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Phenotypical mapping of TP53 unique missense mutations spectrum in human cancers

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ABSTRACT

The p53 tumor suppressor is one of the most mutated genes responsible for tumorigenesis in most human cancers. Out of 29,891 genomic mutations reported in the TP53 Database (https://tp53.isb-cgc.org/), 1,297 are identified as unique missense somatic mutations excluding frameshift, intronic, deletion, nonsense, silent, splice, and other unknown mutations. We have comprehensively analyzed all these 1,297 unique missense mutations and created a phenotypical map based on the distribution of mutations in each domain, the functional state of the protein, and their occurrence in different types of tissues and organs. Our mutation map shows that almost 118 unique missense mutations are reported in the transactivation and proline-rich domains, 1,065 in the central DNA-binding domains, and 113 in the oligomerization and regulatory domains. Based on the phenotype, these mutations are subdivided into 46 super trans, 491 functional, 315 partially functional, and 415 non-functional mutations. The prevalence of these mutations was checked in 71 different types of tissues and found that the mutant R248Q is reported in 51 types of tissues followed by R175H and R273H in 46 types. We correlated the potential impact of mutation in target gene transcription and regulation with nucleosomal DNA and RNA-Pol II complexes. We have discussed the impact of mutation at post-translational modification sites in the structure and function of p53 highlighting the potential therapeutic drug targets with tremendous clinical applications.

Abbreviations: DBD: DNA binding domain; NES: Nuclear export signal; NLS: Nuclear localization signal; OD –: Oligomerization domain; PDB: Protein data bank; PTM: Post-translational modification; PRR: Proline-rich region; RD: Regulatory domain; TAD: Transactivation domain; wt: wild type

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p53; missense mutations; phenotype; tumor suppressor; apoptosis

1. Introduction

According to the Global Cancer Statistics 2020, the estimated number of cancer cases reported from all over the world is approximately 18,094,716 with breast cancer, lung cancer, prostate, skin, colon, stomach, liver, and rectum as the top eight commonly diagnosed cancers (Sung et al., 2021). As per reports in the COSMIC database, p53 is the most frequently reported gene in these top 8 cancers such as lung (29%), stomach (37%), skin (42%), liver, (29%), and rectum (60%) cancers (Tate et al., 2019). In the case of breast cancer, the PIK3CA (29%) gene is at the top followed by the p53 (26%) gene. Similarly, in colon and prostate cancer, APC (53%) and LRP1B (37%) genes respectively are the most frequently mutated genes followed by p53 (Zhu et al., 2020; Kandoth et al., 2013). In normal cells, the transcription factor p53 is negatively regulated by E3 ubiquitin ligase MDM2 to keep it under lower concentration by ubiquitin-mediated proteolysis (Hock & Vousden, 2014; Giaccia & Kastan, 1998). However, the p53 concentration increases in response to various stresses, such as DNA damage, activation of oncogenes, or hypoxia (Lavin & Gueven, 2006; Ljungman, 2000).

In response to this genotoxic stress or cellular stress, the TP53 gets activated by post-translational modifications thereby escaping the MDM2-mediated ubiquitination and translocating inside the nucleus (Meek & Anderson, 2009). Inside the nucleus, p53 binds to the appropriate DNA response element and performs its transcription function by activating the genes involved in cell cycle arrest, DNA repair, senescence, or apoptosis (Zilfou & Lowe, 2009) depending on cell stress signals.

The missense mutation in p53 leads to the elimination of the cell cycle arrest or repair mechanism in a cell leading to tumorigenesis (Malhotra et al., 2023). Mostly the inactivation or deletion of genes coding for crucial proteins like E2F1, RB, WNT signaling regulator APC, and BRCA1 is the prime cause of tumorigenesis while in the case of p53, the occurence of a single missense mutation often leads to cancer progression. Till date, in the TP53 database ((https://tp53.isb-cgc.org/), a total of 29,891 mutations have been reported so far, out of which 2,682 are caused by frameshift, 217 intronic, 49 deletion, 2,417 non-sense mutations, 21,781 missense mutations, 1,230 silent mutations, 730 splice mutants, 658 other types of mutations and 127 are of unknown origin (Bouaoun et al., 2016;

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Olivier et al., 2002). Out of the 21,781 missense mutations, 1,297 are unique or distinct amino acid residue replacements without including intronic, frameshift, deletion, splicing, silent and multiple mutations. All the reported 1,297 unique somatic mutations are majorly distributed in the three functional domains with 82.11% (1,065) in the DNA binding domain (DBD) followed by 6.01% in the oligomerization domain of TP53.

Studies were reported to understand the impact of these mutations in TP53 and their role in tumor progression and metastasis (Malhotra et al., 2023; Tang et al., 2020; Powell et al., 2014). The variants of TP53 either have a loss of function or a gain of function (Oren & Rotter, 2010; Zhang et al., 2020; Alvarado-Ortiz et al., 2020). Various review reports have also described the overall TP53 gene variations, gain of functions (GOF), mutation distribution in different populations, and clinical applications (Zhu et al., 2020; Bouaoun et al., 2016; de Andrade et al., 2022; Olivier et al., 2010; Parkin et al., 2011; Sabapathy & Lane, 2018). In this study, we have performed a detailed analysis of 1,297 unique missense somatic mutations of p53 and created a phenotypical map based on the functional state of the mutant, the position of the mutated amino acid in each functional domain, and the distribution of mutants in various tissues. Upon analysis, only 50 amino acid positions showed no missense mutations out of 393 residues in p53. We have also calculated the propensity of mutation of each amino acid residue in p53 by highlighting the important structural and functional regions like DNA contact region, Zinc binding region, and post-translational modification (PTM) sites. The analyses give an overall summary of these mutations and their role in cancer progression that can be used for the development of a new drug to restore or inhibit the functions of TP53.

2. Methods and datasets

2.1. Collection and distribution of p53 unique missense mutations datasets reported in cancers

The unique p53 missense somatic mutants were extracted (excluding frameshift, intronic, deletion, nonsense, silent, splice, and other unknown mutations) from *TP53* Database (R20, July 2019): https://tp53.isb-cgc.org) (Bouaoun et al., 2016; Olivier et al., 2002; de Andrade et al., 2022; Leroy et al., 2013). A dataset was created with these 1,297 unique missense mutations and the dataset was further separated into five datasets based on p53 domains such as Transactivation domain (TAD) (1 to 63), Proline-rich region (PRR) (64 to 91), DNA binding domain (DBD) (92 to 312), Oligomerization or tetramerization domain (OD or TD) (319 to 360) and Regulatory domain (RD) (361-393). Each dataset of different p53 domains was further divided into four phenotypes as described in Kato et al. where the p53 mutants are categorized based on transcription activity using yeast-based functional assay (Kato et al., 2003). Based on the p53 transcription activity on p53 binding elements/promoters such as p21, MDM2, Bax, GADD45, 14-3-3σ, p53AIP1 Noxa and p53R2, the p53 mutations were classified as super trans with enhanced transcription activity, partially functional with lower activity and non-functional with no activity. Hence, we divided the datasets into super trans, functional, partially functional, and non-functional mutations. A heat map was generated based on the phenotypes of p53 using GraphPad Prism 8. Further, the mutations were examined for their prevalence in different human tissues.

2.2. Propensity calculation of p53 phenotypical mutants

The propensity of amino acid substitution in each p53 domain was calculated using the following equation:

$$Propensity\ of\ X\ in\ domain = \frac{Total\ mutations\ present\ at\ X}{Total\ number\ of\ X\ in\ domain}$$

Where X denotes amino acid residue. Using the Expasy Protparam tool, the amino acid composition in each domain was determined and the total number of substitutions present at position X was calculated. Further, the total number of substitutions in each phenotype was calculated using the equation:

$$Propensity\ of\ X\ in\ domain\ belonging\ to\ Z\ phenotype = \frac{Total\ mutations\ at\ X\ belonging\ to\ Z\ phenotype\ in\ domain}{Total\ number\ of\ X\ belonging\ to\ Z\ phenotype\ in\ domain}$$

Where Z represents phenotypes (Super trans or functional or partially functional or non-functional). The distribution of all unique missense somatic mutations in 71 different topographies was performed using the collected datasets. The domain-wise topographical distribution of p53 mutations in different phenotypes was performed as described in Kato et al. (Kato et al., 2003). The PDBs 3KMD and 1OLG were used to describe the structural features of the p53 DNA binding domain and the tetramerization domain using Pymol 1.7. The bar diagram and checkers were generated using GraphPad Prism 8.

