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Review

Unraveling the unique amyloid-like aggregation behavior of the tumor suppressor p53 mutants in human cancers

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ABSTRACT

Missense mutations in the tumor suppressor p53 significantly disrupt its native structure and functions, playing a pivotal role in human cancer pathogenesis. Oncogenic mutant p53 (mutp53) not only loses its tumor-suppressive capabilities but also acquires oncogenic functions, driving cancer progression, metastasis, and chemoresistance. Despite extensive research on mutp53, the role of missense mutations in triggering amyloid-like aggregation of p53 remains an underexplored and fascinating area of study.

To date, over 50 proteins are known to form amyloid-like aggregates due to abnormal folding, resulting in insoluble protein fibrils that contribute to various protein misfolding diseases, including cancer. However, the precise mechanisms by which aggregated proteins induce cancer remain inadequately understood. Notably, certain p53 mutations promote its aggregation, which has emerged as a critical factor in protein aggregation-induced oncogenesis.

This review delves into the mechanisms underpinning mutp53 aggregation, emphasizing unique properties such as coaggregation, bio-isolation, prion-like cell-to-cell transmission, and chemoresistance promotion. Leveraging diverse in-silico, biophysical, and biochemical approaches, we comprehensively analyzed the aggregating potential of 26 mutp53 variants among 1297 missense mutations identified in human cancers. These findings shed light on the multifaceted roles of mutp53 aggregates in oncogenesis and tumor progression.

Lastly, we present an integrative exploration of emerging therapeutic strategies designed to disaggregate mutp53 aggregates, offering promising directions for targeted cancer therapy. By addressing this enigmatic aspect of mutp53 biology, our review advances the understanding of protein aggregation in cancer and identifies avenues for innovative therapeutic interventions.

1. Introduction

Today, a multitude of neurological disorders, amyloidosis, and protein misfolding diseases are recognized as a result of protein aggregates deposition [1–5]. Amyloidosis encompasses a collection of diseases caused by either protein misfolding and aggregation, or protein unfolding and aggregation, ultimately leading to the formation and accumulation of insoluble protein aggregates, which accumulate as insoluble amyloid fibrils [2,6–8].

Intriguingly, the role of protein aggregation in oncogenesis and tumor progression has garnered substantial attention and investigation since 2010. Yet, compared to other amyloid-related diseases, cancer remains the least explored area.

The behavior of proteins expressed uncontrollably due to genetic mutations, leading to structural aberrations, is crucial for decoding the cancer-mediated pathway. Genetic mutations can endow proteins with either gain-of-function or loss-of-function activities that promote tumor formation. One of the key master regulatory proteins often found mutated in more than 50 % of cancers is p53 [9–11]. The tumor suppressor p53 is a key master regulator of the cell, which can control several crucial functions related to the cell cycle, cell stress, cell growth and senescence, DNA repair, aging, apoptosis and more [12–14]. Therefore, mutations that abrogate the wild-type functions of p53 and transform this guardian of the genome into an oncogene [9,11,15].

Today, 1297 different combinations of missense mutations have been identified in all five domains of p53, and have been widely reported in 71 diverse types of human cancers [16]. Among all the p53 domains, the highest number of missense mutations are found in the core DNA Binding Domain (DBD) (82.11 %), followed by Oligomerization Domain (OD) (6.01 %), N-terminal Trans-Activation Domain (TAD) (5.01 %), Proline-Rich Region (PRR) (4.08 %), and C-terminal Regulatory Domain (RD) (1.39 %). Out of these 1297 different unique

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combinations of missense mutations, 264 have also been identified as germ-line mutations, showing the potential to affect humans across generations [16].

With the continuous assessment of different mutant p53 (mutp53) over the last two decades, it is now understood that several types of p53 mutations could lead to the formation of amyloid-like aggregates [17–20]. Recently, these mutp53 aggregates have been identified as the major contributors to oncogenesis and tumor progression. These investigations have provided potential ways to identify and study mutp53 aggregates under *in-vitro* and *in-vivo* conditions, aiding in unveiling the non-canonical oncogenic function and pathways associated with different p53 mutations in human cancer. Furthermore, these studies have provided substantial evidence to inhibit mutp53 aggregation, which ultimately leads to the suppression of mutp53-mediated oncogenic function.

In this review, we have established how certain missense mutations can transform the guardian of the genome, "p53", into an amyloid-like aggregate. These aggregates bestow unusual properties responsible for oncogenesis, tumor progression, and chemoresistance. Consequently, this information will not only aid in understanding the oncogenic functions of mutp53 amyloid-like aggregates as prions but also provide insights to counteract this issue by exploring various mutp53-disaggregation strategies. These strategies could lay the foundation for developing potential anti-cancerous therapies.

2. Amyloid aggregates in normal cellular functions and protein misfolding diseases

Amyloid aggregates are abnormal structures that form when proteins and peptides misfold and accumulate within cells or tissues. These aggregates are usually insoluble and have a fibrous nature. The term "amyloid" specifically denotes a type of cross-beta structure [21].

Matthias Schleiden coined the term amyloid to denote the amylaceous component of plants in 1838 [21,22]. The amyloid proteins have three characteristic features: they exhibit fibrillar morphology, are formed by β-sheet enriched proteins, and can bind to specific dyes such as Thioflavin (ThS or ThT) and Congo Red [23,24]. These amyloid proteins are generally associated with a plethora of diseases, often referred to as Protein Misfolding Diseases (PMDs). Among these, prions represent a unique subset of amyloidogenic proteins distinguished by their ability to act as infectious agents through self-propagating misfolding. Unlike conventional amyloid deposits, prion proteins can induce conformational changes in normally folded counterparts, perpetuating an irreversible aggregation cycle that disrupts cellular homeostasis. In humans, prions are known to cause more than 50 diseases, including obesity, neurodegenerative diseases and cancer [25–30].

Amyloid aggregates are linked to various human PMDs, including Alzheimer's disease, Parkinson's disease, systemic amyloidosis, and Huntington's disease. Each of these is associated with different types of aggregates: Amyloid-beta and tau aggregates, alpha-synuclein, amyloid light chains, and huntingtin protein aggregates. The formation of amyloid aggregates involves a process where monomers self-assemble into oligomers, protofibrils, and fibrils of varying molecular weights [31,32]. In the brain, Amyloid-beta aggregates contribute significantly to neurodegenerative processes. Alpha-synuclein aggregates emerge in dopaminergic neurons, while systemic amyloidosis causes amyloid accumulation across organs like the heart, kidneys, and liver, leading to organ dysfunction [33,34].

The formation of Amyloid-beta aggregates involves a nucleation-dependent polymerization process, where Amyloid-beta peptides come together to form a nucleus, thereafter generating fibrils [35]. Amyloids are not just fixed as disease-causing protein aggregates; certain physiological roles associated with amyloids include acting as natural reservoirs for storing peptide hormones in pituitary-secreting granules, being involved in skin pigmentation by initiating the biogenesis of melanosomes and contributing to long-term memory fixation [36–38].

