutp53 interacts with the transcription factor E2F4 and binds to the promoter region of BRCA1 and RAD17, key proteins involved in DSB DNA repair, to repress BRCA1 and RAD17 expression and impair DNA repair [58]. Mutp53 was also reported to enhance the association of the DNA repair protein PARP1 with chromatin and increase the levels of nuclear replication proteins MCM4also been suggested to contribute to mutp53 GOF in inducing genomic instability [82,83].

Cell differentiation and stemness:

p53 promotes differentiation and restrains proliferation of stem cells, acting as a barrier of the formation of cancer stem cells (CSCs). In contrast, mutp53 displays a GOF activity to regulate dedifferentiation processes and facilitate CSC maintenance [84]. It was reported that bone-marrow mesenchymal stem cells in Li-Fraumeni syndrome patients are tumorigenic and can induce sarcomas. Similarly, accumulation of mutp53 in progenitor-like cells in the brain subventricular zone-associated areas leads to the initiation of glioma[85]. Mutp53 enhances the expression of colorectal CSC markers (e.g. CD44, Lgr5, and ALDH) by binding to CD44, Lgr5, and ALDH1A1 promoter sequences in colorectal cancer cells [86].

Metabolic reprogramming:

Metabolic reprogramming is a hallmark of cancer, which sustains the needs of energy and macromolecules for the rapid growth and proliferation of cancer cells. While p53 plays a critical role in maintaining metabolic homeostasis of normal cells, GOF mutp53 promotes metabolic reprogramming in cancer cells [82-84]. The enhanced aerobic glycolysis (namely the Warburg effect) is the most well-characterized metabolic change in cancer cells. Wildtype p53 has been reported to repress the Warburg effect in cancer cells through transactivating target genes that are required for oxidative phosphorylation, such as SCO2 [65]. as well as genes such as TIGAR and Parkin to negatively regulate glycolysis [12,19,66, 87]. In contrast, mutp53 enhances glucose uptake and glycolysis by promoting trafficking of glucose transporter GLUT1 to the plasma membrane through activation of the small GTPase RhoA and its direct downstream kinase ROCK both in cultured cancer cells and in R172H mutp53 knock in mice, which promotes tumorigenesis [21].

Tumor microenvironment and immune response regulation:

Cancer cells actively shape a permissive microenvironment for cancer progression. Growing evidence has shown that mutp53 remodels the tumor microenvironment and promotes adaptation of cancer cells to the microenvironment [68]. Mutp53 affects the expression of various secreted proteins to remodel the tumor microenvironment. For instance, mutp53 activates PKC to increase VEGF expression to promote angiogenesis [69]. Mutp53 forms a complex with E2F1 and binds to the promoter of inhibitor of DNA-binding 4 (ID4) to induce ID4 expression, which in turn enhances the expression of pro-angiogenic factors IL8 and GRO-a to promote angiogenesis [70]. Mutp53 binds to the lncRNA MALAT1 to promote the association of MALAT1 with chromatin and induce VEGF expression in breast cancer cells . In addition, R248W mutp53 increases exosome secretion of miR-1246 to reprogram macrophages to tumor-supporting macrophages [6,87]. Thus, through the mutp53 GOF mechanism, cancer cells can reprogram macrophages and other myeloid subsets to support cancer development.

Cancer therapy resistance:

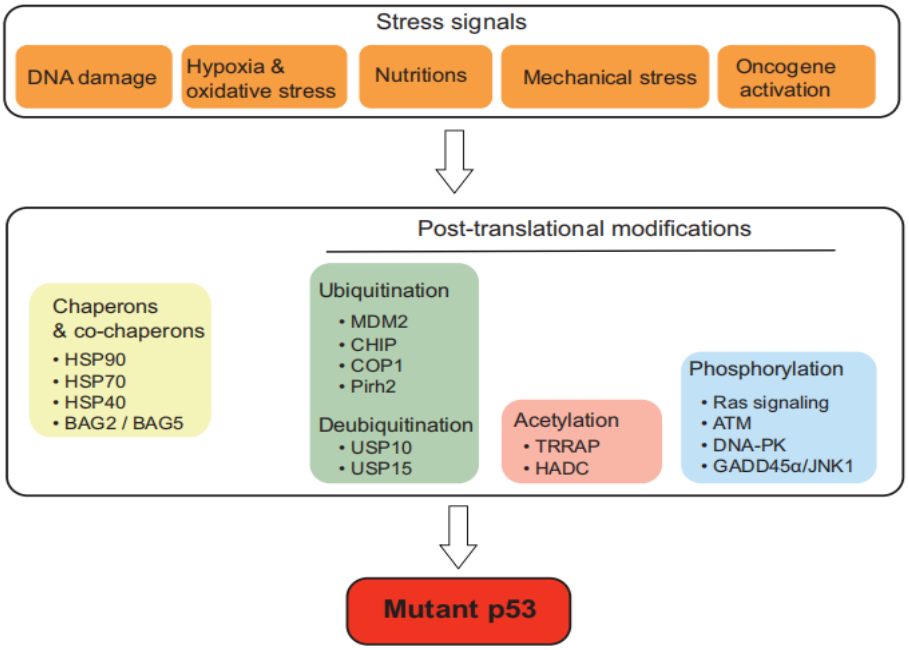
p53 induces apoptosis, cell cycle arrest, senescence, and other biological processes to mediate cancer cell response to therapies. In contrast, GOF mutp53 has been reported to promote therapeutic resistance in cancer [82-90]. Enhanced drug

efflux through upregulation of ATP-binding cassette (ABC) transporters that extrude drugs out of cells is an important mechanism for multidrug resistance. While p53 represses the expression of ABC transporter ABCB1, GOF mutp53 induces ABCB1 expression to mediate the ATPdependent efflux of drugs from cells to promote chemoresistance [75]. Mechanistically, mutp53 is recruited to the ABCB1 promotor through interacting with ETS1 to activate ABCB1 transcription [91]. Mutp53 interacts with NF-Y to induce the expression of ephrin-B2, a ligand for the receptor tyrosine kinases ephrin receptors, which in turn upregulates the expression of the ABC transporter ABCG2 to promote chemoresistance [89].Cytochrome P450 (CYP450) family members are key enzymes in drug metabolism, mediating the process of drug oxidation. Mutp53 (e.g. R282W) induces CYP450 enzyme 3A4 (CYP3A4) expression to promote resistance to several CYP3A4-metabolized chemotherapeutic drugs [82].

Mutp53 protein accumulation and regulation:

p53 protein is exquisitely regulated by many different mechanisms to maintain its proper levels and function in cells. Among these mechanisms, post translational modifications represent a very efficient and critical one for p53 regulation. The posttranslational modifications include ubiquitination, phosphorylation, acetylation, methylation, sumoylation, etc., which affect p53 protein stability, conformation, localization, and interaction with other proteins [79-81]. The E3 ubiquitin ligase MDM2, which directly binds to p53 and ubiquitinates it for proteasomal degradation, is the most critical negative regulator of p53 in cells. Meanwhile, MDM2 is a direct target of p53; p53 transcriptionally induces MDM2. Thus, MDM2 and p53 form a negative feedback loop to tightly regulate p53 protein levels [27, 91]. Mutp53 protein is frequently stabilized and accumulated to very high levels in tumor tissues, which is required for the execution of it GOF activities [80-88] .Recent studies have shown that mutp53 can be regulated by post translational modifications (e.g. ubiquitination, acetylation, phosphorylation, etc.), chaperones and co-chaperone proteins, and different stress signals [Figure 2]. The regulation of mutp53 protein in cancer. Mutp53 protein accumulates to very high levels in cancer cells. Mutp53 protein levels in cancer cells are regulated by different mechanisms, including post translational modifications (such as ubiquitination, acetylation, and phosphorylation), chaperones and co-chaperone proteins, as well as different stress signals [95].

Figure-5: Chaperones and co-chaperone proteins, and different stress signals



Therapeutic strategies to target mutp53:

Given that the p53 gene is mutated in >50% of all human cancers and mutp53 frequently displays GOF activities, mutp53 has become an attractive target for cancer therapy. Based on the facts that mutp53 is frequently accumulated to very higher levels in tumor tissues, loses transcriptional activity of wildtype p53, and frequently acquires GOF activities through interacting with other proteins and/or regulating critical downstream signaling pathways, different strategies have been developed to target mutp53 for cancer therapy. These therapeutic strategies can be classified into two major categories (Figure-6) The first is to target mutp53 directly by restoration of the wild-type tumor-suppressive function of p53 or deprivation of mutp53 through inducing its degradation. The second is to target specific mutp53-binding proteins or critical downstream signaling pathways of mutp53 to inhibit mutp53 GOF activities[89-93].

Restoring wild-type p53 function:

Since majority of p53 mutations in cancers are missense mutations, the idea to convert mutp53 to the wild-type p53 conformation and restore its transcriptional activity has attracted many studies. CP-31398, a styrylquinazoline compound, was identified to be able to restore the wild-type p53 conformation and transcriptional activity in cancer cells expressing mutp53 and inhibit their proliferation in 1999 from a high-throughput screen [94].

Figure-6: Therapeutic strategies targeting mutp53 in cancer:

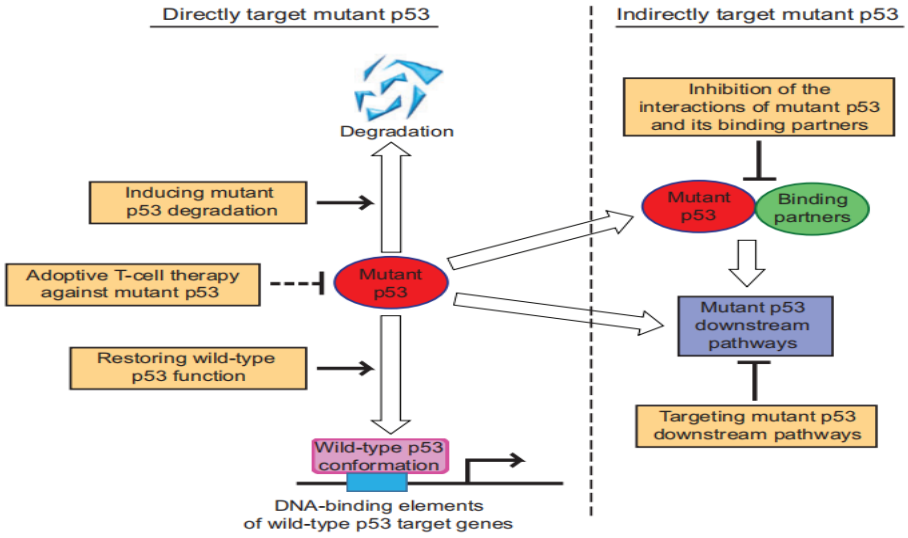


Figure-6, illustrating that the therapeutic strategies targeting mutp53 in cancer include targeting mutp53 directly or indirectly. The direct strategies include restoring wild-type p53 function to mutp53, inducing mutp53 degradation, and adoptive T-cell therapy against mutp53. The indirect strategies include inhibition of the interactions between mutp53 and its binding partners and targeting critical downstream pathways of mutp53.