3. Results and discussion

Of the identified 1,297 unique missense somatic mutations (Figure 1a, Table 1), 264 are also found as germline mutations which can pass on to the next generations (Table 2). Upon sub-dividing, this distinct missense somatic mutants into each functional domain of TP53, a total of 118 mutations are reported in the transactivation domain (TADs) and proline-rich domain (PRR) (1- 91), 1,065 in the central DNA-binding domain (DBD) (92-312), and 95 in the oligomerization (OD) and regulatory domain (RD) (319-393), 18 in DBD-OD linker region (Table 1 and Figure 1b and c). A total of 46 super trans mutations were found in the p53, with 40 in the DBD, 5 in OD, and 1 in TAD (Table 1) (Figure 1d). Surprisingly, no p53 mutations with super trans phenotype was found in the RD and PRR domains. Interestingly, 445 non-functional mutations are reported in p53 out of which 426 are in the DBD, 12 are in OD, 5 in TAD, 1 in PRR, and 1 in the DBD-OD linker region (Table 1, Figure 1d). In most cancers, the non-functional and the super-trans mutants promote tumor progression by loss of function and gain of

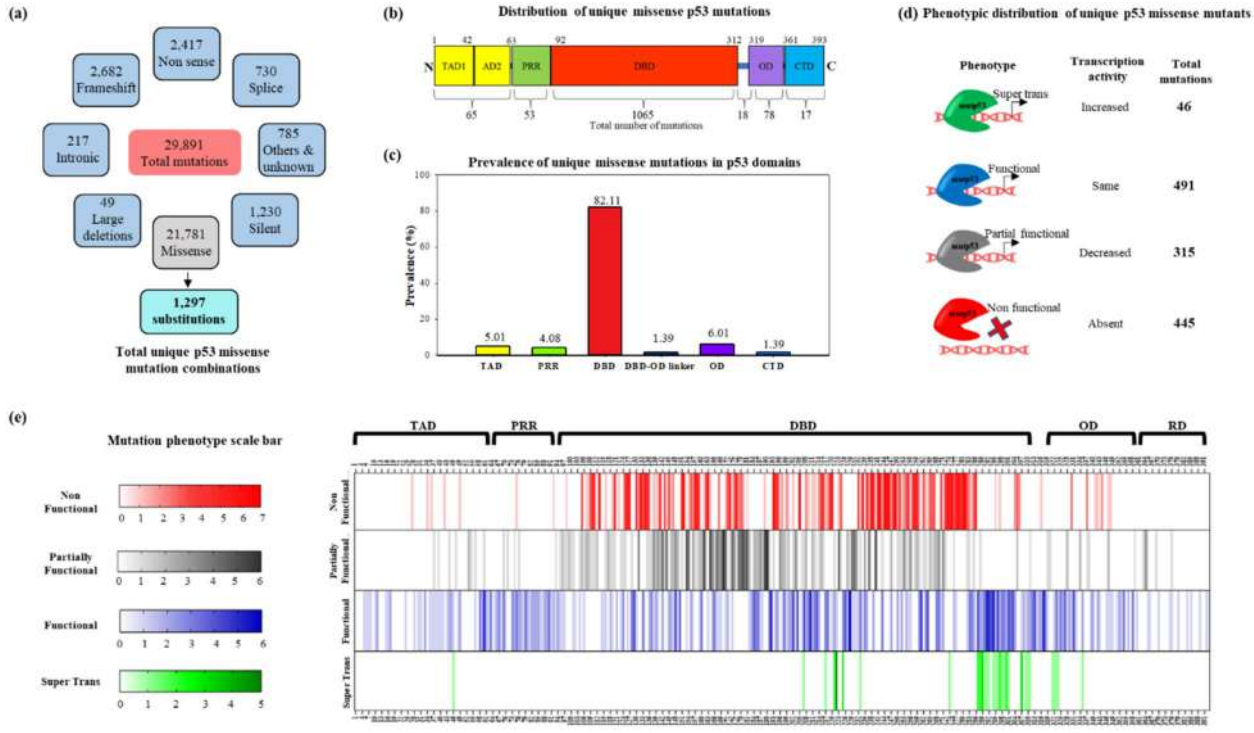

Figure 1. Mutation spectrum of p53. (a) Diverse mutations of p53. (b) Domain-wise distribution of unique missense mutations. (c) Prevalence of unique missense mutations in p53 domains (d) Phenotypic distribution of unique p53 missense mutations. (e) Heat map of p53 phenotypic mutations.

Table 1. Distribution of p53 missense somatic mutations in different domains.

	Types of somatic missense mutations					
Domains	Super trans	Functional	Partially functional	Non-functional	Total mutations	p53 mutations (%)
TAD	1	50	9	5	65	**5.01**
PRR	0	48	4	1	53	**4.08**
DBD	**40**	**315**	**284**	**426**	**1065**	**82.11**
DBD-OD linker	0	17	0	1	18	**1.39**
OD	5	50	11	12	78	**6.01**
CTD/ RD	0	11	7	0	17	**1.39**
Total	**46**	**491**	**315**	**445**	**1297**	-

Table 2. Distribution of p53 missense germline mutations in different domains.

	Types of germline missense mutations					
Domains	Super trans	Functional	Partially functional	Non-functional	Total mutations	p53 mutations (%)
TAD	0	2	0	2	4	**1.51**
PRR	0	2	0	0	2	**0.75**
DBD	**3**	**28**	**43**	**164**	**238**	**90.15**
DBD-OD linker	0	2	0	0	2	**0.75**
OD	0	8	2	5	15	**5.68**
CTD/ RD	0	3	0	0	3	**1.13**
Total	**3**	**45**	**45**	**171**	**264**	-

function, respectively (Alvarado-Ortiz et al., 2020; Rivlin et al., 2011). On the other hand, a total of 491 functional phenotypes (37.86% of total p53 mutations) are reported in the database, with 315 in the DBD followed by 50, 50, 48, 17, and 11 in OD, TAD, PRR, DBD-OD linker region, and RD, respectively (Table 1 and Figure 1d). The different phenotypic variants in p53 domains are shown in the p53 mutation heatmap (Figure 1e). In the case of germline missense mutants, around 238 mutations (90.15%) reside in the DBD followed by OD (15), TAD (4), CTD (3), DBD-OD linker (2), and PRR (2) domain (Table 2). After dividing the dataset based on functional domains, we have analyzed the overall distribution of these mutations in each domain of p53.