On the other hand, Alpha-synuclein aggregates form through liquid-liquid phase separation and undergo a phase transition to a condensed aggregation phase [39]. Unlike prions, which can transmit their misfolded structures between cells, amyloid aggregates do not have this infectious characteristic [40]. In the 1980s Stanley Pruviner developed the concept of Prions and was awarded the Nobel Prize in 1997 for his discovery [41]. Prion aggregates form through a templated conversion mechanism, where a misfolded PrPSc protein (Scrapie isoform of the prion protein) induces the misfolding of the normal PrPC protein, continuing a self-propagating cycle of aggregation [42].These prions or Prion-like proteins have amyloid-like characteristic structures and can convert cellular proteins into amyloid compounds (by co-aggregation) distributed across the cell [43]. Prions are amyloid proteins with the unique ability to self-transmit from a donor cell to a recipient cell.

3. The origin of mutp53 as an amyloid-like aggregating protein

To date, more than 50 proteins are known to exhibit amyloid characteristics, with p53 being one of them, especially in certain missense mutations [44–46]. Mutant p53 can form amyloid-like aggregates and template the misfolding of wild-type p53 protein, leading to the creation of amyloid-like aggregates. These mutp53(s) can acquire new deleterious function(s) (gain-of-function) and persist in tumors, providing a selective advantage that drives cancer development and progression [47]. The characteristics of specific p53 mutations forming misfolded & aggregation-prone conformations, and gaining oncogenic functions, make the mutp53 a new unique member of the prion family.

The accumulation of p53 in an aggregation state has been reported in several cancers, such as breast, retinoblastoma, neuroblastoma, and colon [48,49]. This aggregation weakens the cell's defence against tumorigenesis, endowing p53 with oncogenic properties. The occurrence of these mutations in p53 could lead to both loss-of-function (LOF) and gain-of-function (GOF) phenotypes.

The LOF function of mutp53 includes the inability of mutp53 to function like wild-type protein, thereby inhibiting apoptosis. Additionally, LOF mutp53 could aggregate into large masses of insoluble protein that lose the ability to move from the cytoplasm into the nucleus and become resistant to proteasomal-mediated degradation [50–52]. This inability of mutp53 to execute wild-type functions results in increased expression of pro-cancerous genes, leading to the abolishment of cell cycle arrest and apoptotic functions ([50,52]. Mutp53 accumulation as high-molecular-mass aggregates is capable of exhibiting dominant negative (DN) effects (DNE), by suppressing the wild-type-like functions of p53 and inhibiting the Mdm-2-mediated ubiquitination [53–57].

On the other hand, the aggregating mutp53 exhibits a variety of GOF functions such as transactivating genes that are not the target of wtp53, co-aggregating other proteins such as p63, p73, Hsp70, acetyltransferase p300, wtp53, etc.; promotes inflammation, chemoresistance, cell migration, tumor invasion, and metastasis. [9,15,52,58–66].

In the 1990s, it was discovered that p53 could also exist as self-aggregating higher molecular mass aggregates [62–64]. Out of its five functional domains, only three—namely, the central core DNA binding domain (DBD), the tetramerization domain (TD), and the transactivation domain (TAD) — are known to form amyloid aggregates [16,53,65–68]. The 393 amino acid tumor suppressor p53 has always been a key target of investigation for numerous researchers over the past five decades. With an exorbitant 1297 different types of missense substitution mutations, p53 holds the title for the most mutated human protein, often found mutated in human cancers. Interestingly, 26 of these mutations have been reported with aggregation-forming ability. As of now, these mutations are confined to just 21 residues of the p53, as shown in Table 1 and Fig. S1.

In 1995, the p53 was first mentioned as a prion [22,69]. In 1991, Milner et al. found that certain p53 mutations, such as R151S, R247I, R273L, and R273P, cause changes in wtp53 conformation to mutp53

Table 1
List of aggregating p53 missense mutations.

S. No.	Residue present in wtp53	Residue location	Substitution mutation(s)	Reference
1.	R	110	L P	[52] [53]
2.	R	151	S	[54]
3.	Y	157	F	[55]
4.	R	158	L	[56]
5.	R	175	H	[52]
6.	H	193	L	[53]
7.	I	195	L	[59]
8.	Y	220	C	[60–70]
9.	Y	234	C	[59]
10.	M	237	I	[58]
11.	G	245	S	[58,71]
12.	N	247	I	[57]
13.	R	249	G W	[17,20,52,59,72–75] [52,75]
14.	R	249	S	[52]
15.	P	250	L	[53]
16.	R	258	Y	[52] L [57]
17.	R	273	P H R	[57] [76] [75]
18.	R	280	T	[60]
19.	R	282	W	[52]
20.	G	334	V	[18]
21.	R	337	H	[61]

conformation during co-translation [57]. Later studies showed the efficient antibody-based determination of wt or mutp53. The Antibody pAb1620 recognizes wtp53; on the other hand, the mutp53 is recognized by the pAb240 antibody [90]. Aggregating mutp53 were reported as transcriptionally inactive high-molecular-mass complexes. The aggregation of mutp53 can be triggered by increasing temperature (from 30 to 37 °C), pH and pressure. [17,91].

X-ray crystallography and NMR studies have revealed similarities between misfolded mutant p53 conformations and prions [17,92]. Cellular assays have shown that mutant p53 can aggregate and template the misfolding of wild-type p53 [93]. Additionally, *in-vivo* studies have illustrated that GOF mechanisms in mutant p53 are linked to cancer development [19,52,86]. The elucidation of the oncogenesis mechanism of different mutp53 aggregates will add tremendous significance to cancer research. Targeting mutp53 aggregates might serve as a promising tumoricidal strategy.

4. Unusual properties of Mutp53 aggregates

Mutp53 has been reported as the newest member belonging to the class of aggregating proteins causing numerous diseases, as reported in Amyloid Nomenclature 2020 [44].

The following are some unusual properties exhibited by mutp53 aggregates in different human cancers:

1. Mutp53 can form high-molecular-mass aggregates

In 2006, Higashimoto et al. conducted a study on the tetramerization domain of various p53 mutants associated with lung cancer (Fig. 1a) [18]. They discovered that the mutant p53G334V possesses a non-ordered structure that undergoes a thermal conformational change, primarily involving β-dominant structural alterations as estimated by Circular dichroism (CD) spectroscopy. The study found that the p53G334V mutant could form amyloid-like fibrils through a two-step process under physiological pH and temperature conditions, as evidenced by gold-standard Congo Red and Thioflavin T staining [23,24]. Additionally, the atomic force microscopy (AFM) analysis indicated that mutp53G334V forms amyloid-like fibrils with a thickness greater than 1 nm, a width of 25 nm, and a length ranging from 150 to 300 nm. Moreover, globular aggregates with a thickness of 3 nm and a diameter of 60 nm were observed.