Inducing mutp53 degradation:

The mutp53 is frequently accumulated to high levels in cancer cells to exert its GOF activities, inducing mutp53 degradation should be an effective strategy for cancer therapy. Since the interaction of mutp53 with the HDAC6/HSP90 chaperone complex is critical for mutp53 stabilization in cancer cells, disruption of the HDAC6/HSP90 complex by inhibitors of HSP90 or HDAC6 has been shown to be a promising strategy to induce mutp53 degradation [95-98]. Geldanamycin is the first HSP90 inhibitor used for targeting mutp53 to induce its degradation [36, 97]. 17-AAG, an analog of Geldanamycin, induces proteasomal degradation of mutp53 through MDM2 and CHIP-mediated ubiquitination [99]. Ganetespib, another HSP90 inhibitor,has a much higher potency in mutp53 degradation.

Targeting critical downstream pathways of mutp53:

Mutp53 frequently displays GOF activities through regulating different downstream signaling pathways in cancer cells. Therefore, targeting some critical downstream pathways of mutp53 provides an alternative strategy for treating cancers expressing mutp53. For instance, mutp53 upregulates EGFR/ integrin recycling and PDGFRb to promote tumor metastasis, and thus inhibition of EGFR by cetuximab or inhibition of PDGFRb by imatinib blocks cancer metastasis [98-100]. The Rac1 inhibitor NSC23766 inhibits mutp53 GOF activity in tumor growth and metastasis through blocking the activation of Rac1 signaling by mutp53 [6, 24,100]. The ROCK inhibitor Y27632 blocks RhoA/ ROCK pathway activated by mutp53 and inhibits mutp53 GOF in promoting glycolysis and tumorigenesis [28, 101].A growing body of evidence has demonstrated that mutp53 often renders cancer cells dependent on some downstream pathways for survival, and inhibition of these pathways leads tosynthetic lethality, providing new therapeutic targets for tumors expressing mutp53.

Mutp53 types in cancer:

TP53 is located on the short arm of human chromosome 17 (17p13.1) and consists of 11 exons, 10 introns and 393 amino acid residues. p53 protein is a transcription factor that is usually divided into three functional domains: the amino-terminal domain, the DNA binding domain and the carboxy-terminal domain [21, 102].

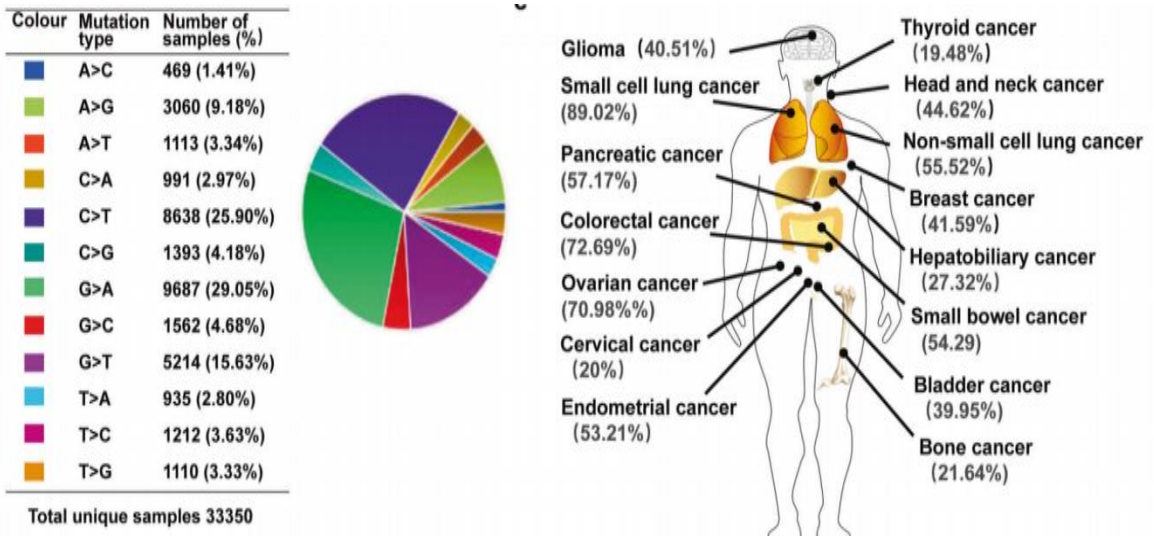
Wild-type p53 (wtp53) plays pivotal role in many important biological processes by regulating the transcription of several target genes [12]. However, mutp53 not only loses the tumor suppressor function of wtp53, but also acquires new functions that contribute to the progression of malignant tumors [33, 103]. The main mutant types of TP53 include missense mutations,

truncating mutations, inframe mutations, and splice mutations . Missense mutations result in single amino acid substitutions, which can display gain-of-function activity during tumorigenesis, such as p53 R175H and R273H mutants that promote tumor cell invasion and migration [9, 44, 104]. Approximately 80% of TP53 mutations are missense mutations [88]. It is mainly located in exons 5–8 , which encode the DNA binding domain, with the most common mutation sites occurring at R175, G245, R248, R249, R273 and R282 . Using the COSMIC Database (https://cancer.sanger.ac.uk/ signatures/) showed that the most substitution mutations are G to A transitions, followed by C to T transitions . Missense mutations are usually divided into two categories. One category is DNA contact mutations, which occur in amino acids in contact with DNA, resulting in the inability of p53 to bind to DNA, such as p53 R273H and R248Q mutants. The other category is conformational mutations, which occur in amino acids that maintain structure, resulting in unfolded proteins, such as p53 R175H, Y220C and R249S mutants [29].

Mutp53 spectrum in cancer:

Evidence suggests that the TP53 mutational spectrum differs between tumors [38,39]. The cBioportal for Cancer Genomics Database (https://www.cbioportal.org/) showed that frequency of TP53 mutations in tumor tissue samples from 10,000 cancer patients is 42%. However, the mutation frequency varies across different types of tumors, with mutation frequency of 89.02% in small cell lung cancer and 72.69% in colorectal cancer. In contrast, the frequency of TP53 mutations is lower in malignancies such as thyroid cancer, cervical cancer and bone cancer . In lung and liver cancers, G:C to T:A transversions are the most common substitutions. In colorectal cancer, brain tumors, and leukemia, transition mutations mostly occur in CpG dinucleotide hotspots. In esophageal cancer, A:T base pair mutations are more common [39, 104]. Furthermore, mutation spectrum of TP53 also varies among tumor subtypes in the same organ [9]. For example, Dumay et al. studied the mutational spectrum of TP53 in 572 breast cancers and found that luminal breast cancers were predominantly missense mutations, particularly A:T to G:C transitions, whereas basal breast cancers showed a higher incidence of truncating mutations [40]. Moreover, the mutational spectrum of TP53 in tumors is correlated with environmental carcinogens. For instance, ultraviolet light induces CC-TT double base transition in invasive squamous cell carcinomas of the skin [41]. More G to T transitions occur in smokers compared to non-smokers in lung cancer [92]. Aflatoxin B1 induces typical G:C to T:A transversions in codon 249 of p53 in primary hepatocellular carcinoma [39, 105]. Remarkably, mutations in TP53 are associated with poor prognosis in malignant tumors [101]. The cBioportal for Cancer Genomics Database showed that expression of mutp53 is negatively correlated with overall survival of patients in breast cancer, pancreatic cancer, hepatobiliary cancer, bone cancer, non-small cell lung cancer, and thyroid cancer (Figure-7).

Figure-7: Mutp53 spectrum (prevalence and Mutation Type) in Different cancer:



Ferroptosis:

Ferroptosis is an iron-dependent form of cell death that has been reported to inhibit tumor growth as an independent pathway [73–75]. Interestingly, p53 was found to have a critical but complex role for the regulation of ferroptosis. Although most studies have supported the function of p53 in promoting ferroptosis. In certain circumstances, p53 can inhibit ferroptosis . In lung cancer, wtp53 inhibits cystine uptake by suppressing expression of SLC7A11, leading to reduced activity of GPX4 and cellular antioxidant capacity, which causes the onset of ferroptosis [105]. Wtp53 also inhibits the level of H2Bub1 by promoting nuclear translocation of the deubiquitinase USP7, further contributing to the inactivation of SLC7A11 expression [106]. Furthermore, wtp53 induces ALOX12 expression by downregulating SLC7A11 levels, resulting in ALOX12-dependent ferroptosis [107]. In esophageal and lung cancers, mutp53 suppresses SLC7A11 expression by interacting with

the master antioxidant transcription factor NRF2, which promotes the accumulation of ROS and induces ferroptosis . p53 can regulate the ferroptosis pathway through diverse mechanisms. In most cases, p53 promotes ferroptosis. However, in certain circumstances, p53 can inhibit the onset of ferroptosis.