3.1. Transactivation domain (TAD) and proline-rich region (PRR)

The transactivation domain (TAD) of TP53 can be divided into two distinct domains, TAD1 (1-42) and TAD2 (43 - 63) both crucial for tumor suppressor activity of the protein (Baral et al., 2019; Lee et al., 2010) (Figure 2a). Each domain has a distinct role or transcriptional requirements related to the p53 tumor suppressor activity (Brady et al., 2011). Both TADs are majorly comprised of acidic and/or hydrophobic residues crucial for forming interactions with general transcription factors, Chromatin Modifiers p300 and CBP; and its negative Regulators Mdm2, MdmX, and E1B (Collavin et al.,

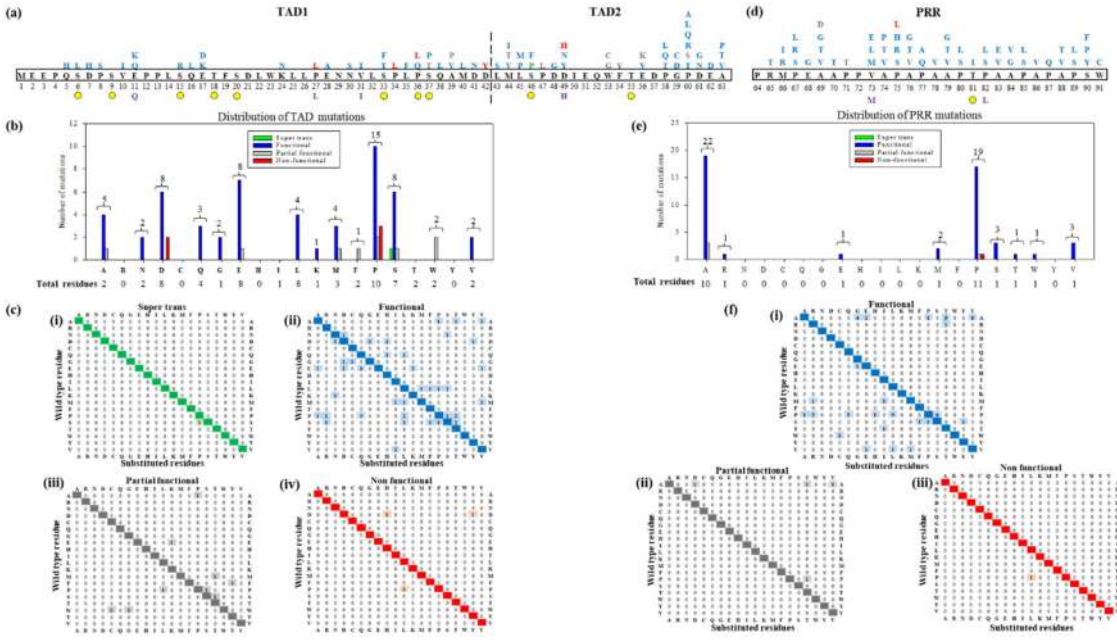

Figure 2. Mapping of missense mutants in Transactivation and Proline-rich domains of p53. (a) and (d) represent the map of somatic mutations in TAD (a) and PRR (d) domains by different colors based on the phenotype (The residues labeled in red: non-functional phenotype, grey: partial functional phenotype, blue: functional phenotype and green: super trans phenotype). The germline mutant residues are shown in violet color below the wild-type residues. The yellow circle represents PTM sites. (b) and (e) shows the amino acid residues present in the domain and the propensity of mutations of each residue in TAD and PRR. (c) and (f) Checkers diagram shows the propensity and frequency of each amino acid substitution in terms of phenotypes (ci) super trans, (cii and fi) functional, (ciii and fii) partial functional, and (civ) non-functional mutations in TAD and PRR.

2010; Fernandez-Fernandez & Sot, 2011). Similarly, the domain also holds the crucial post-translational modifications (PTMs) sites for the activation of p53 (Table S1) (Meek & Anderson, 2009; Gu & Zhu, 2012; Che et al., 2020; Dai & Gu, 2010). In these domains, a total of 65 unique missense somatic mutations were reported. Out of which 5 are non-functional mutations, 9 are partially functional mutations, 50 are functional mutations and one super trans mutation S46P is reported in this domain (Table 1) (Figure 2a). Out of the five non-functional mutations, P27L, P34L, and P36L indicate that proline at these positions is crucial for the function of TP53, apart from D42Y and D49H (Figure 2a, c(iv)). In the partially functional mutations, the W53C, W53G, and F54Y variants are reported as non-functional in combination with other mutations p53[25,26,53,54] (Brady et al., 2011) (Figure 2a and c(iii)). Upon analyzing the propensity of mutation of each amino acid or the phenotypic distribution of the mutations in TAD domains, Proline was found to be the most frequently mutated residue particularly substituted to Leu or Gln (Figure 2c(ii-iv)). All these hydrophobic residues especially proline is important for forming interactions with general transcription factors and regulators (Raj & Attardi, 2017). To regulate genes involved in essential processes like cell-cycle arrest and DNA repair, p53 needs to form a complex with nucleosomes to bind to a specific promoter of the target gene. To initiate the process, first, the N-terminal region (1-93) of p53 binds to the histones H3-H4 tetramer located at the entry/exit region of the nucleosomal DNA and supports the p300 interactions with nucleosomes to induce target gene expression. Mutations at P27L, P34L, P36L, D42Y, D49H, and 9 partially functional mutations in the TAD1 and TAD2 region (Figure 2) might impact the interactions of p53 with histones H3-H4 resulting in reduced or loss transcription of target genes. Similarly, the partial mutations also might impact the interactions formed between p53-RNA pol II to promote the pre-initiation complex assembly by binding to the promoter site (Liou et al., 2021).

On the other hand, in the PRR domain, a total of 53 mutations were reported, out of which 48 are functional, 4 are partially functional and 1 is non-functional (P75L) (Table 1 and Figure 2d(iii)). To date, no super trans mutation has been reported in the PRR domain. Upon analyzing the propensity of amino acid mutation, Alanine followed by Proline is the most frequently mutated amino residue in the PRR domain with maximum mutations reported as either Pro to Ser or Leu (Figures 2e and f(i)). In both domains, there are 24 amino acid residue positions with no mutations reported so far (Figure 2a and d).

3.2. DNA binding domain or core domain

In the case of the p53 DNA binding domain, 82.11% of the missense mutations are predominantly found in exons 4 to 9 of the p53 gene (Bouaoun et al., 2016). Out of 1,297, 1,065 mutants are reported in DBD with 40 as super trans phenotype, 315 as functional, 284 as partially functional phenotypes, and 426 as non-functional phenotypes (Table 1, Figure 3a). On the other hand, 238 are found as germline mutations in human cancers (Table 2, Figure S1). Excluding the amino acid Tyr103, all the residues in the DNA binding domain are reported to be mutated in cancer (Figure 3a). The majority of the p53 hotspot mutants are reported in the DNA binding domain due to their presence in the methylated CpG

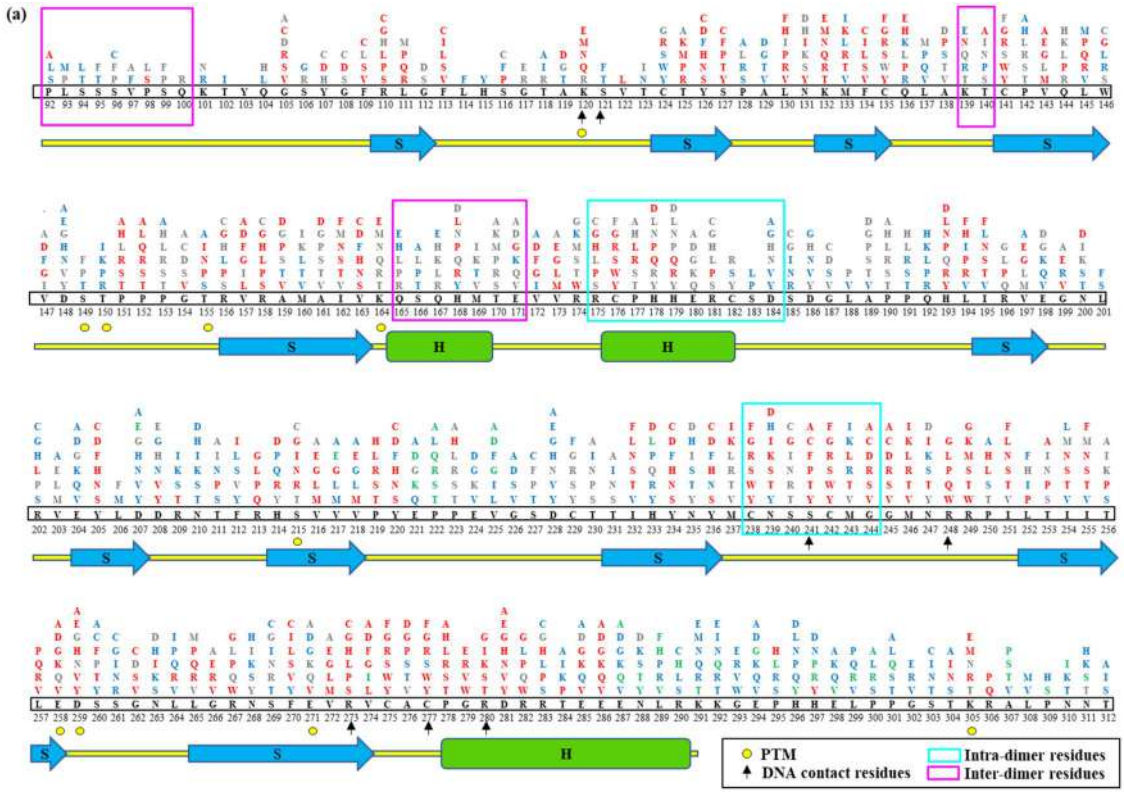

Figure 3. Mapping of missense mutations in the p53 DNA binding domain. The different phenotypic mutations are shown along with the secondary structure of the p53 DNA binding domain. The mutated residues are highlighted on the top of wt residues with different colors based on the phenotype of the mutations. (The residues labeled in red: non-functional phenotype, grey: partial functional phenotype, blue: functional phenotype, and green: super trans phenotype). S-beta strand (Blue arrow), H-alpha helix (green cylinder), and the unstructured region (loops and coils) in a yellow line.