In 2021, Yang et al. reported the unusual property of the mutp53R248Q to form the mesoscopic clusters, distant from amorphous and ordered aggregates. Using the HCC70 breast cancer cell line, they found that these protein clusters facilitate amyloid fibril nucleation [76]. The application of 1,6-hexanediol did not reduce the number of cytoplasmic p53 puncta, confirming that the p53R248Q aggregates present within the cytoplasm were not dense liquid condensates.

Through Oblique illumination microscopy (OIM), the average size of these mutp53 mesoscopic aggregates was identified to be 100 nm, varying from 90 to 150 nm depending on temperature. These clusters could encompass approximately 1000 p53 tetramers in a moderately packed conformation (Fig. 1a).

The diameter of these aggregates was further validated by dynamic light scattering (DLS), and electron microscopy (EM) micrographs confirmed the aggregates' size, range of 60 to 80 nm. Using various biophysical and bioinformatics tools, they proposed a two-step fibrillization process for R248Q mutp53, where the misassembled monomers, native tetramers, and mutp53 oligomers (such as pentamers) assemble to form mesoscopic mutp53 aggregates or clusters (Fig. 1b). Subsequently, mutp53 fibrils, assumed to be formed by the stacking of refolded mutp53 monomers, nucleate from the mesoscopic clusters after mutp53 monomers assembly. The existence of R248Q mutation in p53 results in the destabilization of the p53DBD structure, exposing the ILTIITL motif ranging from 251 to 257 residues, thereby reinforcing mutp53 aggregation (Fig. S1).

2. Mutp53 sequester wtp53 during aggregation

MutpS3 has a characteristic amyloid-like structure and typically exhibits seed aggregation [26,68]. By 2012, it was already known that the p53R248Q seeds could accelerate the aggregation of wtp53 [17], where the free energy of unfolding of mutp53R248Q was determined to be 34.6 kJ/mol, compared to 42.5 kJ/mol of wtp53 [78,94]. Thus, indicating that a single missense mutation could induce severe alterations in the protein conformation sufficient to cause aggregation (Fig. 2).

Later studies found that mutp53 can co-localize with other amyloid-like proteins in breast cancer [59]. Levy et al. discovered that p53 mutants such as H193L, I195L, G245S, and R248Q could moderately form aggregates, whereas the p53Y234C mutation resulted in abundant aggregate formation. Using A11 antibodies, they identified aggregated mutp53 existing with wtp53 which was characterized by DO1 antibody in breast cancer tissue samples [59]. Further studies, including X-ray diffraction and EM studies analysis, revealed that mutp53R248Q and wtp53 co-aggregate to form amyloid fibers [17]. The size of these aggregates was found to be approximately 132 nm at pH 6.0, increasing to 160–250 nm at pH 7.2. Interestingly, Wang and Fersht demonstrated that mutp53 could sequester not only wtp53 but also p53 homologs such as p63 and p73 [68]. They proposed that this mutp53-wtp53 coaggregation process is predominantly due to trapping rather than seeding and induced propagation (Fig. 2).

3. Mutp53 aggregates promote bio-isolation of various tumor suppressors: a curious case of Co-aggregation

In 2011, Xu et al. discovered that mutp53 aggregates result from the assembly of a conserved sequence existing in the hydrophobic core of the protein's DNA-binding domain. Transient expression of various mutp53 proteins (R175H, R282W, R249S, R248Q, P250L, E258V, R110L, and R110P) in a p53 null osteosarcoma cell line revealed a predisposition of mutp53 as cytoplasmic aggregates, identified as punctate cytoplasmic spots. These mutp53 aggregates were