Figure-8: Schematic representation of the mechanism of mutp53 in ferroptosis:

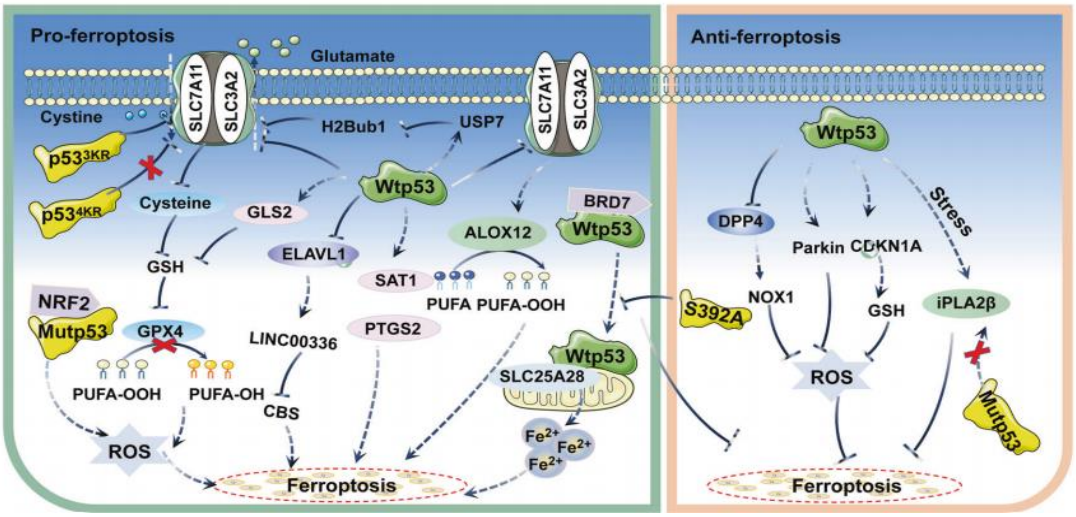


Figure-8: Schematic representation of the ferroptotic process. Lipid peroxidation resulting in the generation of Lipid-ROS is considered the point of no returnin the execution of ferroptosis. The current hypothesis is that peroxidized PL-PUFAs destabilize the membrane thus compromising its barrier functions. PUFA are introduced into cell membranes, as PL-PUFA, through the combined activity of ACSL4 and LPCAT3, while lipid peroxidation is catalyzed by increased available iron (LIP) through Fenton reactions, or by lipoxygenases (ALOX), which use iron as a cofactor.

p53 and stress conditions triggering ferroptosis:

Hypoxia:

Hypoxia is chronic in most tumors, and this condition is often exploited by cancer cells to sustain proliferation, metabolism, tumorinvasion, and metastasis [108]. In this context, a key role is played by HIF1, a transcription factor activated by low oxygen and frequently overexpressed in cancer [109]. Interestingly, HIF1 inhibits ferroptosis by: i) upregulating SCD1 to increase MUFA synthesis; ii) inhibiting the expression of ACSL4 to reduce Lipid-ROS generation, and iii) inhibiting the degradation of SLC7A11 [33]. Therefore, the reduced efficacy of radiation or drug-based therapies in solid tumors has been, at least in part, associated with HIF1-mediated inhibition of ferroptosis [110].p53 is activated by hypoxia, driving a cellular response that also involves modulation of cell metabolism [22].

Oxidative stress:

ROS production is associated with both physiological and pathological conditions. Proper ROS production contributes to differentiation, immunity, and cell signaling, but uncontrolled accumulation leads to damage of proteins, lipids, and nucleic acids, causing “oxidative stress”, involved in cardiovascular and neurodegenerative diseases, obesity, aging, and cancer [44,89]. Oxidative DNA damage is one of the stimuli driving tumorigenesis and was detected in cells dying through ferroptosis [11-14]. Therefore, in addition to being an integral part of the molecular mechanism of ferroptotic death, oxidative stress might regulate the process itself [21]. p53 is activated by oxidative stress, and can reduce ROS to promote cell survival, or increase ROS to facilitate cell death, depending on its gene targets or binding partners. The cellular response to oxidative stress is mainly regulated by NRF2, a transcription factor that controls expression of several antioxidant proteins [110-111]. Notably, depending on cellular context, p53 can increase NRF2 levels by preventing its degradation, or reduce NRF2 levels by repressing its transcription.

Endoplasmic reticulum stress:

Nutrient deprivation, proteasome dysfunction, sustained secretory activity, and somatic mutations in ER client proteins cause dysregulated proteostasis in proliferating tumor cells, thus triggering activation of the unfolded protein response (UPR)[19,21]. Accumulation of unfolded/misfolded proteins in the ER is sensed by the receptors PERK, IRE1, and ATF6, that trigger activation/upregulation of transcription factors: ATF4, induced by PERK activation, XBP1s, produced by IRE1-dependent cytoplasmic splicing of XBP1 mRNA, and ATF6f, generated by proteolytic cleavage of activated ATF6. These factors orchestrate a transcriptional response aimed to: i) increase ER folding capacity; ii) inhibit cap-dependent translation;

iii) degrade misfolded/unfolded ER client proteins (ERAD). Overall these activities sustain cell survival ("adaptation phase" of UPR), but acute or unresolved ER stress stimulates apoptosis ("cell death phase") [112].

Nutrient deprivation and autophagy:

Autophagy is an evolutionarily-conserved process responsible for lysosomal degradation of intracellular cargoes, sustaining cell survival under nutrient shortage conditions [113]. Autophagy plays a paradoxical role in tumorigenesis, depending on the stage of tumor development; it is suppressive in early stages, mainly through degradation of potentially oncogenic molecules, but becomes oncogenic in advanced stages, promoting cell survival and ameliorating stress in the microenvironment [11,23]. Evidence of autophagy has been detected in cancer cells dying by ferroptosis, suggesting a potential connection between the two pathways Indeed, NCOA4 mediates autophagy-dependent degradation of FTH, thus releasing iron (ferritinophagy) and triggering lipid peroxidation and ferroptosis [24,114].

Recently, other factors linking ferroptosis to specific autophagic processes have been identified, in particular affecting Lipid-ROS generation: Wild-type p53 modulates autophagy both directly and indirectly [21, 22,28]. When activated by DNA-damage, nuclear p53 upregulates autophagy-associated genes, contributing to cancer cell death upon chemotherapy [115]. In contrast, cytoplasmic/ mitochondrial p53 can suppress autophagy [13]. Additionally, p53 controls autophagy via interaction with key metabolic pathways, for instance positively modulating AMPK activity and negatively regulating AKT and mTOR [115-117].

Figure-9: Relationship between stress-related signaling pathways, ferroptosis, and tumor growth, from a p53 status-centered (wt or mut) perspective.

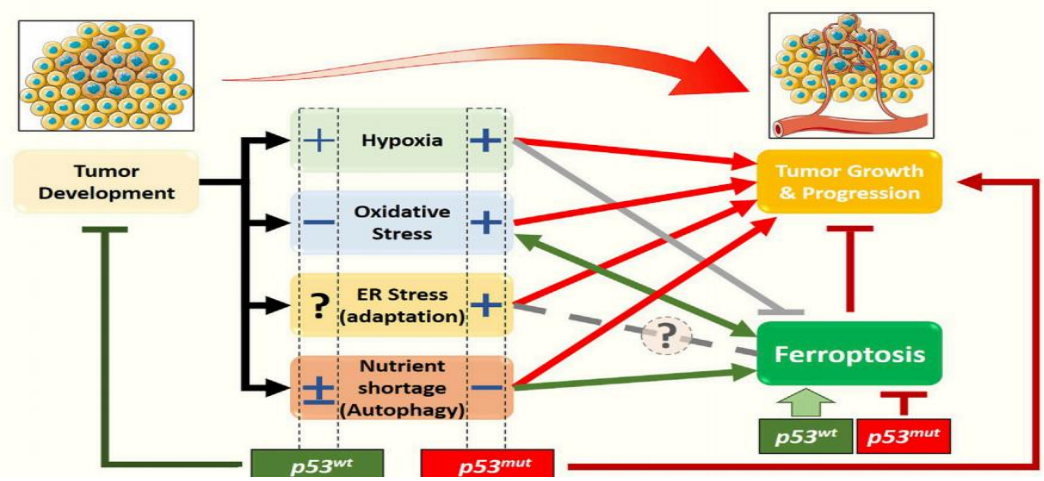


Figure-9, revealed that, in the early stages of solid tumor development, cancer cells are subjected to oxygen and nutrient shortage, oxidative stress, and dysfunctional proteostasis. The molecular pathways activated in response to those stimuli will define the fate of the early tumor: survival (red arrows) or death. Beyond apoptosis, very recently, the new form of cell death named ferroptosis has been described to have a role in preventing/limiting the early tumor formation and growth, although the precise molecular mechanisms are still elusive. The p53 status (wt vs. mut) might have a significant impact on ferroptosis and tumor growth through a positive (+) or negative (−) effect on cancer-associated stress-related signaling pathways. [117]

A study,directly examine the interplay between mutant p53 or Mdm2 and wild type p53 in gene occupancy and expression, an integrated RNA-seq and ChIP-seq analysis was performed in vivo using isogenically matched mouse strains. Response to radiation was used as an endpoint to place findings in a biologically relevant context. Unexpectedly, mutant p53 and Mdm2 only inhibit a subset of wild type p53-mediated gene expression. In contrast to a dominantnegative or inhibitory role, the presence of either mutant p53 or Mdm2 actually enhances the occupancy of wild type p53 on many canonical targets. The C-terminal 19 amino acids of wild type p53 suppress the p53 response allowing for survival at sublethal doses of radiation. Further, the p53 mutant 172H is shown to occupy genes and regulate their expression via non-canonical means that are shared with wild type p53. This results in the heterozygous 172H/+ genotype having an expanded transcriptome compared to wild type p53 + /+.[12, 111,118].

P53-immune response:

A study also The importance of cancer-cell-autonomous functions of the tumour suppressor p53 (encoded by TP53) has been established in many studies, but it is now clear that the p53 status of the cancer cell alsohas a profound impact on the immune response. Loss or mutation of p53 in cancers can affect the recruitment and activity of myeloid and T cells, allowing immune evasion and promoting cancer progression. p53 can also function in immune cells, resulting in various outcomes that can

impede or support tumour development. Understanding the role of p53 in tumour and immune cells will help in the development of therapeutic approaches that can harness the differential p53 status of cancers compared with most normal tissue.