dinucleotides island which is prone to spontaneous mutation by certain environmental carcinogens (Rosendahl Huber et al., 2021), such as polycyclic aromatic hydrocarbons in tobacco, which preferentially binds to guanines in methylated CpG sites (Peto et al., 2000; Pfeifer et al., 2002); and UV irradiation often modifies methylated cytosines (Parkin et al., 2011). To assess the propensity of mutation in each amino acid residue in the DNA binding domain, we divided the residues based on their location in the domain such as the DNA contact region, Zinc binding region, and conformational mutants that lead to the loss of thermostability of p53 (Figure 4a-c).

TP53 recognizes DNA with a loop-sheet-helix motif and the residues involved in direct interactions with DNA are Lys120, Ser121, Ser241, Arg248, Arg273, Ala276, Cys277, and Arg280 (Figure 4c). Interestingly all the arginine residues that are involved in DNA contact were found to be mutated and all the mutants belong to the Non-functional phenotype (Figures 3a and 4e(iv)). On the other hand, the mutations reported at the Lys120 position are majorly non-functional except for K120R which turns out to be partially functional (Figure 3a). Surprisingly, the mutations occurring at Ser121 (S121F, S121T) and Ala276 (A276S and A276T) belong to the functional category (Figure 3a). The DNA contact mutants are considered as first hotspot mutants. Remarkably, apart from DNA contact residues, substituting any residue that is part of the loop-sheet-helix motif makes TP53 a non-functional protein. The TP53 recognizes the promoter sequence as a tetramer (dimer of dimers) and the dimerization interface in the DNA binding domain is the 3rd hot spot mutation site (Figures 3a and 4a). Dimerization is crucial for DNA binding and regulating the transcription of genes. The dimer interface consists of a Zinc binding motif formed by two loops (L2 and L3) where the zinc atom forms a tetrahedral coordination bond with C176 and H179 of the L2 loop and C238 and C242 of the L3 loop (Figure 4b). TP53 is extremely unstable in the absence of zinc and it is predicted to be in an unfolded state in the cell (Blanden et al., 2020). Out of these four amino acid residues, C176 and H179 are the 7th and 8th most frequently mutated residues in p53 (Table 3).

Apart from these zinc-coordination residues, the p53R175 and G245 are also reported as high-frequency hotspot mutations in almost all cancers (Table 3). The R175 and G245 are located near the zinc-binding site in the DBD dimer and DNA binding interface maintaining the stability of the zinc-binding motif (Figure 4b). Arg175 forms a salt-bridge interaction with Asp184 and G245 is part of the 3_{10} helix which holds the Zinc ion (Figure 4b). Mutations of Arg and Gly lead to non-functional proteins (Figure 3a). Apart from DNA-contact and Zinc-binding mutants, several structural mutants are also reported to cause non-functional phenotypes. The mutations lead to either distortion in the DNA binding surface or cause the formation of water-accessible crevices or lead variations in dimer-dimer interacting interfaces involved in tetramerization (Figures 3a and 4a).

Many structural p53 mutations are considered therapeutic targets rescued by small molecule drugs to restore protein

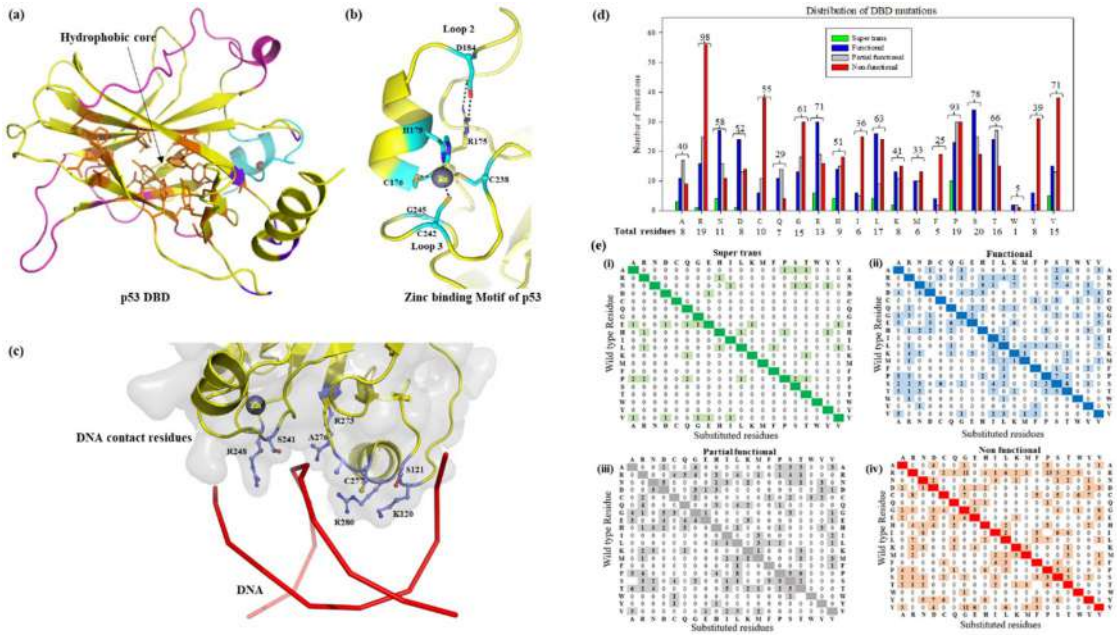

Figure 4. Structure of p53 DNA binding domain and the mutation distribution (a) p53 DBD cartoon highlighting Zinc binding motif in cyan, DNA contact region in blue, hydrophobic core residues shown in orange, and dimerization region in magenta. (b) Represents the Zinc binding motif and the residues involved in Zinc coordination are shown in cyan. (c) The DNA contact residues of p53DBD are shown in blue. (d) Phenotypical distribution of mutations for each type of amino acid in DBD. (e) Phenotype-wise checker diagrams ((i) super trans, (ii) functional, (iii) partial functional, and (iv) non-functional mutations) represent the propensity of a particular amino acid to get mutated in p53 DBD.

Table 3. Top 10 most frequently mutated residues of p53.