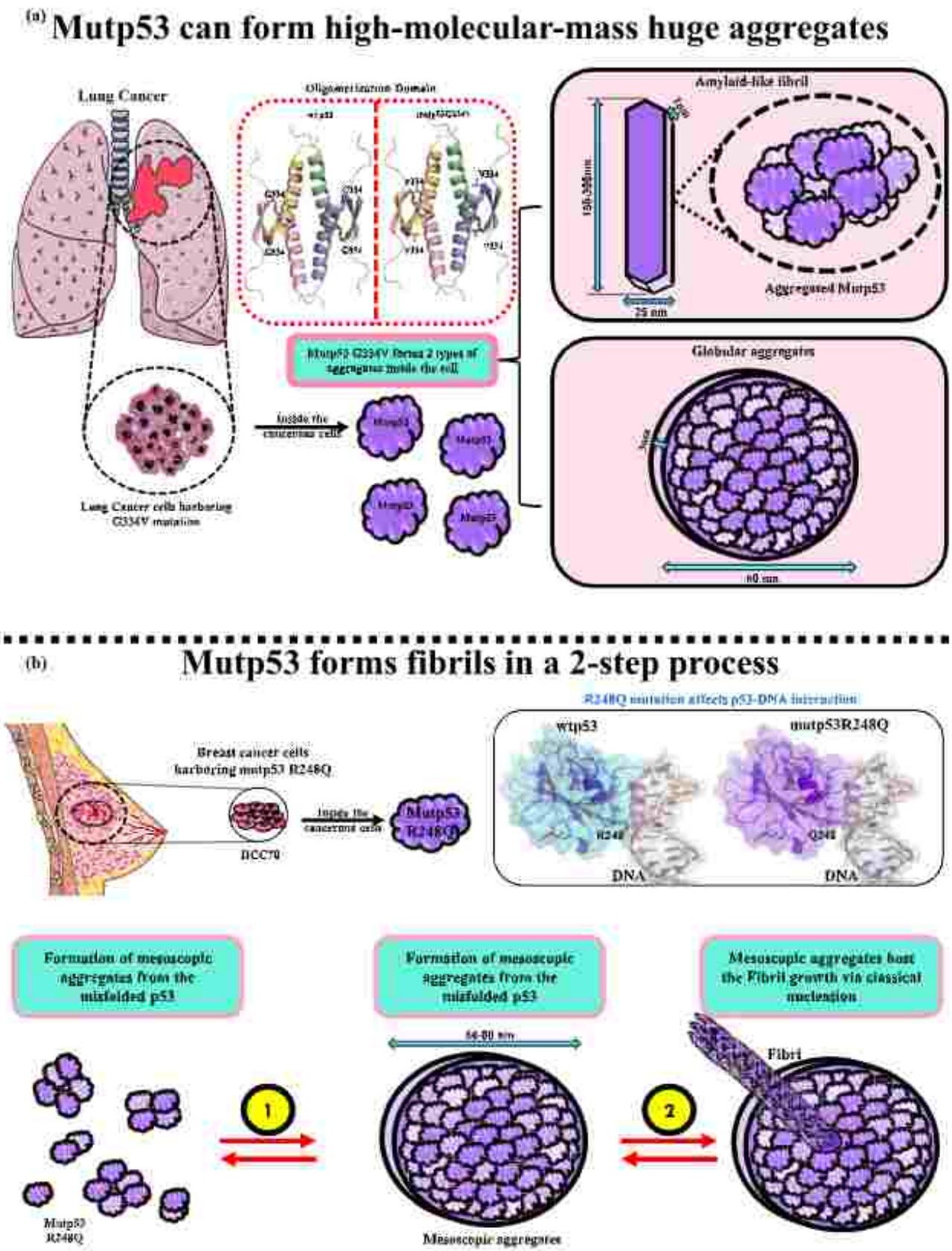

Fig. 1. Illustration showing Mutp53 can form high-molecular-mass aggregates: (a) The p53G334V mutant facilitates the formation of amyloid-like fibrils and globular aggregates within the lung cancer cells. (b) In breast cancer cells, the p53R248Q DBD mutant undergoes a 2-step aggregation process where the misfolded mutp53 initially forms mesoscopic aggregates. These aggregates then act as nucleation sites for the fibril growth.

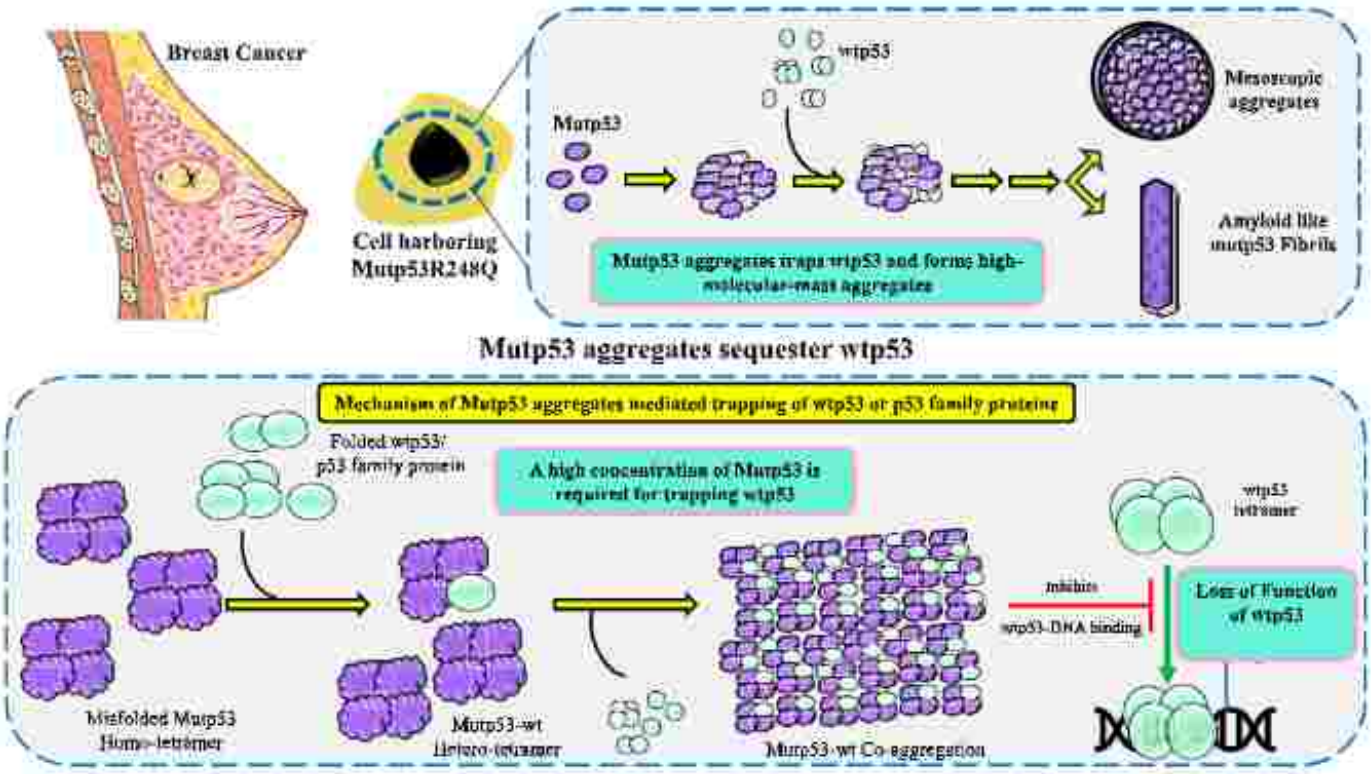

Fig. 2. Illustration showing Mutp53 aggregates sequester wtp53: In breast cancer cells harboring the mutR248Q, misfolded mutp53 aggregated into both mesoscopic and amyloid-like fibers. These pathological aggregates trap wtp53 or p53 family proteins, leading to loss of wtp53 functionality and contributing to tumor progression.