Figgure-10a: Functions of p53 in tumour cells that regulate interactions with the immune system:

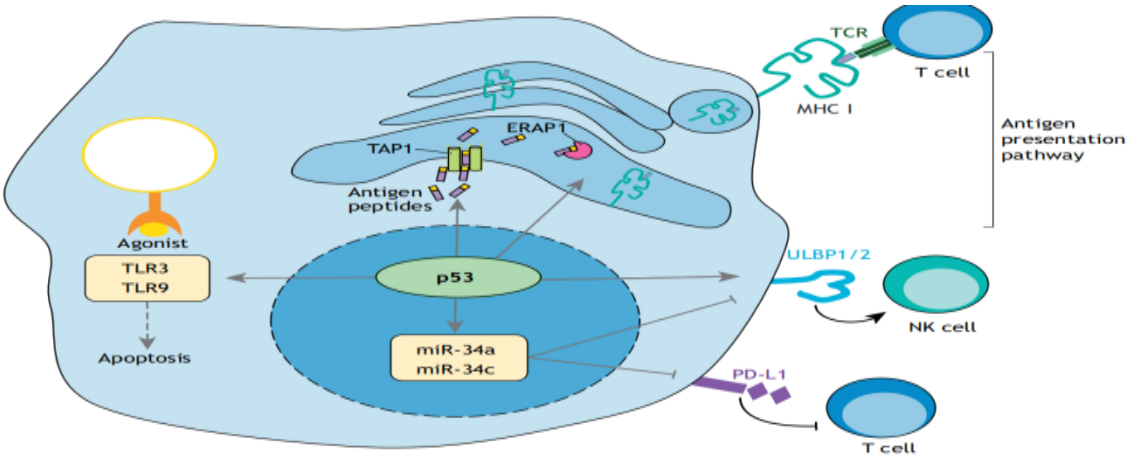


Figure-10a, revealed that the p53 regulates endogenous antigen presentation through transcriptional control of ERAP1 and TAP1. In addition, p53 regulates the expression of the NKG2D ligands ULBP1 and ULBP2, either positively as a transcriptional target or negatively through the upregulation of miR-34a. The miR34 family also represses PD-L1 expression, an inhibitor of T cell activity. TLR3 and TLR9 are transcriptional targets of p53 that promote agonist-induced cell death.

Figure-10b: Loss of p53 in cancer cells modifies the immune environment:

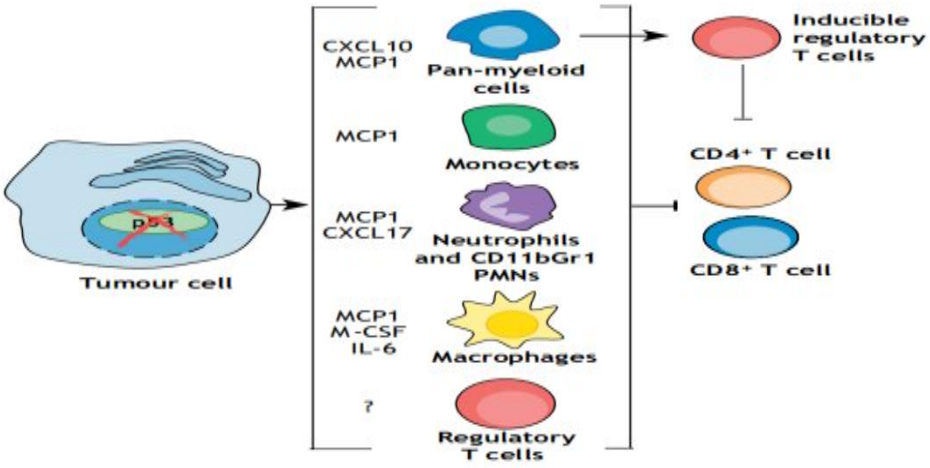


Figure-10b, illustrated that the loss of p53 in cancer cells modulates their cytokine production, which influences various tumour-associated immune populations (myeloid cells, neutrophils, macrophages, monocytes and regulatory T cells). MCP1 recruits all myeloid cells – neutrophils, macrophages and monocytes – while CXCL17 attracts PMNs (CD11bGr1), and CXCL10 is a general myeloid chemoattractant. M-CSF promotes the homeostasis and differentiation of macrophages. Regulatory T cells are enriched in p53-null tumours, a response that is in part mediated through de novo generation of Tregs by PMNs. Overall, the recruitment of these immune populations reduces T cell responses to favour tumour growth.

The role of wild-type and mutant p53 in pro-inflammatory cytokine signalling:

As discussed above, cytokines can both inhibit or induce p53 function. p53, in turn, modulates pathways that are activated in response to cytokine signalling. Wild-type p53 regulates inflammation through signal transducer and activator of transcription 3 (STAT3), which acts downstream of the inflammatory cytokine IL-6 (Fig. 12). Loss of p53 in mouse models of pancreatic and prostate cancers results in increased STAT3 phosphorylation, which is mediated, in part, through enhanced autocrine/paracrine IL-6 signalling [33,44,113, 118]. p53 deficiency in pancreatic cancer cells increases reactive oxygen species (ROS), which inhibits Src homology region 2 (SHP2) phosphatases and drives STAT3 activity. In addition, p53

ablation in PTEN-null mouse embryonic fibroblasts (MEFs) leads to enhanced STAT3–Myc pro-growth signalling [11, 33, 119]. STAT3 activation is regulated through a negative feedbackloop by suppressors of cytokine signalling (SOCS) proteins. SOCS1, an inhibitor of STAT3, binds to the N-terminal transactivation domain of p53 to induce cell cycle arrest and senescence.

Figure-11: Functions of p53 in response to immune signalling:

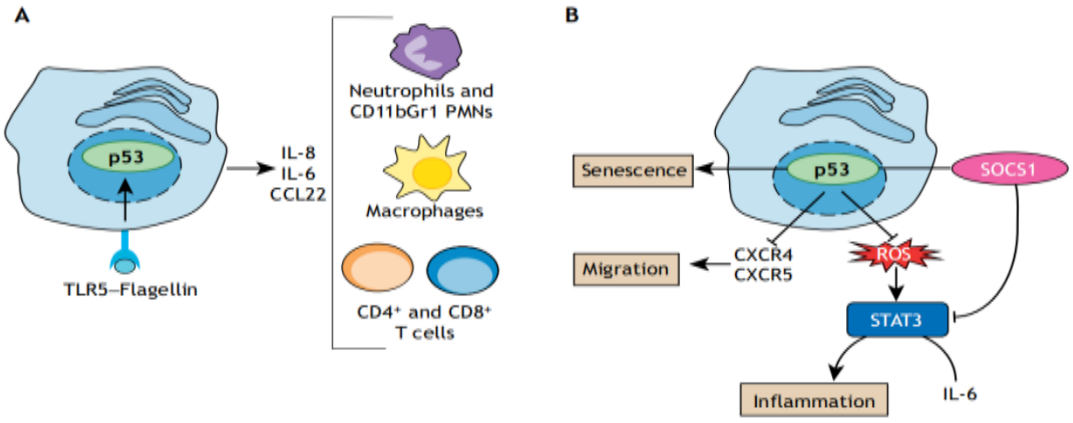


Figure-11, revealed that,A) Activation of p53 downstream of TLR5 ligation by bacterial flagellin increases the secretion of IL-6, IL-8 and CCL22, which can regulate the recruitment of leukocytes including macrophages, neutrophils and T cells. (B) p53 suppresses STAT3 signalling (which drives inflammation) by downregulating ROS. SOCS1, a modulator of STAT3 activity, interacts with p53 to induce senescence. Migration-mediated chemokine signalling is also regulated by p53 through its inhibition of the chemokine receptors CXCR4 and CXCR5.

Functions of p53 in stromal populations during tumour development and progression:

The tumour–stromal network is a heterogeneous population of cells, originating from mesenchymal or lymphoid origins, that directly or indirectly interact with tumour cells [118,119]. While there has been a focus on how alterations of p53 in the tumour cells contribute to cancer progression, tumour cells expressing wild-type p53 show accelerated growth when transplanted into p53-null hosts [25, 114]. demonstrating a role for p53 in the non-cancerassociated stromal cells in modulating tumorigenesis.rogression.

Figure-12: Functions of p53 in stromal cells.

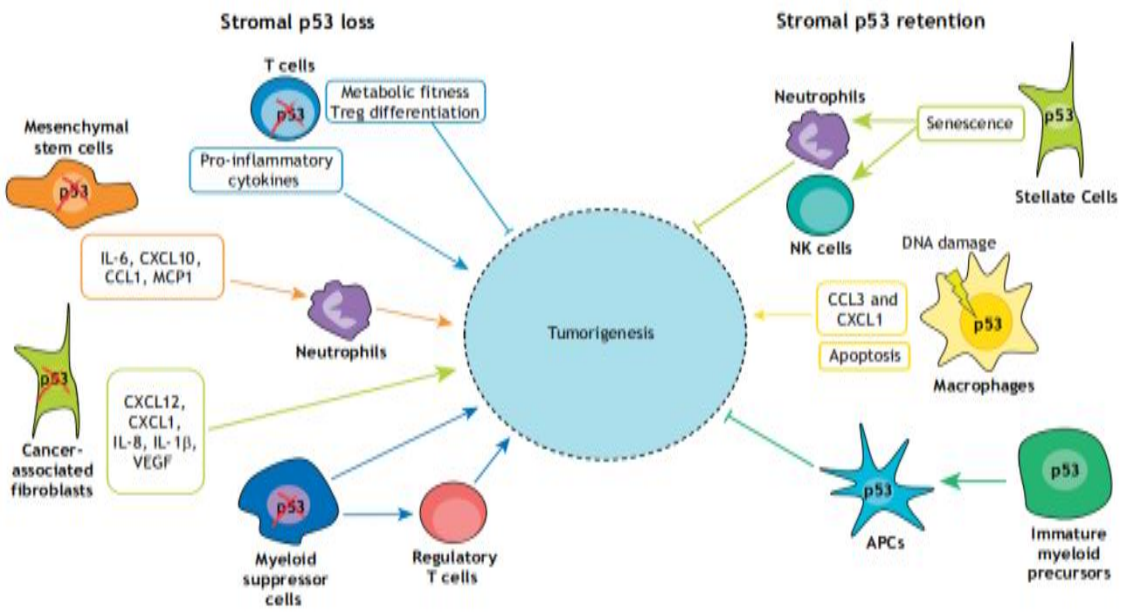


Figure-12, revealed that the tumour–stromal network is a heterogeneous population of cells, originating from mesenchymal or lymphoid origins, that directly or indirectly interact with tumour cells .