S.No.	Amino Acid Residue	Somatic	Germline	Type
1	R248	G, L, P, Q, W	L, Q, W	DNA contact
2	R273	C, G, H, L, P, S	C, G, H, L, S	DNA contact
3	R175	C, G, H, L, P, S	C, G, H, L	Zinc binding motif
4	G245	A, C, D, R, S, V	C, D, S, V	Zinc binding motif
5	R249	G, K, M, S, T, W	K, T	DNA contact
6	Y220	C, D, F, H, N, S	C, S	Structural
7	C176	F, G, R, S, W, Y	G, Y	Zinc binding
8	H179	D, L, N, P, Q, R, Y	D, Q, R, Y	Zinc binding
9	V157	A, D, F, G, I, L	–	Structural
10	M237	I, K, L, R, T, V	–	Structural

function in tumors, especially Y220C (Malhotra et al., 2022; Malhotra et al., 2021; Raghavan et al., 2019; Joerger et al., 2006; Bauer et al., 2020; Bauer et al., 2019; Malhotra et al., 2024) (Table 3). The other structural p53 mutants are occuring at the residues present in the hydrophobic core of the DBD (Figure 4a). The hydrophobic core holds and stabilizes the domain upon DNA binding. The core is formed by the residues F109, L111, F113, C124, Y126, M133, C135, C141, V143, L145, V157, A159, I195, V197, V216, V218, I232, Y234, Y236, T253, I255, L257, F270 and V274 (Figure 4a). The mutations in these residues lead to mostly non-functional proteins (Figure 3a). Interestingly, upon analyzing the phenotypic distribution of all amino acid residues in the DNA binding domain, a total of 98 mutations are reported for Arginine (19 in the domain) (Figure 4d and e). The 19 residues are found to be most frequently reported with an average of 5.2 mutations per arginine in DBD and the maximum number of mutations are non-functional (Figure 4d and e(iv)). Out of all Arginine mutants, only Arg → His (1) mutation leads to super trans phenotype (Figures 3a and 4e(i)). On the other hand, Arg → Cys (3), Arg → Gly or His or Ile (1), Arg → Leu or Ser (4), and Arg → Lys (2) (Figure 4e(ii)) are functional, whereas in the case of p53 mutations elucidating partially functional phenotype, Arg → Cys or Gly or Leu or Ser (4), Arg → Gln or His (3), and Arg → Met or Pro or Thr (1) (Figure 4e(iii)). The non-functional mutations are Arg → Pro (13), Arg → Gly (11), Arg → Leu (6), Arg → Ser or Trp (5), Arg → His (4), Arg → Thr or Gln (3) each, Arg → Ile or Met (1). Similarly, the analysis was performed for other amino acid residues shown in Figure 4e. It is also reported that post-translational modifications in the DBD enhance the DNA binding affinity of p53 (Table S1). The domain is reported to have 10 potential PTM sites (Meek & Anderson, 2009; Gu & Zhu, 2012) and mutations are observed at these sites (Figure 3a).

3.3. Mutants in the p53 tetramerization (TD) and the regulatory domain (RD)

The C-terminus of TP53 consists of tetramerization (TD) and the regulatory domain (RD). The oligomeric form of p53 is crucial for its transcription function and it is reported that the DNA binding affinity increases several folds upon tetramerization (Lubin et al., 2010). The post-translational modifications (23 PTM sites) in the C-terminal domain also dictate many functional roles of p53 in protein-protein interactions (Laptenko et al., 2015) (Figures 5a and 6a and Table S1). The TD also has important sequence motifs like nuclear localization signal (NLS1) and nuclear export signal (NES) (Figure 5a).

The active TP53 forms a tetramer, a dimer of dimers where the TD plays an important role (Figure 5b) (Clore et al., 1994; Chène, 2001). Structurally the monomeric tetramerization domain (319-360) of p53 consists of a β-strand (residues 326 to 333) and an α-helix (residues 335 to 355)

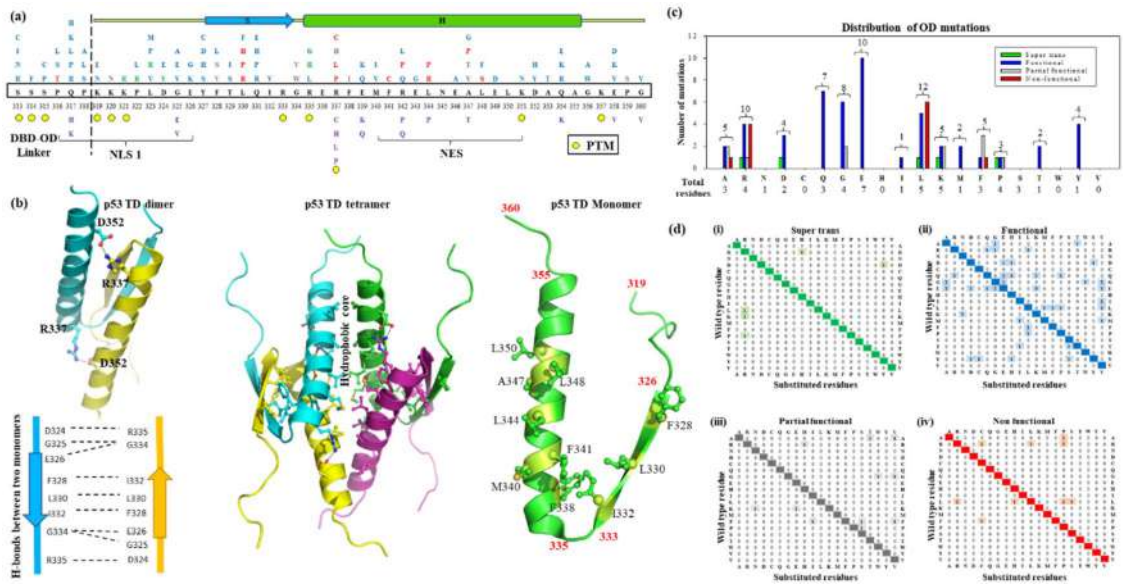

Figure 5. Mutation mapping and distribution of p53 oligomerization mutations. (a) The mutation map represents somatic mutations in the Oligomerization or tetramerization domain by different colors based on the phenotype (The amino acid single letter code with red color represents non-functional phenotype, grey color represents partial functional phenotype, blue color denotes functional phenotype and green color represents super trans phenotype). The germline mutations are also shown in violet color below the wild-type residues. (b) Cartoon diagram OD of p53 showing the structure of p53 tetramer formed by OD (the monomers are shown in cyan, yellow, neon, and magenta), the residues involved in the formation of the dimer (cyan and yellow), and the hydrophobic residues involved in the formation of a dimer of dimer or tetramerization of p53 (green). (c) Represent the propensity of mutations of amino acid residues in p53 TD. (d) Phenotype-wise checkers diagrams ((i) super trans, (ii) functional, (iii) partial functional, and (iv) non-functional mutations) representing the propensity of a particular amino acid to get mutated in p53 DBD, where the Y-axis denotes the wild-type residue whereas the X-axis denotes the total number of different combinations present.

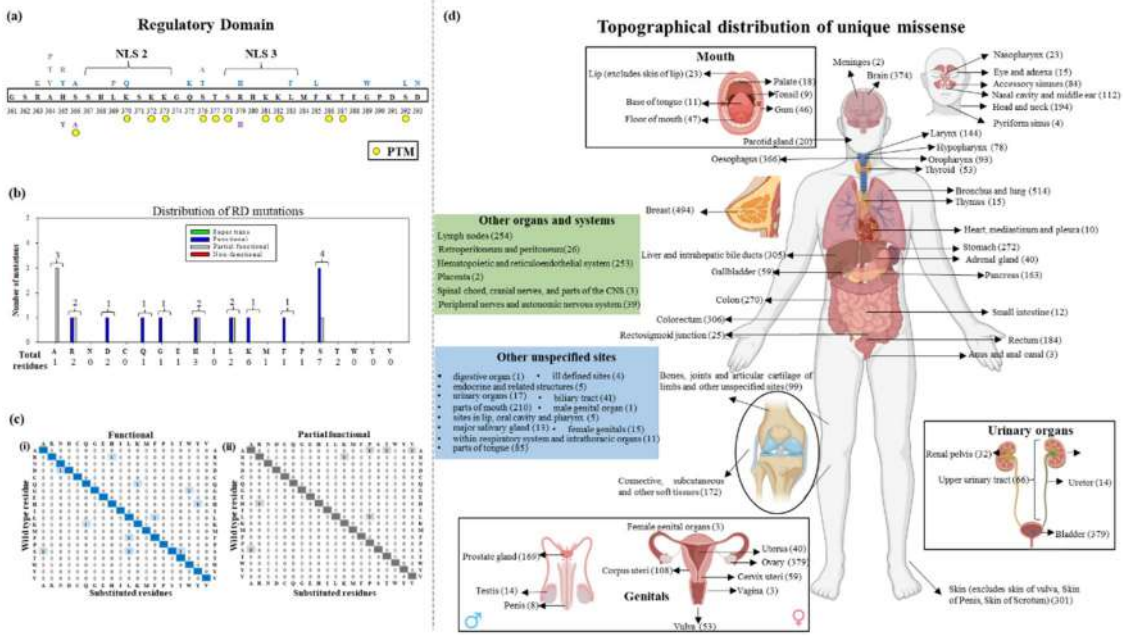

Figure 6. Mutation mapping and distribution of p53 Regulatory domain: (a) The mutation map represents somatic mutations in the regulatory domain by different colors based on the phenotype (The amino acid single letter code grey color represents partial functional phenotype; blue color denotes functional phenotype). The germline mutations are also shown in violet color below the wild-type residues. (b) Represent the propensity of mutations of amino acid residues in p53 RD. (c) Phenotypical distribution of mutations at each type of amino acid of p53 RD shown by checkers diagrams (i) functional and (ii) partial functional mutations. (d) Represents topographical distribution of p53 unique missense mutations in 71 different tissue topographies. The total number of mutations observed in each tissue type is given in brackets.