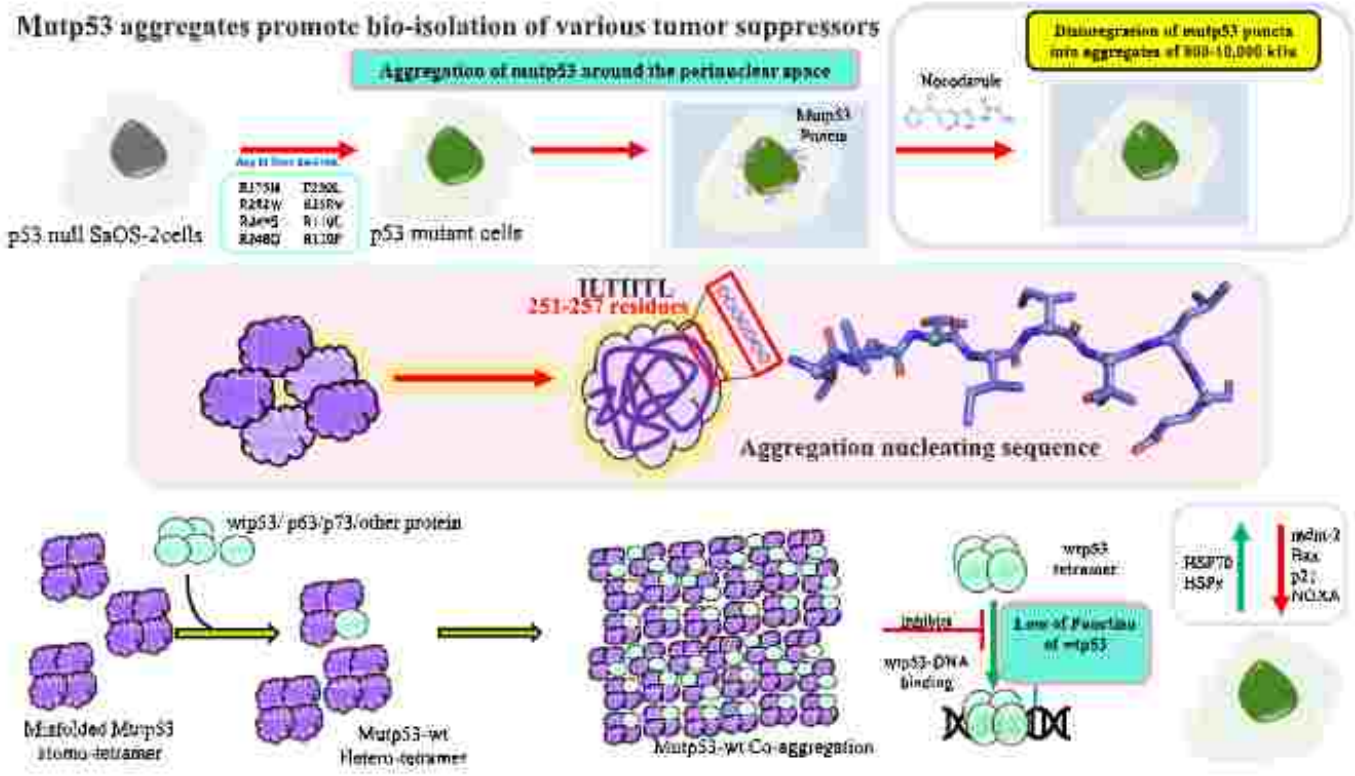

Fig. 3. Graphical representation showing Mutp53 aggregates promote bio-isolation of various tumor suppressors. The transient expression of various mutp53 variants results in the formation of mutp53 puncta around the perinuclear space in the p53-null cells. Upon Nocodazole treatment, these puncta disaggregate into large protein complexes ranging from 600 to 10,000 kDa. The p53 residues 251–257, coding for ILTIITL, act as an aggregation nucleation sequence. Furthermore, mutp53 aggregates can trap p53 family proteins and other tumor suppressor proteins via co-aggregation, ultimately inhibiting wtp53 tetramer formation and impairing tumor suppression.

predominantly found in the perinuclear space, leading to impaired mutp53 nuclear import [52].

Using a microtubule assembly disrupter, Nocodazole, they have observed the spontaneous disintegration of the cytoplasmic punctate-mutp53 aggregates, thus highlighting the aggresomal nature of the mutp53 aggregates. Unlike the normal oligomerization state of wild-type p53 (monomeric, dimeric, tetrameric, and octameric), the oligomerization state of the p53 mutants R175H, R282W, R248Q, R249S, P250L, E258V, R110L, and R110P was found within the molecular weight range between 800 and 10,000 kDa. Therefore, indicating the formation of high molecular weight multimeric mutp53 assemblies (Fig. 3).

The p53 mutants R175H, R282W, P250L, and R110P were found to exist as aggregates in human cancer cell lines harboring these mutations (MOG-G-CCM astrocytoma for p53R110P, HT-1376 bladder carcinoma for p53P250L, Detroit 562 pharynx carcinoma for p53R175H, and 1301 T-cell leukemia for p53R282W). Thioflavin T staining confirmed the presence of β-aggregates in the cells. Further, the use of the Biophysical technique FTIR also confirmed the aggregating nature of p53 mutants such as P250L, E258V, and R110L (Peak 1615 and 1683) under *in-vitro* conditions. Electron microscopy cross-validated the aggregation nature of these p53 mutants, confirming that mutp53 can form high molecular weight multimeric assemblies.

Additionally, the Tango server predicted that residues 251–257 could serve as an aggregation nucleus, which was further confirmed by ESI-MS of the proteinase-treated mutp53 fragments, as the β-pleated 251–257 was protected due to the involvement in aggregation. Moreover, it was also established that mutp53 could co-aggregate with wild-type p53, suggesting the possibility of oncogenesis in heterozygous conditions for p53, where both wt and mutant p53 coexist in the cell. These findings indicated that mutp53-mediated aggregation is mutation-specific and does not rely on subcellular localization. Additionally, these aggregates were found to trap not only wtp53 but also have the tendency to trap other family members, such as p63 and p73 and other proteins in them, disrupting their tumor suppressor activity [20]. Interestingly, mutp53 aggregates (especially R175H) were shown to increase the expression of Hsp70 and other heat shock proteins. Consequently, the loss-of-function phenotype of mutp53 was confirmed for p53 mutants R175H, R282W, R248Q, R249S, R248W, P250L, E258V, R110P, and, R110L due to their decreased transcription activity for mdm-2, bax, p21 and NOXA [95].

Wiech et al. established that recurrent transient interaction of Hsp70 with aggregating mutp53 results in increased half-life of the mutp53, promoting aggresome formation from the pseudoaggregate state of mutp53-mdm2-HSP70 [96]. Subsequently, Li et al. showed that the aggresomal p53R280T mutant interacts abnormally with Hsp90, which results in disruption of p53 nuclear transport, which will lead to decreased cell cycle arrest and apoptosis in nasopharyngeal carcinoma [80] (Fig. 3).

Interestingly, the p53I254R mutation tends to suppress the aggregation. The p53 mutants such as I254R, I254D, I255R, and I255D disrupt the aggregation phenomenon of these mutp53 and prevent wtp53 entrapment.

4. Mutp53 fibril displays prions-like cell-to-cell transmission

Mutp53 aggregates could display prion-like properties & morphology and could undergo cell-to-cell transmission. In 2020, Iwahashi et al. evidenced that the prion-like behavior of mutp53 aggregates is mediated by sulfated-glycosaminoglycans (GAGs) (Fig. 4). GAGs are linear, unbranched heteropolysaccharides, such as heparin sulfate (HS), chondroitin sulfate (CS), and keratin sulfate (KS), which are linked to the core of a protein to form proteoglycans with varied net charges. Sulphated-GAGs are associated with the disease pathology of amyloidosis and cancer [25,97,98]. They have investigated sulfated GAGs-mediated cellular interaction and extracellular release of the