Immune recognition of p53 in cancers:

Wild-type p53 levels are very low in normal cells where as, mutant p53 proteins tend to accumulate at high levels in cancer cells. These observations raise the possibility that the tumour-specific expression of p53 could stimulate a B cell (humoral) response, providing diagnostic value, as well as activating T cells that may be harnessed for vaccination (Fig. 13).

Figure-13:. Immune responses to p53 expression in tumour cells:

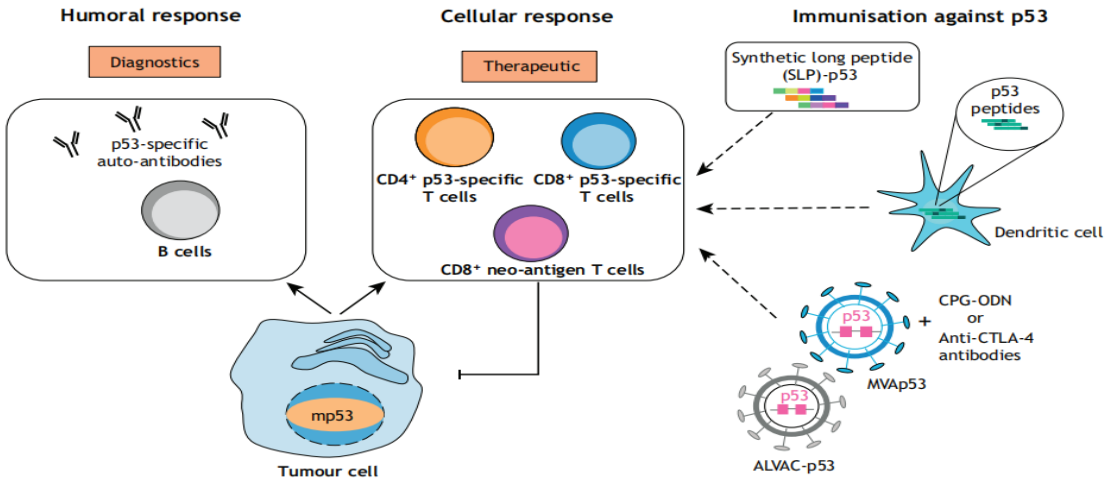


Figure-13, illustrated that the p53 expression in cancer cells can provoke immune recognition, most commonly in response to the accumulation of high levels of mutant p53 protein. p53 can elicit T-cell-specific responses both in CD4+ and CD8+ T cells, including those recognising neo-antigens derived from point-mutated p53 proteins. Therapeutic approaches using p53 vaccines or synthetic long peptides of p53 drive T cell responses, and have shown some efficacy in early clinical trials. Vaccine-based therapies include use of ALVAC-p53 and MVAp53. Combination therapy with either CPG-ODN or anti-CLTA-4 antibodies and MVAp53 elicits T cell responses that might reduce tumour growth. SLP-p53 alone and dendritic cells (DCs) pulsed with mutant p53 peptides can also drive p53-specific T cell responses.[120].

Tumor microenvironment:

Increasing evidence suggests that mutp53 can regulate the tumor microenvironment. Tumor-associated macrophages (TAM) are the hallmark of solid tumors. Wtp53 suppresses tumorigenesis by promoting an anti-tumor microenvironment and modulates M1 polarization pattern in neighboring macrophages [91]. Interestingly, in colon cancer, mutp53 selectively releases miR-1246-rich exosomes that are taken up by surrounding macrophages, leading to miR-1246-dependent reprogramming into a tumor-promoting M2 state [102].CAFs (cancer-associated fbroblasts) are an essential part of the TME and modulate infammatory and leukocyte recruitment signals [112]. When CAFs come into contactwith cancer cells, they trigger the IFN-β pathway, which interacts with wild-type p53 in fbroblasts to inhibit cancer cell migration and decrease tumor development (Fig-14) [113, 114]. In contrast to its wild-type counterpart, mutant p53 in cancer cells regulates and inhibits the tumor-suppressive response to IFN-β via inhibiting STAT1 phosphorylation and downstream targets of IFN- β. IFN-β produced by CAFs, in turn, can lower the amounts of mutant p53 RNA in tumors [125] Te infammatory microenvironment can disrupt the equilibrium of this regulatory network, causing a molecular stop that both suppresses and enhances the tumorigenic efects of mutant p53 in cancer cells [116]. Reactivating wild-type p53 activity might be a synergistic opportunity for targeting IFN-related therapy, as the mutational state of p53 is important for targeting IFN-related therapy [121].

Figure-14 :Mutant p53 and tumor microenvironment (TME)

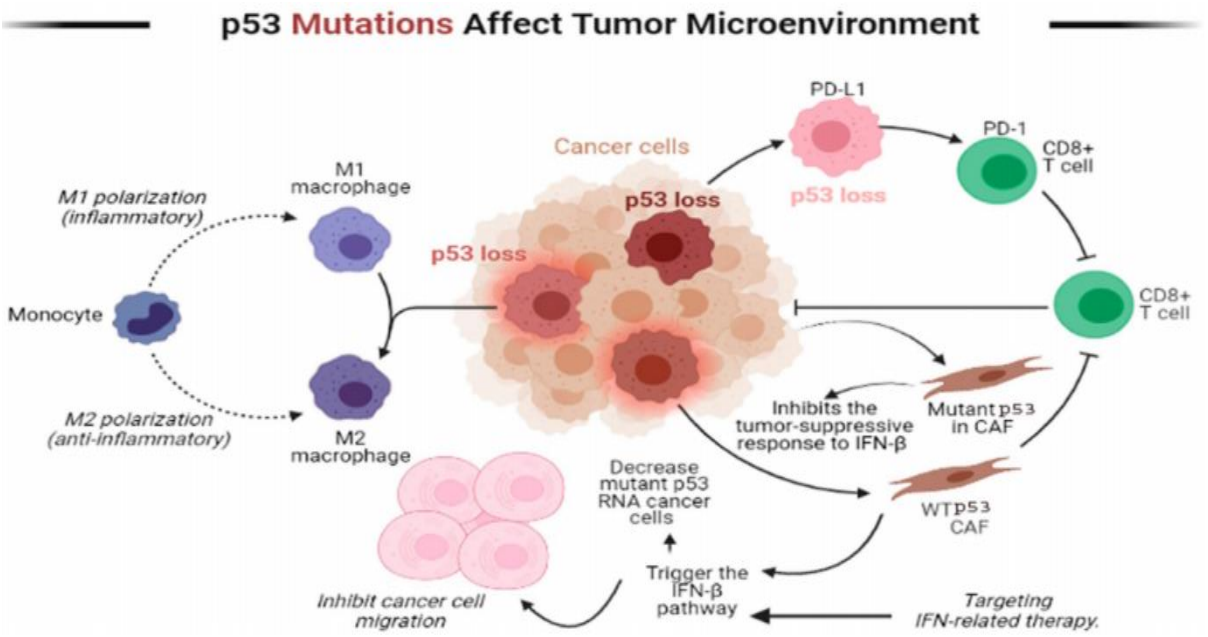


Figure-14, illustrated that the Mutant p53-expressing tumors can reprogram M2-type macrophages (M2) and increase tumor invasion. High wild-type p53 activity acts as a brake on M1-like macrophage and, decreased M1-like gene expression. When cancer associated fbroblasts (CAFs) come into contact with cancer cells, their Interferon-β pathway is triggered and interacts with wild-type p53 in fbroblasts to inhibit cancer cell migration, decrease tumor development, and response to stress. In contrast, the function of CAFs is impaired in the presence of mutated p53, where they promote cancer cell proliferation. p53 transactivates programmed death-ligand 1 (PD-L1) and its receptor programmed death-1 (PD-1) in cancer cells and normal T cells in response to stress leading to suppression of CD8+ T cells

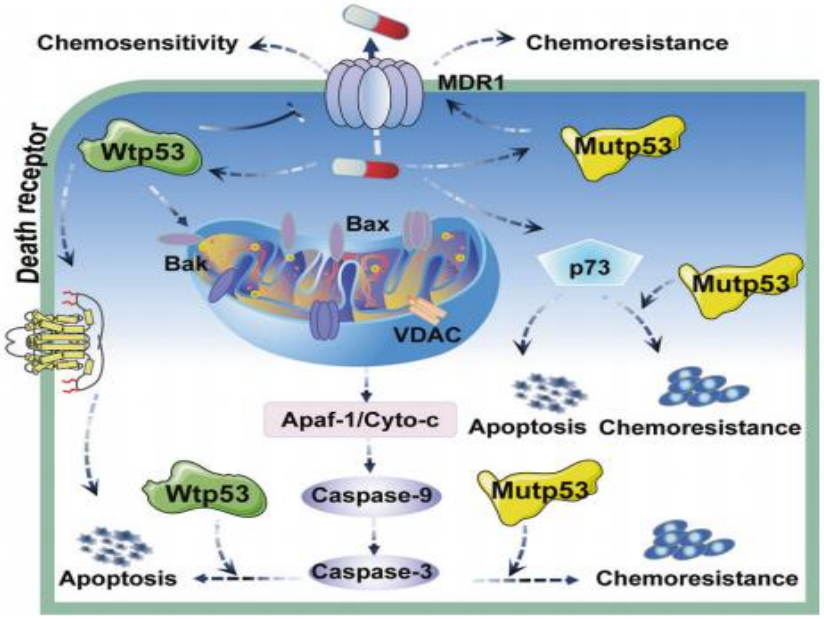
2. CLINICAL IMPACT OF MUTP53 IN CANCER:

Chemotherapy:

Chemotherapy is an integral part of cancer treatment, but chemoresistance has become a major barrier to treatment. Plenty of evidence suggests that expression of mutp53 is positively correlated with increased chemoresistance in different tumors .