connected by a flexible residue G334 (Jeffrey et al., 1995) (Figure 5a, b). Two monomers interact with each other through an antiparallel double-stranded β-sheet and antiparallel helices to form a double-helical bundle (Figure 5b). Four monomers of p53TD form a V-shaped fold with a hydrophobic core formed by F328, L330, I332, F338, M340, F341, L344, A347, L348 and L350 (Figure 5b) from each monomer. The four monomers are also held by four salt bridges formed by R337 and D352 from different monomers (Figure 5b). The mutations at positions F341 and L344 are non-functional while L330, R337, R342, A347 and L348 are reported to be mostly non-functional highlighting the importance of these

hydrophobic residues in stabilizing the domain and p53 (Figure 5a). The OD domain was reported to have a second maximum number of mutants in p53, accounting for 78 unique missense mutants in human cancers (Table 1). Among these mutations, 50 are functional, 12 non-functional mutants, 11 are partially functional and 5 are super trans (Figure 5a, Table 1). Upon analyzing the propensity of amino acid mutations in this domain, Leucine followed by Glutamic acid is the most frequently mutated residue in the TD domain (Figure 5c and d). The mutations at the DBD-OD linker region between 313 and 318 were mostly reported as functional and this region contains 3 PTM sites (Figure 5a).

Finally, the regulatory domain (RD) is known to be the least mutated domain in p53 to date with 18 mutations, out of which 11 are functional and 7 are partially functional. RD is the only domain that lacks non-functional and super trans mutation (Table 1, Figure 6a-c). The most important feature is the 12 post-translational modification sites particularly phosphorylation and acetylation at K370, K381, and K382 which converts the p53 into active form upon cellular stress (Figure 6a, Table S1). The RD is also proposed to regulate the DNA binding of p53 where residues K373, K381, and K382 are predicted to be involved in DNA binding (Weinberg et al., 2004). The RD also has NLS2 and NLS3 regions where the mutations are reported at these sites but found to have functional phenotypes (Figure 6a). Upon analyzing the propensity of amino acid mutations in this domain, Serine and Alanine are the most frequently mutated residues in the RD domain (Figure 6b and c). Although the p53 binding to its target sequence is primarily mediated through to its DNA binding domain, however, the regulatory domain also has a role in binding to specific DNA promoter sequences. The p53 C-terminal domain slides along DNA until it finds its target promoter sequence and binds to the DNA cooperatively along with other domains. The TAD and DBD interact with the nucleosomal DNA to promote the transcription of specific target genes (Murata et al., 2017). The 6 partially functional mutants R363K, A364V/T/P, H365R and L369P in the regulatory domain (RD) affect its p53 DNA sliding mechanism and its interactions with other binding partners involved in target gene transcription. While mutation of S376 to Ala affects the PTM hence it leads to functional loss of the protein.

3.4. Mutation in functionally associated post-translational modifications (PTMs)

p53 is commonly regulated by post-translational modifications (PTMs), which affect its conformation, increasing its capacity to adopt multiple structural and functional states. In p53 PTMs like phosphorylation, SUMOylation, acetylation, and other novel modifications are key steps in signal transduction. As most of the PTMs are reversible, it is used as a switch ON and OFF signal for p53 to decide cell fate. Upon mutation, various pathways involved in cell survival, and proliferation gets affected, resulting in abnormal proliferation of cells. A total of 88 missense mutations are reported at 44 residues which can be post-translationally modified in wtp53, out of these 30 are non-functional, 23 are partially functional, 32 are functional and 3 super trans (Table S1, Figures 2, 3, 5a and 6a).

The phosphorylation of Ser15 in the TAD1 is a key in activation, increasing the stability and the dissociation of MDM2 from p53. The only mutation reported at this position is Ser15 to Arg which is functional. Similarly, the acetylation at Lys120 in the p53DBD is crucial for the activation of genes involved in apoptosis. Mutation of Lys to Glu, Met, Asn and Gln are reported as non-functional where replacement with Arg is partially functional (Figure 3 and Table S1). The Lys120 is involved in forming interactions with the promoter DNA hence acetylation might increase p53 affinity towards the sequence. Positive charge at this position is essential for acetylation to increase binding to the promoter, therefore replacement of positive amino acid residue Lysine to hydrophobic or polar residues interferes with DNA binding hence functional loss is observed. These non-functional mutations are reported in many solid malignant tumors, Ewing's Sarcoma and Esophageal squamous cell carcinoma (Zhang et al., 2015). Likewise, absence of acetylation at K164 due to missense mutation was reported in glioblastoma and bladder carcinoma cancer (Kruse & Gu, 2008). Interestingly, mutation of Lys164 to Glu is reported to be non-functional whereas, the replacements to Met, Asn, Gln, Arg, and Thr is partially functional. Lys164 forms interactions with Glu285 and stabilizes the S10 β-strand and H2 helix which are involved in DNA binding. The acetylation at Lys164 increases the hydrophobic interactions with the H2 DNA binding helix, whereas, mutation of Lys to Glu destabilizes the S10 and H2 binding region and hence p53 becomes non-functional. Similarly, PTM at 6 lysine residues K370, K372, K373, K381, K382, and K386 in the C-terminal regulatory domain activates transcriptional activity and stability of p53 (Figure 6 and Table S1). No non-functional mutations are reported at these positions.

Like other PTMs, the poly ADP-ribosylation of p53 is an important factor in the regulation of its transcription activity. The Poly(ADP-ribose) polymerase-1 (PARP-1) acts as a DNA damage sensor that regulates p53 function in response to DNA damage. The PARP-1 senses only ADP-ribosylated p53 (Wieler et al., 2003; Godoy et al., 2011; Alvarez-Gonzalez, 2007). PARP, poly (adenosine diphosphate-ribose) polymerase, is a damage-sensing protein, which is essential for the repair of DNA single-strand breaks (Herceg & Wang, 2001; Shall & de Murcia, 2000). PARP and p53 function synergistically in repairing DNA damage and suppressing chromosomal rearrangements. The residues E254, D259, and E271 are reported as sites for poly-ADP-ribosylation in p53. Mutation of E254 to Ala, Asp, Gly, Lys, and Val leads to non-functional p53, interestingly, replacement with Gln makes p53 partially functional. Similarly, the replacement of Asp at 259 position with Ala, Glu, Gly, His, Val, and Tyr is non-functional but Asn is partially functional. Hence, the length of the side chain is crucial for poly-adenylation at this position in p53. A recent study has also shown the effect of PTMs on p53 conformation with prominent involvement in Alzheimers's Disease pathogenesis (Clark et al., 2022).