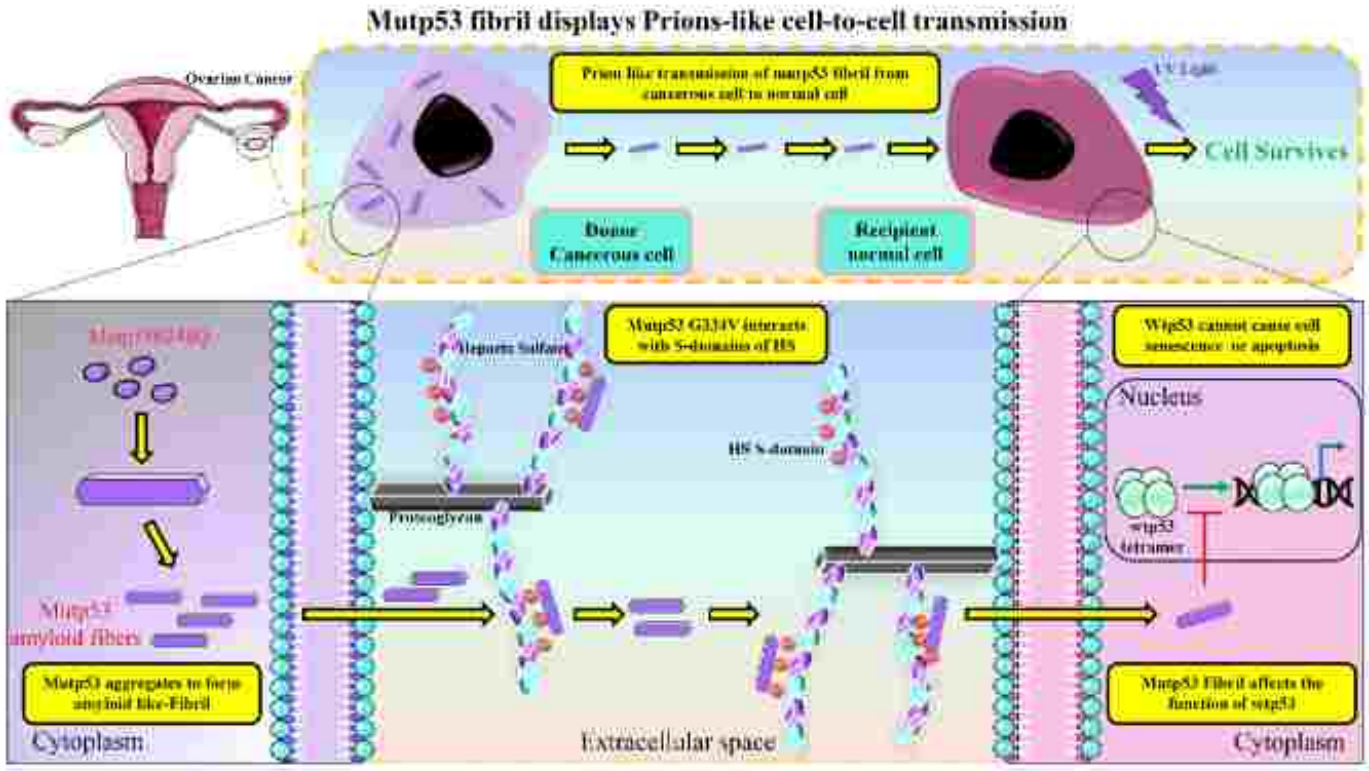

Fig. 4. Schematic diagram representing that Mutp53 fibril displays Prions-like cell-to-cell transmission: In ovarian cancer cells harboring the p53R248Q mutation, misfolded mutp53 aggregates into self-transmissible amyloid-like fibrils that exhibit prion-like behavior. These fibrils interact with HS-S domains of normal cells, facilitating cellular entry and promoting cancerous transformation. As a result, wtp53 loses its ability to induce senescence or apoptosis, further contributing to tumorigenesis.

aggregated mutp53. They identified the extracellular release of mutp53-amyloid aggregates using the OVCAR-3 ovarian cancer cells harboring p53R248Q mutation [35,75]. These aggregates were readily taken up by the CHO (Chinese hamster ovarian cancer) cells harboring wtp53. The cellular uptake of aggregated mutp53 was found to be less efficient in cells (pgsA-745 and pgsD-677 cells) lacking sulfated GAGs and HS (Heparin sulfate). These findings were corroborated by the pre-treating cells with β-xyloside and sodium chlorate, which suppress proteoglycan synthesis or sulfation modification, resulting in decreased uptake of mutp53 aggregates secreted by ovarian cancer cells [99,100]. Additionally, the use of HS S-domains structural analog "Heparin" highlighted the competitive inhibition of cellular uptake of mutp53 aggregates, implicating the involvement of HS's highly sulfated domains as non-protein components in mutp53 aggregation in ovarian cancer cells (Fig. 4).

Although the role of mutp53 aggregate from the donor mutant cell in the recipient wtp53-containing cell was not discussed in this study. However, it was found that the recipient cells taking up p53 aggregate secreted from the donor cells gained chemo-resistant properties. This was evidenced by reduced Caspase-3 activation and decreased damage from UV radiation exposure.

5. Relationship between amyloid-like mutant p53 and chemoresistance

In 2020, a study conducted by Oliveira group revealed that in glioblastoma, the entrapment of mutp53 within amyloid-like aggregates is accountable for endowing chemoresistance as an unusual GOF [58]. They observed increased chemoresistance against TMZ-treatment (Temozolomide) in glioblastoma T98G cells expressing p53M237I compared to U87 cells expressing wtp53 (Fig. 5). These glioblastoma cells with mutp53, found as aggregates in perinuclear and nuclear regions, exhibited enhanced transcription levels of MGMT (O6-methylguanine DNA-methyltransferase) and PTEN. Thus, indicating accelerated cell proliferation as well as migration.

While the mechanism behind the mutp53 aggregation causing TMZ chemoresistance in glioblastoma has not been fully explored. However, they gave an account of pressure and hydration effect in promoting mutp53 aggregation, along with in-silico studies where mutp53 was found susceptible to water infiltration.

In another study, the mutp53R248W-mediated Cisplatin-resistance was investigated in lung cancer H1299 cells. Here, the mutp53 aggregates were found to be involved in upregulating UPR (unfolded protein response), and ERP29, aka Endoplasmic reticulum protein-29 [77]. Thus, reflecting the elevated ER stress, impaired protein secretion, folding, and trafficking. The mutp53 was shown to interact with the ERP29 gene promoter, thereby triggering chemoresistance (Fig. 5). This aggregated mutp53-ERP29-mediated chemoresistance was revoked using an artificial peptide ReACp53 [77].

6. Inhibition of p53 aggregation

The best strategy to combat the oncogenic function of aggregated mutp53 is to disaggregate it [43,56,73,101,102]. A large diversity of molecules has been discovered to target the disaggregation of mutp53 by employing different strategies, which are discussed (Tables 2 and S1, Figs. 6 and 7).

1. The effect of non-aggresomal p53 mutants

Interestingly, the p53I254R tends to suppress the aggregation. The p53 mutants such as I254R, I254D, I255R, and I255D disrupts the aggregation phenomenon of mutp53 and prevent the entrapment of wtp53 (Fig. 6a) [52].