Induction of apoptosis is one of the most important functions of p53, and disruption of this function can promote tumor chemoresistance [104]. Wtp53 can induce apoptosis through mitochondrial and Fas-mediated apoptotic pathways [105, 106]. As shown in Fig. 5, wtp53 induces oligomerization of Bax, Bak and VDAC, increases the permeability of the outer mitochondrial membrane and promotes the release of cytochrome c. Chemotherapeutic agents such as 5-fluorouracil and oxaliplatin sensitize colorectal cancer cells carrying wtp53 to Fas-mediated apoptosis [117]. In contrast, p53 R175H, L194F, R249S, and R280K mutants lose the ability to activate the Bax/Bak lipid pore and alter VDAC multimerization state, which inhabit apoptosis in cancer cells [135]. In osteosarcoma, p53 R273H mutant reduces expression of procaspase-3, resulting in failure of chemotherapeutic agents such as methotrexate and doxorubicin to induce apoptosis [128]. In colon cancer, mutp53 does not bind to PUMA promoter to activate its transcription, which helps tumor cells evade apoptosis and reduces sensitivity to 5-fluorouracil [119]. Furthermore, in tumor cells lacking functional p53, various chemotherapeutic agents can cause apoptosis by inducing expression of p73. Yet, mutp53 can inactivate p73 in colon cancer, and downregulation of mutp53 enhances chemosensitivity [110]. In colorectal cancer, mutp53 activates EFNB2 in response to DNA damage, while silencing EFNB2 increases the sensitivity of cancer cells to 5-fluorouracil [121]. Expression of mutp53 is positively correlated with increased resistance to chemotherapy in different tumors.

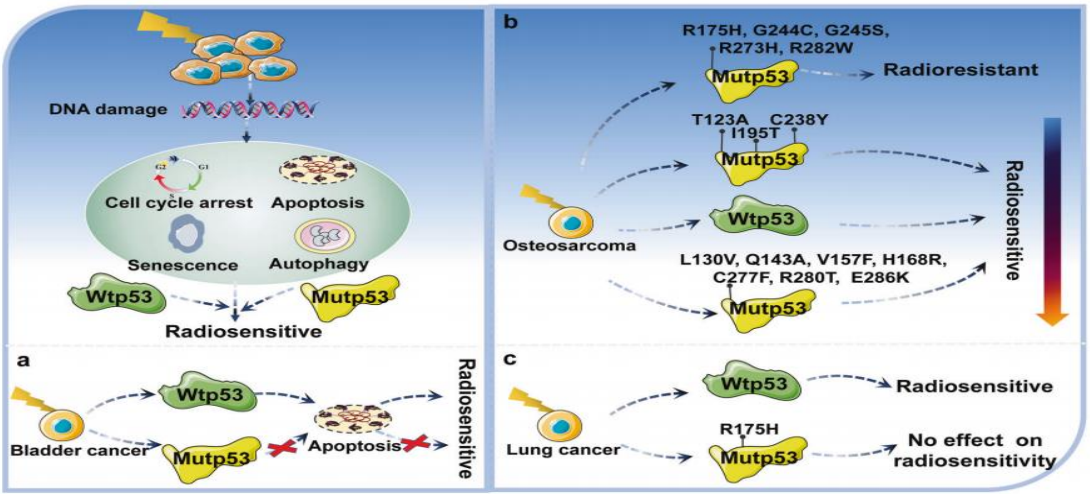
Figure-15: Schematic representation of the mechanism of mutp53 in chemotherapy:



Radiotherapy:

Radiotherapy is now considered to be one of the effective approaches to cancer treatment. However, many tumors exhibit resistance to radiation [114]. Hence, it is critical to determine the role of p53 status in radiotherapy (Figure15). In diffuse intrinsic pontine gliomas, mutations in p53 are a major driver of increased radiation resistance, with mutp53 carrying patients less responsive to irradiation and relapsing earlier after radiotherapy with a worse prognosis [122]. O'Connor et al. studied the response of p53 status to radiation in 60 different cancer cell lines. In contrast to cell carrying wtp53, most tumor cells carrying mutp53 failed to induce expression of CIP1/WAF1, GADD45 and MDM2 mRNA, as well as G1 phase arrest after γ-irradiation, resulting in radioresistance [115]. In bladder cancer, ionizing radiation can induce tumor cells carrying wtp53 to undergo G1 phase arrest and apoptosis, resulting in a higher radiosensitivity. In contrast, it is not significantly observed in tumor cells carrying mutp53 (Fig. 15) [116]. The previous reports are also demonstrated that mutp53 lost the ability to induce G1 phase arrest after γ-irradiation [117,119]. In glioblastoma, clonogenic survival assays have shown that U87 cells carrying wtp53 and T98 cells carrying mutp53 exhibit essentially identical sensitivity to fractionated radiotherapy. But cells carrying wtp53 in response to ionizing radiation exhibit accelerated senescence [123]. In ovarian cancer, cells carrying wtp53 are very sensitive to irradiation, which leads to p53 accumulation after irradiation, whereas cells carrying mutp53 show varying degrees of radiation resistance and do not lead to p53 accumulation after irradiation [119]. In head and neck cancer [120], hepatocellular carcinoma [121], cervical cancer [12], and endometrial cancer [123], cells carrying mutp53 are also more resistant to radiation. Furthermore, transgenic mice carrying mutp53 increases resistance of various hematopoietic cell lineages to γ-irradiation, and overexpression of p53 R193P or A135V mutants increases radiation resistance of mouse hematopoietic cell by 45–57% [124].

Figure-16: Schematic representation of the mechanism of mutp53 in radiotherapy



Mutp53 can regulate radiotherapy through various mechanisms. In most cases, expression of mutp53 leads to radiotherapy resistance. However, under a certain context, mutp53 expression can promote radiotherapy sensitivity or have no effect on radiotherapy sensitivity.

Figure-17: Schematic representation of the mechanism of targeting mutp53 for tumor therapy.[125]

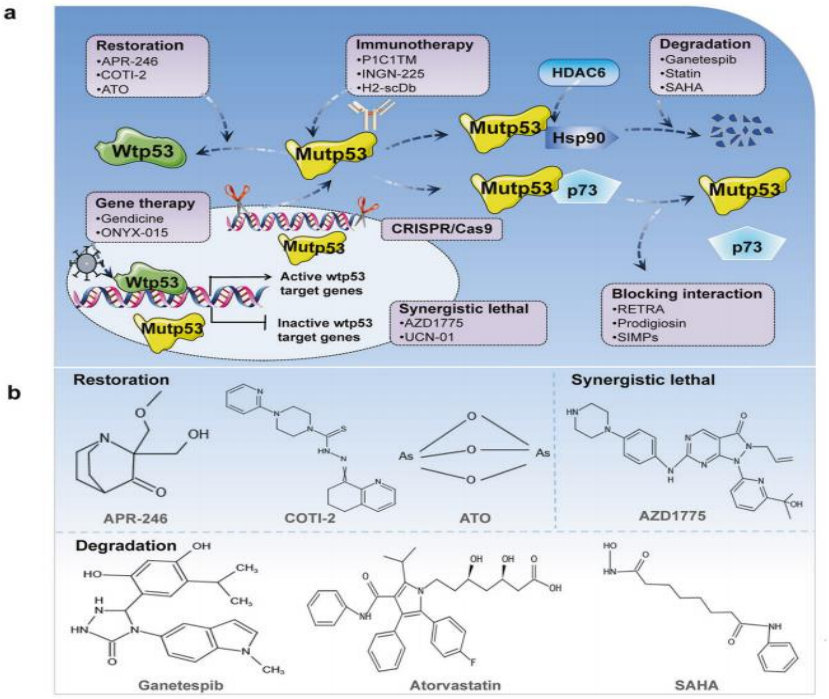


Figure-17, illustrating that , a) Treatment strategies for tumor cells carrying mutp53. b) Chemical structures of common drugs used in clinical trials.

Consequences of Mutant p53 Expression to Tumor therapy:

The realization that loss of p53 and expression of mutant p53 may not be analogous has also raised the question of whether the presence of a mutant p53 protein may affect the response to therapy. Whereas there is evidence that the presence of mutant p53 may dampen the response to restoration of wildtype p53 [2,11], reflecting a dominant negative activity of mutant p53, more recent studies have indicated that the retention of wild-type p53 can be detrimental to the therapeutic response in breast cancer. This effect is seen in tumors that express both mutant and wild-type p53 alleles [122]. Such studies highlight the possibility that in sometumor types wild-type p53 can be dominant over mutant, and that studies of patient response based on p53 status must take into account heterozygosity at the TP53 locus, as well as the presence of mutant or wild-type p53[125].

Therapeutic Strategies to Restore Wild-Type Activity to Mutant p53:

With so many different mutations and phenotypes it is not surprising that a variety of strategies are being explored to target tumors expressing mutant p53s (summarized in Figure 1). Wild-type p53 is a potent inducer of apoptosis and senescence when expressed in tumor cells, making the reactivation of some level of wild-type function in mutant p53 (which is generally expressed at high levels in cancer cells) an attractive therapeutic avenue. Interestingly, loss of wild-type function introduced by some destabilizing tumor-derived mutations can be rescued by additional point mutations that serve to stabilize the conformation of p53 protein,showing that the loss of structure is intrinsically reversible [138]. In addition, a variety of compounds that might restore wild-type p53 function have been characterized and are reviewed in several recent publications [22,38,126]. Small molecules that bind to a site in p53 formed in the Y220C mutant (PhiKan083 and PK7088) function by stabilizing the structure of this mutant p53, and so increasing the level of p53 with a wild-type conformation and activity [109, 110]. Other compounds bind to multiple mutant p53 proteins (e.g., PRIMA-1, or the soluble derivative PRIMAmet/APR-246, CP-31398, and SCH29074; interacting with the DNA binding domain, thereby promoting proper folding of the mutant protein and restoration of p53 function. However, the precise mechanistic function of these compounds and others, such as maleimide analogs and STIMA-1, remain to be elucidated [66,81, 127]. Whereas wild-type p53 requires binding to the metal ion Zn(2+) to fold correctly the R175H p53 mutant was found to be impaired in zinc binding [112]. Loss of metallothioneins that chelate and store intracellular of misfolded p53 and addition of zinc to the conformational mutants G245C and G245D p53 partially restored the wild-type conformation. The potential use of zinc to recover wild-type folding

has therefore been explored and this approach has been shown to restore chemosensitivity to anticancer drugs in cells expressing endogenous mutant p53 [120-123].