3.5. Distribution of TP53 mutants in the tissues

The phenotypic distribution of all the mutants from different domains in various tissues showed that the variants are

reported in almost 71 different types of tissues (Figure 6d) (Table S2). Out of these, the DBD mutations are reported in 71 different types of tissues while DBD-OD-linker region mutations in 18 types, OD mutations in 29 types, RD in 13 types, and TAD-PRR domain mutations in 33 types of tissues (Tables S3-7). Out of these, R248Q is reported in 51 different types of tissues, R175H and R273H in 46 types, and mutations R248W, R273C, R282W, G245S, Y220C, E285K, and C176F are reported in more than 30 types of tissues (Table 4). The phenotypical distribution of these mutations is shown in Supplementary Tables S3-7.

3.6. Impact of mutations on p53 interactome aided transcriptional activity

The master regulator p53 mediates a plethora of cellular functions by performing its transcription function upon binding to specific promoter sequences. However, interactions with other partners/proteins also play a role in p53 activation, oligomerization, and inactivation by imparting post-translational modifications or altering its conformation (Collavin et al., 2010; Fernandez-Fernandez & Sot, 2011). One of the most important roles of p53 is to mediate the changes in the chromatin environment coupled with histone modifications and chromatin remodeling (Vukojevic et al., 2013; Brázda & Fojta, 2019; Gulve et al., 2022). To execute the transcription function, the p53 has to bind to the genomic DNA present in the nucleus of the cell. To initiate the process, first, the p53 N-terminal region (1-93) binds to the histones H3-H4 tetramer located at the entry/exit region of the nucleosomal DNA. The mutation at residue positions 27, 34, 36, 42, 49, and 75 in the TAD1 and TAD2 region might impact the interactions of p53 with histones H3-H4 essential for p53-nucleosome complex formation (Nishimura et al., 2020). The replacement of Proline with Leucine might lead to a loss of flexibility that is required for forming crucial interactions with H3-H4 tetramer in the entry region of nucleosome. Upon analyzing the cryo-EM structure of the p53DBD-nucleosome complex (PDB ID: 7XZX), we also observed that the mutations of residues K120, S121, S241, R248, R273, A276 and R280 in p53DBD also significantly impact the interactions with nucleosomes (Nishimura et al., 2022). Mutation of Lys to Arg at the 120th position does not have an effect whereas, the mutation of the same residue to Glu, Met, Asn, and Gln leads to the loss of interactions with the nucleosome promoter binding site (Figures 3 and 7). Similarly, mutation of Ser at 121 to Tyr, Thr, Pro, Phe, Cys, and Ala leads to loss of interactions. Mutation of Arg at 248 position to Trp, Gln, Pro, Leu, and Gly, and mutation of Arg at 273 to Ser, Pro, Leu, Gly, and Cys affect the interactions with the nucleosome. Another two important mutation sites are Alanine at 276 and Arg at 280 where Ala276 to Asp, Gly, and Phe, and Arg280 to Gly, Ile, Ser, and Thr (Figure 3), reduce p53 affinity towards the promoter sequence in the nucleosomal DNA (Figure 7) (Nishimura et al., 2022). Out of all p53DBD residues that were found interacting with nucleosomal DNA possessing a p53 binding site, mutations reported at R248, R273, and R280 belong to the non-functional phenotype (Figure 3). Although p53 binding to target promoter sequence is primarily mediated by p53DBD, the C-terminal regularity domain also plays an important role in binding to the nucleosomal DNA. The RD slides through the non-specific DNA sequence until it reaches target promoter sequence. Once the target promoter is found p53 forms a stable complex with nucleosome and provides a platform for the nucleosome to interact with other transcription activators like p300. Therefore, non-functional mutants in p53DBD and RD affect the formation of the p53-nucleosomal complex as these positively charged residues are crucial for the interaction with negatively charged DNA thereby abolish the transcription activity of p53.

Once the wild-type p53 tetramer binds to its DNA promoter region, it turns on transcription of diverse genes involved in tumor prevention or inhibition processes (such as cell cycle arrest, DNA repair, and apoptosis) (Beckerman & Prives, 2010). To activate these genes, the p53 directly or synergistically needs to promote the pre-initiation complex assembly by binding to the promoter site. RNA Pol II is the core member of the pre-initiation complex. However, the impact of p53 mutations on RNA Pol recruitment or activity has never been explored. The recent CryoEM structures have shown that p53 induces conformational changes in TFIID to aid recruitment to the target gene and also plays an important role in transforming RNA Pol II from the poised state into the elongation phase (Liou et al., 2021; Singh et al., 2016). The p53/Pol II co-complex structure showed that the p53 DNA binding domain binds at the top of the RNA Pol II subunit 2 (Rpb2) to introduce a large structural change in the Pol II to form a closed conformational state. The structure also revealed that the H2 DNA-binding helix in the p53 interacts with the Rpb1 clamp, to engage with response elements within target genes (Liou et al., 2021; Singh et al., 2016). The p53 N-terminal transactivation domains also bind to the Pol II jaw and can contact downstream DNA and TFIIH during PIC formation to promote transcription activation.

Table 4. List of most dominant missense somatic mutants observed in $\geq$ 25 different types of human cancers.

Mutation	Total number of tissues where the mutation is reported
R248Q	51
R175H	46
R273H	46
R248W	43
R273C	41
R282W	40
G245S	36
Y220C	33
E285K	33
C176F	32
H179Y	31
Y205C	30
G245D	30
R249S	28
V173M	27
M237I	26
G266E	26
R280T	26
P151S	25
Y163C	25
Y234C	25
G244D	25

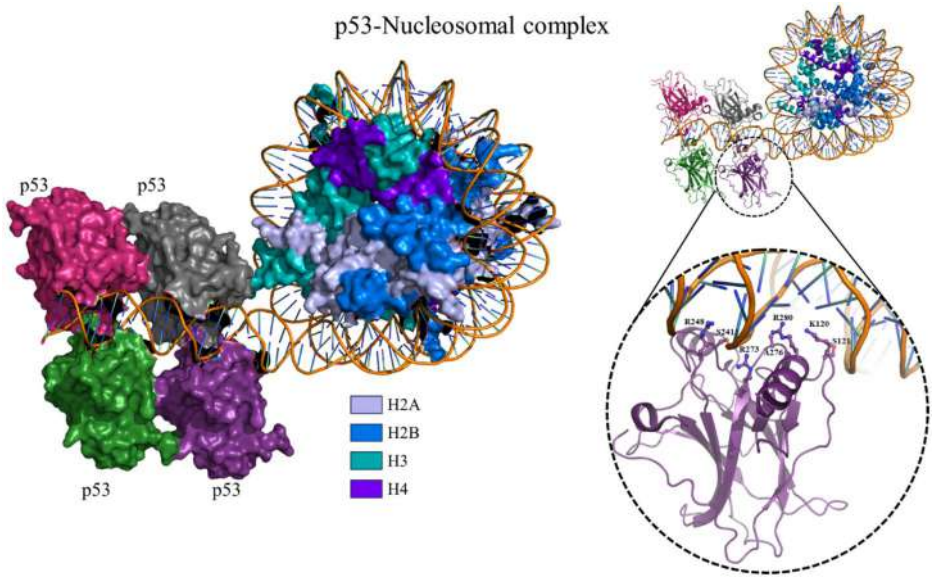

Figure 7. p53-Nucleosomal complex: The cartoon diagram represents the active p53DBD tetramer bound to the DNA promoter site in the nucleosomal complex (PDB ID: 7XZX and 7XZZ) (LHS). The cartoon diagram shows the p53 residues involved in DNA binding (RHS).