2. Acetylation inhibits tumor aggregation and rescues the tumor suppressor function of mut53

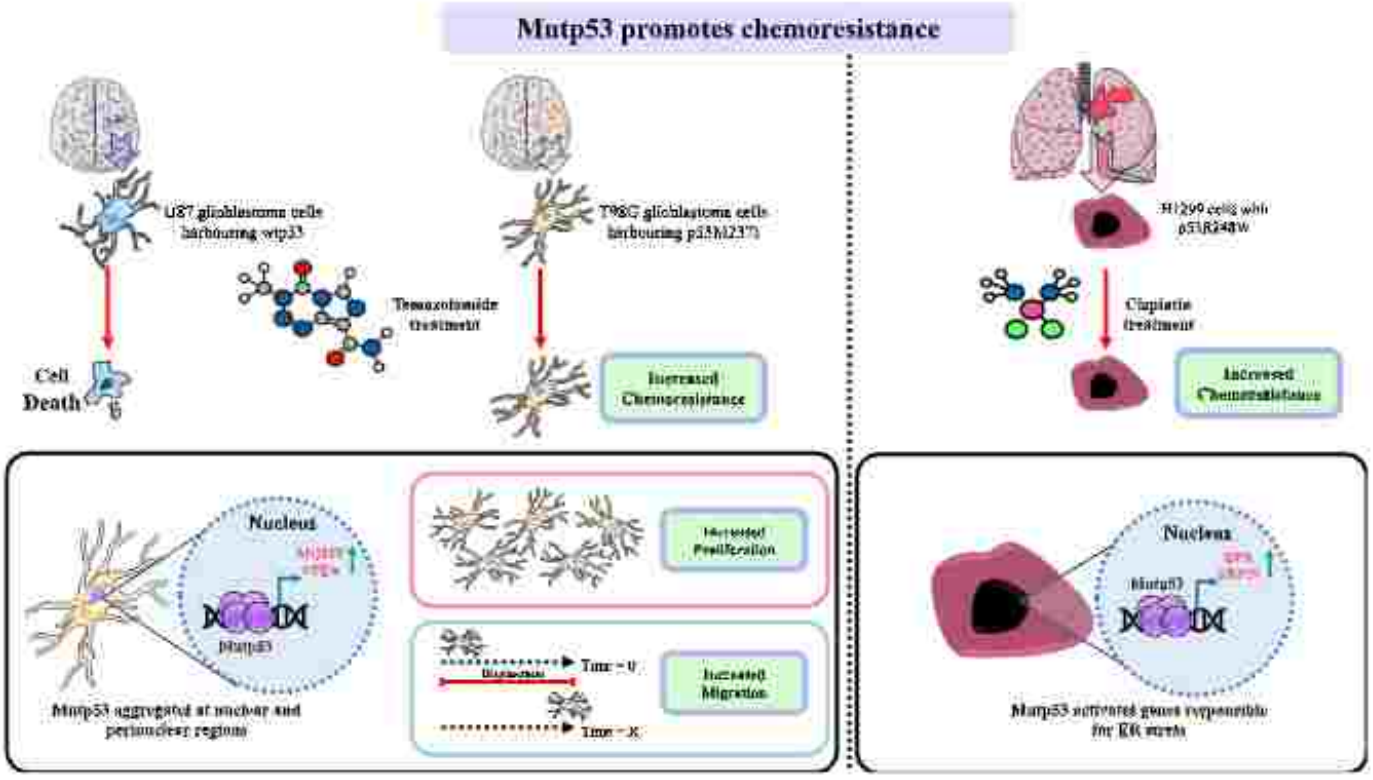

Fig. 5. Schematic displaying Mutp53 promotes chemoresistance: In glioblastoma cells, the mutant p53M237I variant confers temozolomide resistance by inducing MGMT and PTEN overexpression, leading to enhanced proliferation and migration. Similarly, the transient expression of p53R248W mutant in p53-null small-cell carcinoma cells results in cisplatin resistance due to mutp53-induced UPR and ERP29 ER stress activation, thereby fostering a chemoresistant phenotype.