Figure-18. Strategies that Are Currently Being Explored to Target Mutant p53:

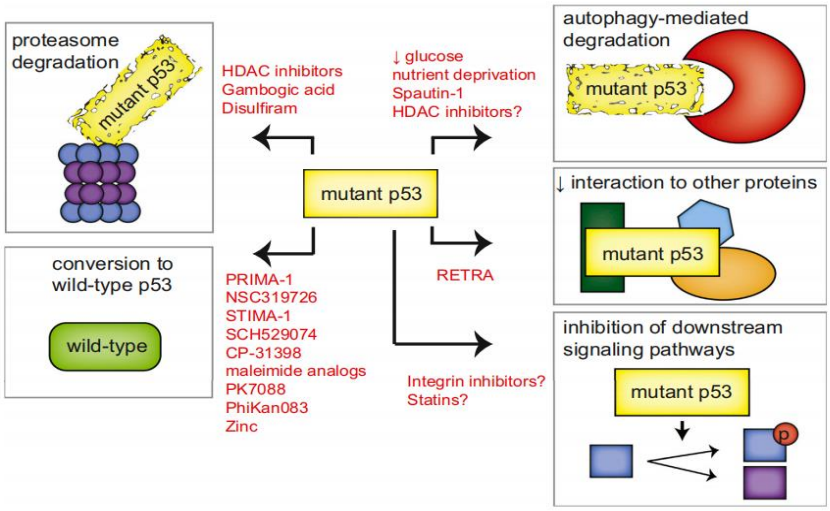


Figure18, illustrating that the depicted in red are schematics of the strategies that are currently being explored to target p53 mutant-expressing cancers. These strategies include promotion of mutant p53 degradation through the proteasome and autophagy pathways, restoration of wild-type p53 activity, interference with the interaction between mutant p53 and other proteins, and interference in signaling pathways downstream of mutant p53.

Mutant P53/ Wild type Expression for Diagnosis in cancer therapy:

Protein Analysis:

A study determined the prognostic value of p53 in ovarian cancer using a novel method of compartmentalized in situ protein analysis. In this study tissue array composed of 141 advanced stage ovarian cancers uniformly treated was constructed to evaluate of p53 protein expression, immunofluorescence-based method of automated situ quantitative measurement of protein analysis (AQUA)[tissue microarray construction, quantitative IHC,automated image acquisition and analysis] was used (figure-17). It was observed that the high nuclear p53 expression levels were associated with better outcome for overall survival (OS) (P = 0.0023) and disease-free survival (P = 0.0338) at 5-years subsequently High cytoplasmic p53 expression levels were associated with better outcome for OS (P = 0.0002). In multivariable analysis, high nuclear and high cytoplasmic p53 level with International Federation of Gynecology and Obstetrics (FIGO) stage were the most significant predictor variables for OS and high nuclear p53 level with FIGO stage were the significant predictor variables for disease-free survival. It was concluded as the assessment of the prognostic value of p53 protein levels using conventional immunohistochemistry is limited by the nonquantitative nature of the method. AQUA provides precise estimation of p53 protein levels and was able to elucidate the association of p53 protein levels and ovarian cancer prognosis[127].

Figure-20: Protein expression of p53 was determined using AQUA analysis on the basis of immunofluorence:

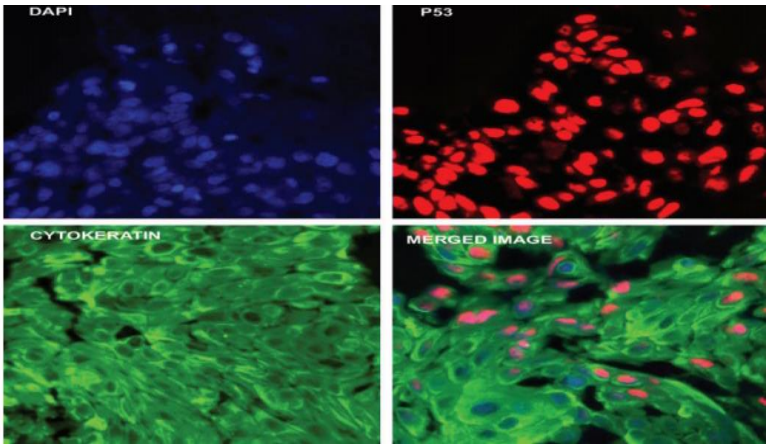


Figure-20, illustrated that the digital images of each tumor spot were captured using Cy3 anticytokeratin antibody to generate a tumor mask. 4,6-Diamidino-2-phelynindole (DAPI) was used to visualize nuclei and Cy-5 was used to visualize p53. A three-color merged image for each tumor is also shown.

Mutant p53 protein accumulates in tumours and affects pathology:

As p53 does not accumulate in normal unstressed cells, positive p53 immunostaining may serve as a biomarker for the identification of precancerous cells. Analysis of resected human pancreatic cancer tissues revealed no correlation between p53 accumulation and tumour progression, but p53 accumulation did correlate with lymph node metastasis and increased liver metastasis [18]. A study revealed that theMutations in the TP53 (p53) gene are present in a large fraction of human tumours, which frequently express mutant p53 proteins at high but heterogeneous levels. The clinical significance of this protein accumulation remains clouded. Mouse models bearing knock-in mutations of p53 have established that the mutant p53 proteins can drive tumour formation, invasion and metastasis through dominant negative inhibition of wild-type p53 as well as through gain of function or 'neomorphic' activities that can inhibit or activate the function of other proteins. These models have also shown that mutation alone does not confer stability, so the variable staining of mutant proteins seen in human cancers reflects tumour-specific activation of p53-stabilizing pathways. Blocking the accumulation and activity of mutant p53 proteins may thus provide novel cancer therapeutic and diagnostic targets, but their induction by chemotherapy may paradoxically limit the effectiveness of these treatment [128].

The role of mutant p53 protein in human cancer -diagnosis:

Immunohistochemical:

IHC analysis of human breast cancer sections of known p53 status using the p53 antibody DO-1 reveals a striking heterogeneity in mutant p53 protein levels (Figure-18). The background is very low in p53-null tumours (Figure 3A), but staining in different tumours expressing mutant p53 is highly variable (Figure-18B, C). Although the increased expression of mutant p53 in the tissues of Mdm2-null and p16INK4a-null mice suggests that wild-type and mutant p53 are regulated by similar mechanisms [22], the regulation of mutant p53 is still poorly understood. The mechanism by which it is stabilized in human tumours and the causes of such heterogeneous expression (Figure-18) remain unknown. Mutant p53 can have a dominant negative effect on wild-type p53 Mutant p53 may contribute to human cancer by exerting a dominant negative effect on wild-type p53. Early studies of LFS patients showed that p53 mutations with such properties are associated with cancer development [27], a correlation that has been confirmed by a more general analysis of >200 p53 mutants [28]. This dominant negative effect arises from the fact that p53 binds DNA as a tetramer consisting of a dimer of dimers [29]. The wild-type and mutant p53 proteins form heterooligomers that show impaired DNA association and transcriptional activity [30–32]. Mutant p53 can thus inhibit wild-type p53 induction of target gene transcription and tumour suppressor function. The inhibition of wild-type p53 by mutant p53 has been demonstrated by many in vitro studies, often using exogenously expressed protein. The conclusions are supported by data from experiments using genetically engineered mouse models bearing knock in mutations of p53, in which the expression of the mutant protein is subject to physiological regulation. These mice express p53R172H or p53R270H, which are the murine equivalents of the LFS-associated p53R175H and p53R273H mutants. Irradiation of mouse embryos in utero caused significant p53-dependent apoptosis in the brain, which was suppressed in p53-null, p53R172H/R172H as well as p53R172H/+ mice [21]; therefore, the p53R172H allele inhibited the DNA damage response induced by the wild-type p53 allele. Cell cycle analysis of primary mouse embryonic fibroblasts (MEFs) prepared from p53R270H/+ and p53R172H/+ mice revealed that their phenotype is more similar to that of p53-null MEFs than p53+/− heterozygotes [129].

Figure-21: Human tumours accumulate high but heterogeneous levels of mutant p53 protein.

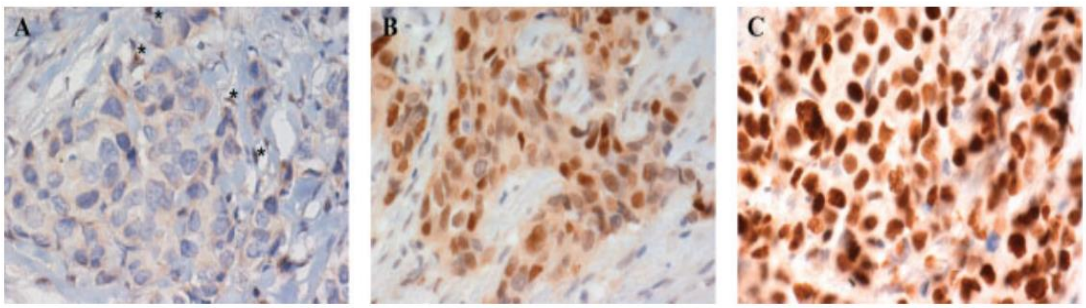


Figure-21, illustrated that the Immunostaining of breast tumours expressing mutant p53 with the p53 antibody DO-1, showed p53 accumulation in the nuclei (images provided by Borivoj Vojtesek and Rudolf Nenutil). (A) A tumour with a frameshift mutation in p53 that results in no protein expression was shown weakly positive staining only in stromal cells (marked by

asterisks). (B) A tumour expressing p53C135Y showed strong heterogeneous staining. (C) A tumour expressing p53E285K showed strong homogeneous staining.