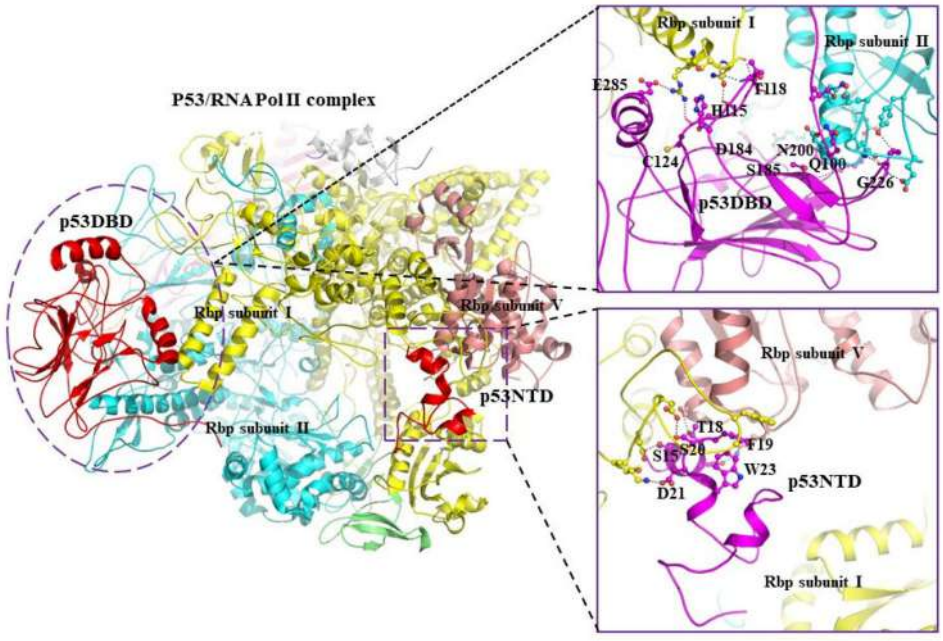

Figure 8. p53-RNA/Pol II complex: The cartoon diagram represents the overall molecular structure and interactions formed between the p53/RNA/Pol II complex. The residues involved in interactions between p53DBD (pink) and Rbp subunits I (yellow) and II (Cyan) (RHS) and the p53NTD (pink) residues involved in interactions with Rbp subunit V (peach) are shown (RHS) PDB ID (6XRE).

To understand the mutation effect on the p53/RNA Pol II complex we analyzed the cryoEM structure of the p53/RNA Pol II assembly complex (PDB ID: 6XRE). We found almost 12 residues in the p53DBD are actively involved in the formation of H-bond with the RNA Pol II subunit I and II (Table S8) (Figure 8). Similarly, 6 residues in the p53NTD form interactions with Rbp subunit V. By comparing the residues with our generated mutation profiles, in DBD His115 to Tyr, Thr118 to Ile/Ala, Asp184 to Tyr/Val/Gly/Ala, Cys124 to Tyr/Trp/Ser, Ser185 to Arg/Asn/Ile, Gly226 to Val/Ser/Ala/Asp, and Asn200 to Thr/Ser are found to be functional mutants indicating that the mutations are not directly affecting the interactions between p53 and PIC complex. The DBD mutants Thr118 to Arg, Asp184 to Asn/His, Ser185 to Gly/Cys, Cys124 to Gly/Arg, Gln100 to Arg, and Asn200 to Lys/Ile/Asp belonging to partial or non-functional could signify the impact of these mutations on the inability of p53 to stably interact with RNA Pol II assembly (Figures 3 and 8). In the p53NTD, no mutation has been reported to date for the residues interacting with Rbp subunit V and subunit I. Altogether, the p53-RNA Pol II axis requires further studies for the correlation of the impact of p53 mutations on RNA Pol II recruitment and functioning. Therefore, the mutant p53-RNA Pol II axis remains an unexplored research highlight that can be addressed by the researchers.

3.7. Geographical distribution of p53 missense mutations in different cancers

It is well known that in more than 50% of cancer cases, p53 exists in a mutated form. Based on the datasets available from the TP53 database ((https://tp53.isb-cgc.org/), we have found 1,297 unique missense mutations which we have characterized in 71 different topographies or cancer types. However, some of the 1,297 missense mutations are abundantly present across the various geographical locations of the world. The dominance of these certain mutations is a major concern for clinicians and strongly requires the development of therapeutic strategies targeting these p53 mutants.

A recent geographical analysis of 689 gastric cancer cases in Eastern Europe, China, and Japan by the Sugimura group showed the complete dominance of p53 in 41% (285 cases) of gastric cancer cases. Out of 285 of these gastric cancer cases having p53 mutations, around 60% were found to belong to the missense mutations category (Natsume et al., 2023). According to their finding, the R175H, R175G, R273C, R273H, R273P, R248W, and R248Q were repeatedly found in more than 5-10 cases per geographic location. Surprisingly, all these mutations belong to the non-functional phenotype category and are also known as the most frequent p53 mutations found in different human cancers (Figure 3, Table 4). The p53 R175H, R248W, and R248Q mutations were reported dominant in Eastern European and Japanese gastric cancer cases. The p53 R248Q mutation was recorded in 5.7% of the gastric cancer cases from Japan as compared to 3.9% in Eastern Europe. Surprisingly, no gastric cancer case was found with R248Q in China. On the other hand, R273C (5.5%) and R273H (4.1%) were the most predominant p53 mutations existing in gastric cancer cases from China. The p53 R237H mutation was also found in 3.9% and 1.6% of gastric cancer cases from Eastern Europe and Japan.

Another geographical p53 mutation analysis of colorectal cancer cases from the Romanian population revealed that out of 81.8% (18 cases) of the p53 mutant positive cancer cases belong to the missense mutation category (Natsume et al., 2023). The mutations such as P152L, Y163H, H179Y, I195N, V203M, S215N, C238Y, C242Y, G244C, G245V, R248W, R248Q, R249S, R273C and R273L were found in Romanian colorectal cancers. Out of all these mutations, only H179Y and V203M belong to partial functional phenotype whereas the rest of the mutations possess non-functional phenotype (Figure 3).

Thus, these two studies provide a classic example to highlight the prevalence of non-functional p53 missense mutations in different cancers of different geographical regions. Additionally, some p53 missense mutations are predominately found in a specific population. An earlier study conducted by the Thorlacius group showed the occurrence of p53 missense mutations such as R175H, R173L, Y163C, R249S, R248G, M237I, S241F, R273C, and C141Y in the Icelandic population (Thorlacius et al., 1993). Among all these mutations, the most frequently occurring mutation in Icelandic populations was R248G. All these reported mutations belong to the category of non-functional p53 missense mutations. Moreover, a recent study conducted on breast cancer cases showed the prevalence of p53 hotspot non-functional missense mutations such as R175H, Y205C, Y220C, R248Q, R273C, and R273H in the Malaysian population (Ragu et al., 2023).

Furthermore, there are a lot many p53 missense mutations that have been reported in various cancers around the world as shown in Table S9. However, most of these reported mutations belong to non-functional mutations. Therefore, all the above data indicates the impact of non-functional p53 missense mutation in tumorigenesis.

4. Conclusions

The tumor suppressor p53 is the master regulator of a cell which regulates several pathways specifically apoptosis. Therefore, the inability of p53 to perform its transcription function is the main cause of tumorigenesis and tumor progression. It has been well understood that certain missense mutations make p53 an oncogene and loses its conventional guardian of the genome feature. Also, it is commonly observed that in several cancers harboring heterozygous states where both wildtype and mutant p53 co-exist, the mutant p53 is found to be in the dominant state where it hijacks the normal function of p53 (Milner & Medcalf, 1991; Milner et al., 1991; Cuddihy et al., 2008). Therefore, it becomes a major necessity to understand the impact of each unique mutation on the structure and function of p53. However, there is no such study that showcases the possible different types of p53 missense mutation existing in different human cancers.

Our map shows the list of all potential p53 mutants which have been found in numerous human cancers to date. In addition, the map also describes the list of all super trans, functional, partially functional, and non-functional p53 mutations. Our analysis significantly provides an overall proposition of all potential therapeutic targets in p53 that can be used to identify drugs, small molecule chaperones, or small peptides to restore its wild-type function or to eliminate mutant p53 by immunotherapies to prevent the progression of tumor cells.

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Author contributions

L.M: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data Curation, Graphics, Writing, Review & Editing, and Visualization. **A.S:** Validation, Investigation, Formal analysis, Review & Editing, Data Curation. **P.K:** Data Curation. **A.S.E:** Methodology, Validation, Formal analysis, Investigation, Data Curation, Writing - Original Draft, Writing - Review & Editing, and Visualization.

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Ethical approval

The article does not involve any study with human participants or animals to be performed by any of the authors.

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Data availability statement

The data underlying this article will be shared on reasonable request to the corresponding author.

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