Strategies for Boosting Wild-Type p53 Activity in Cancer:

Gene Therapy, Cytotoxic Chemotherapy, MDM2/MDMX(MDM4) Inhibitors, p53-Binding Compounds, Targeting p53 PTMs:

Chemotherapies used for the treatment of cancer include DNA damaging agents such as doxorubicin, 5-FU, irinotecan, actinomycin D, etoposide, mitomycin D, bleomycin, daunomycin, and cisplatin. These agents induce the DNA damage response and p53, ultimately resulting in apoptosis mediated by p53 target genes. A majority of clinical studies have demonstrated a correlation between adverse clinical outcomes after treatment with chemotherapy in patients with mutant p53-expressing tumors compared to patients with wild-type p53 tumors. Thus, it is thought that wild-type p53 function is at least partly responsible for the clinical efficacy of conventional chemotherapy [73]. Treatments such as conventional chemotherapy and γ-radiation activate p53, which mediates apoptosis through activation of a subset of p53 target genes such as Puma, Noxa, Bax, and death receptor 5 (DR5), among others [74]. Though effective, these conventional chemotherapies induce DNA damage that in some cases leads to secondary malignancies, therefore, novel strategies for specifically targeting p53 are needed [75]. Despite this, there are no FDA-approved therapies that target either wild-type or mutant p53, though some are in various stages of clinical trials [50,76,77]. Advanced strategies, including biotherapeutic and pharmaceutic approaches, have been developed for targeting p53 reactivation for cancer therapy. Biotherapeutic approaches mostly focus on the replacement of p53 with wild-type p53 by gene delivery. Wild-type p53 can be transferred into cancer cells to replace endogenous p53 function using a recombinant virus such as a recombinant adenovirus which fails to replicate efficiently with the E1B-55 Kd protein deletion in cells [129-133].

Gene therapy based on p53 delivery is under clinical evaluation. Pharmaceutical approaches using small molecules for reactivation of wild-type p53 function is a major effort for cancer therapy targeting wild-type p53 [6,30,131].

Figure-22:. Strategies for targeting mutant p53 and wild-type p53 in cancer cells.

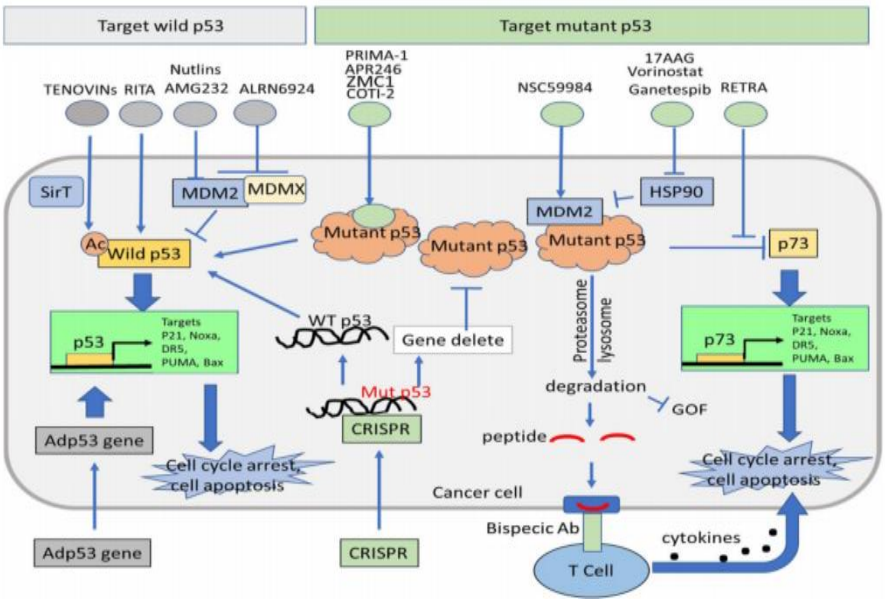


Figure-22, revealed, Pharmacological approaches for targeting wild-type and mutant p53 in cancer cells are focused on small molecules (upper panel). Small molecules targeting wild-type p53 activation via binding to p53 (such as RITA), inhibition of MDM2/X (such as an MDM2 inhibitor nutlin-3 and the dual inhibitor ALRN6924), post-translational modifications (such as tenovin). Small molecules target mutant p53 via restoration of p53 function (such as PRIMA-1), degradation of mutant p53 via activation of MDM2 (such as 17AAG and NSC59984) or interruption of mutant p53-p73 interaction (such as RETRA). Activation of p73 upregulates p53 target gene expression and induces cell death. Biotherapeutic approaches are based on gene transfection and genomic modifications (bottom panel). p53 is transfected into cancer cells with an adenovirus to replace mutant p53, and upregulates p53 signaling (such as rADp53). Genomic editing is used to restore wild-type p53 or delete mutant p53 in cancer cells by genome editing approaches (such as CRISPR). A bispecific antibody with mutant p53-specific peptide and ALH ligands promotes T cells to recognize and kill p53-mutant tumor cells in cancer immunotherapy[12, 128].

Figure-23: Small-Molecule-Compounds-Based Therapy Targeting Mutp53: [133]

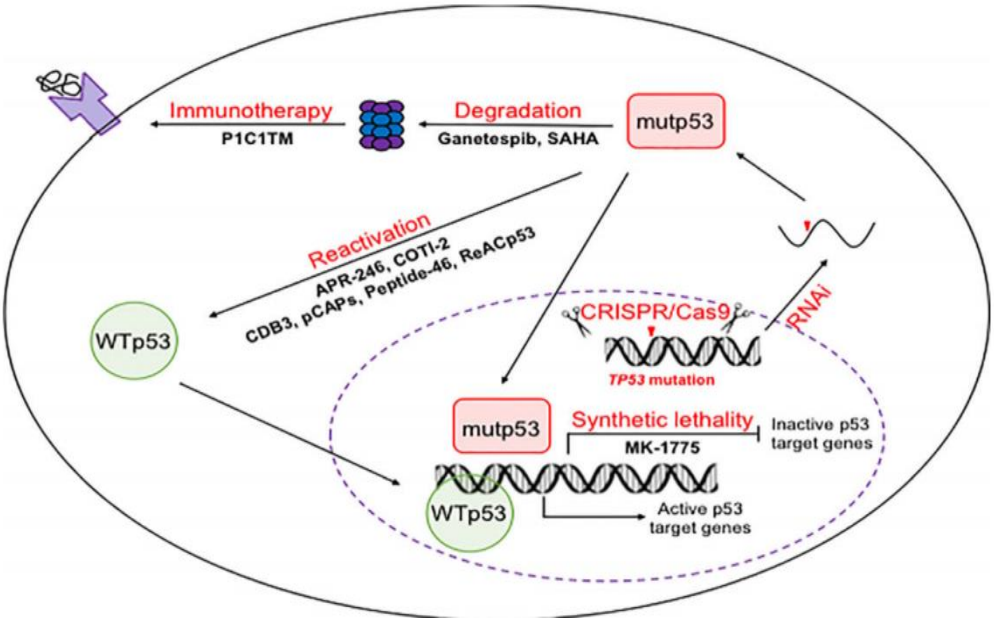


Figure-23: Therapeutic strategies to target p53 mutants. On the DNA level, mutations in TP53 allele could be reversed back to wild-type ones using CRISPR/Cas9 mediated genome editing. One the mRNA levels, mutp53 mRNA could be silenced by RNAi. On the protein level, mutp53 could be reactivated or trageted for degradation by both small molecule compounds and small peptides. The inability of mutp53 to activate its downstream target genes provides an opportunity for synthetic lethality based therapy. The mutant peptides produced by degradation of mutp53 makes immunotherapies possible.

3. CONCLUSION

Since p53 gene is mutated in >50% of all cancers and mutp53 often displays GOF activities in tumorigenesis, mutp53 has become an attractive target for cancer therapy. p53 has been discovered for over 40 years, and p53 is one of the most extensively studied proteins; however, majority of studies on p53 have been focused on the wild-type p53. Although significant progress has been made in our studies on mutp53/Wtp53 in cancer during the past decade, our understanding of the role and mechanism of mutp53/Wtp53 regulation and GOF activities in cancer is still limited, with many questions unresolved. For instance, while well established by tremendous in vitro cell experiments and various animal models, the mutp53 GOF needs to be further validated in clinical studies. Furthermore, majority of mutp53/Wtp53 GOF studies have been focused on several hotspot p53 mutants in cancer, while it remains unclear whether other nonhotspot p53 mutants can exert similar GOF activities through similar mechanisms. Based on the results from previously published studies, mutp53 GOF effects and mechanisms appear to vary in different contexts, potentially depending on mutation positions, cell and tissue types, cancer types, and even the tumor microenvironment and stress signals. This complexity presents challenges in developing some general therapeutic strategies to target different GOF mutp53 in different types of cancers. So far, different mutp53-targeted therapeutic strategies, as summarized above, have been developed, shown to be effective to certain extent and promising in preclinical studies, and some even entered clinical trials; however, there are still unresolved obstacles in mutp53/Wtp53-targeted cancer therapy, and currently, there are no approved drugs for clinical treatment of cancers expressing mutp53/Wtp53. Obviously, more studies on mutp53/Wtp53 regulation and GOF and mutp53/Wtp53-targeted therapies with advanced molecular techniques are necessary, which will lead to more effective and precise therapies targeting mutp53/Wtp53 in cancers.

Genetic Approach to Target Mutp53 CRISPR/Cas9 and RNAi:[12, 130-134]

CRISPR/Cas9-based genome editing appears to be a straightforward therapeutic strategies for tumor cells expressing p53 mutants. By directly replacing the TP53 414delC frameshift mutation locus with a functional copy, Batir et al. Successfully restored the wild-type TP53 genotype and phenotype in prostate cancer cells (119). CRISPR/Cas9 has also also employed in a p53 genetic sensor system which specifically and efficiently killed p53- deficient cancer cells (120). However, the high risk of genome instabiliy induced by CRISPR/Cas9 should be rigorously considered (121, 122). Small interference RNAs could specifically eliminate mutant p53 mRNA without affecting the wild-type one, However, the specificity and in vivo efficacy of such RNAi remains to be elucidated.

However, in recent years, although studies have reported that a variety of small molecule compounds or peptide drugs

targeting mutp53 have been developed, only a few drugs have entered clinical trials, and no drugs targeting mutp53/Wtp53 have been approved for clinical tumor treatment. Obviously, there is still more research to be done on mutp53/Wtp53 in the future.

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