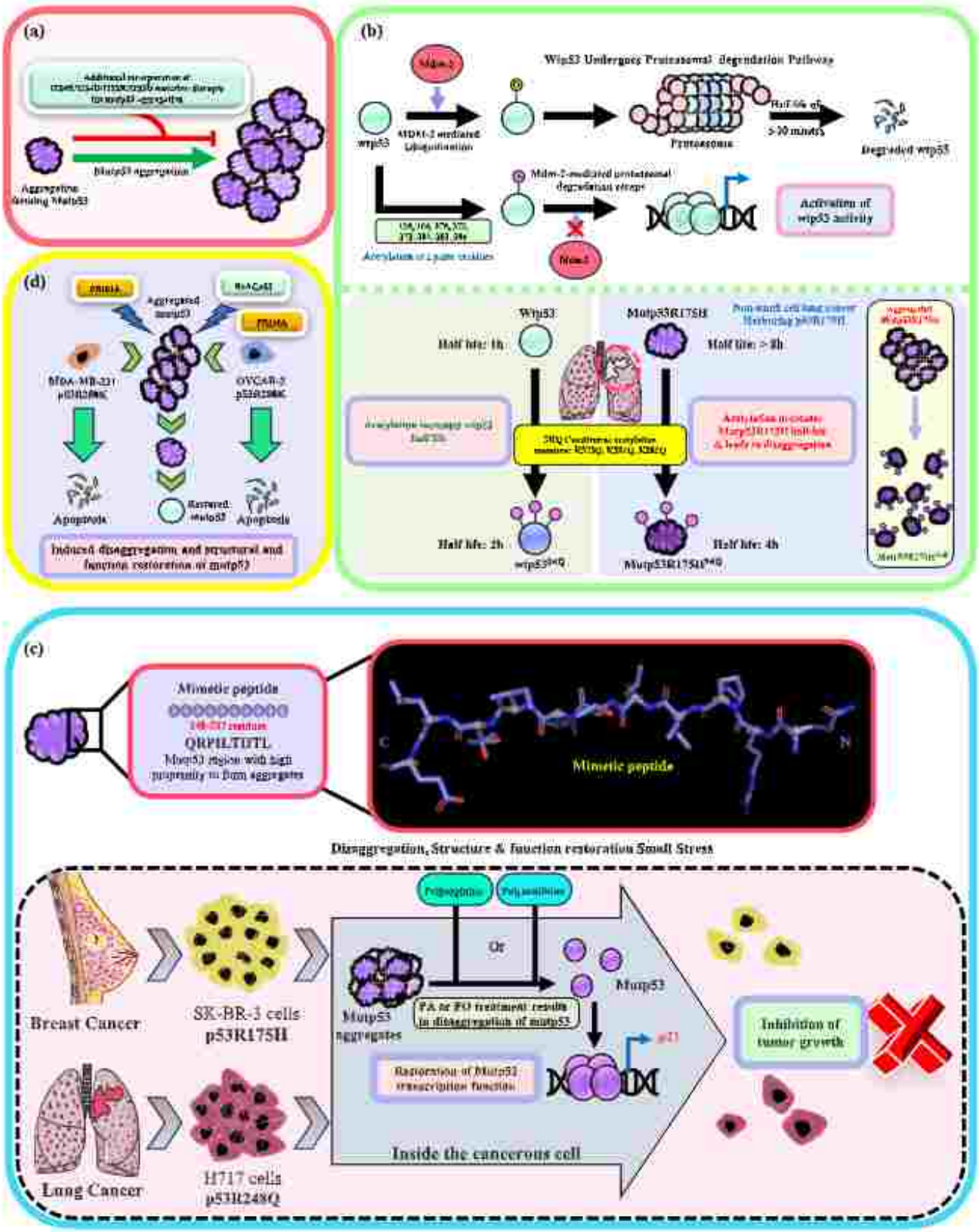

Fig. 6. Illustration showing different strategies to counter Mutp53 aggregation in human cancers. (a) Mutation-induced disaggregation of mutp53. (b) Mutation-induced Acetylation promotes mutp53 disaggregation. (c) Polyarginine and polyornithine mediated inhibition of mutp53 aggregates. (d) PRIMA and ReACp53 facilitate disaggregation as well as structural & functional restoration of mutp53.

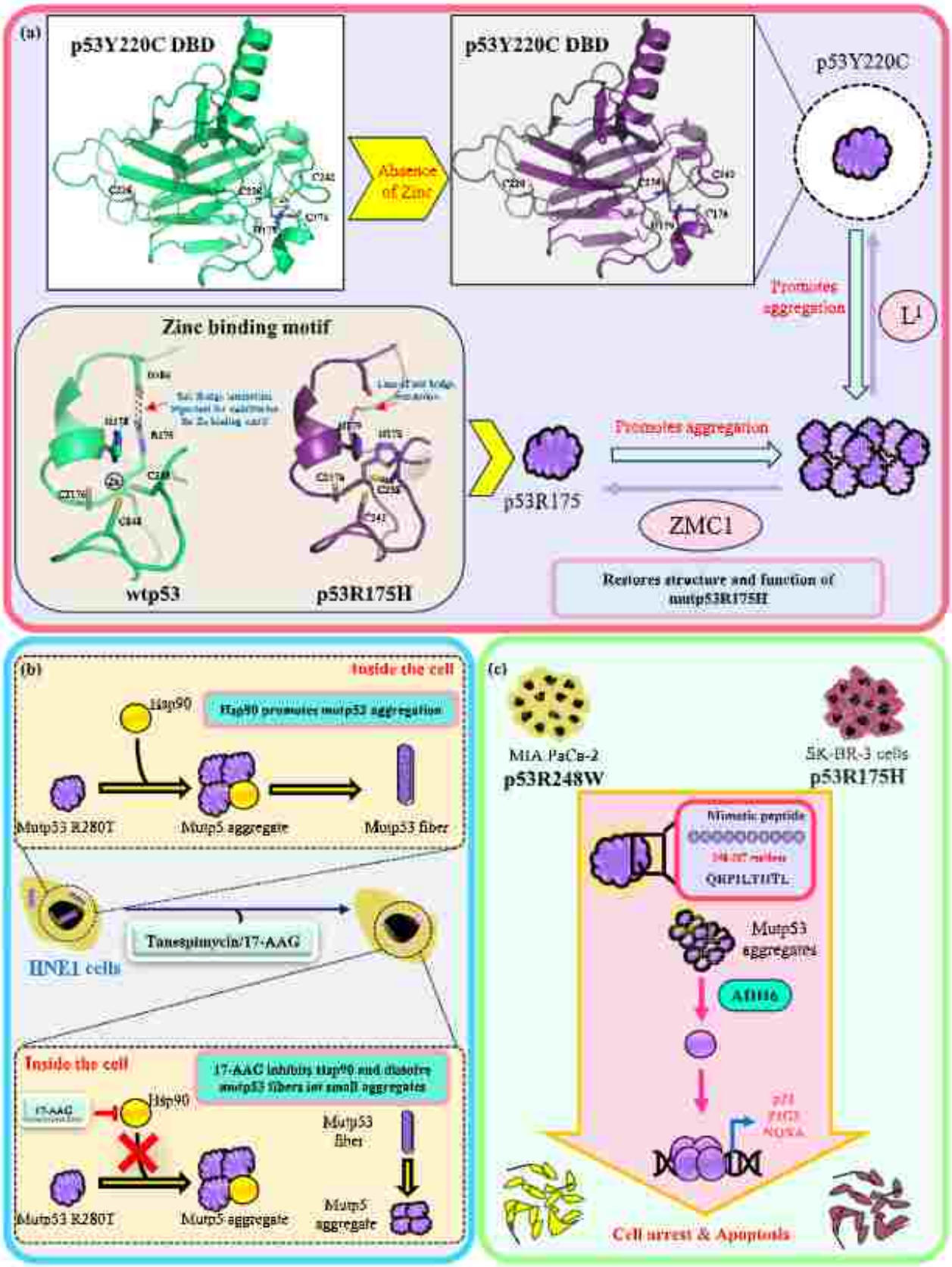

Fig. 7. Graphical illustration showing different mutp53 disaggregation strategies by zinc chaperone ZMC1 & LI (a), Hsp90 inhibitor 17-AAG (b) and ADH-6 (c).

Table 2
Different disaggregation strategies for different aggregating mutp53.

S. No.	Type of inhibitor or mutation or PTM	Inhibitor or mutation or PTM	Type of cell	Cell line	Mutation	Reference
1.	Mutations	I254R, I254D, I255R, and I255D	Bone cancer	Saos-2	R175H and R282V "Transient"	[92]
2.	Acetylation	K373Q, K381Q and K382Q	Non-small cell lung carcinoma cells	H1299	R175H	[103]
3.	Small stress molecule	Polyarginine	Lung cancer	H717	R248Q	[73,74]
			Breast cancer	SK-BR-3	R175H	[73,74]
		Acetylcholine chloride	-	-	R248W	[73,104]
4.	Designer Peptide	ReACp53	Ovarian cancer	OVCAR-3	R248Q	[72]
			Uterine fibroblasts	UtFB	R175H "Transient"	
5.	Designed compound (Quinuclidinone family analog)	PRIMA-1	Ovarian cancer	OVCAR-3	R248Q	[79,105]
			Breast cancer	MDA-MB-231	R280K	
6.	Zinc Metallochaperone	ZMC1, NSC319726	Ovarian cancer	TOV112D	R175H	[106–108]
7.	Bifunctional Zinc Metallochaperone	1[7]	Gastric cancer	HUGC3	Y220C	[109]
8.	HSP90 Inhibitor	Tanespimycin/17-AAG	Nasopharyngeal cancer	HNE1	R280T	[89]
9.	Tripyridylamide ADH	ADH-6	Pancreatic cancer	MIA PaCa-2	R248W	[110]
			Breast cancer	SK-BR-3	R175H	

Wild-type p53 has a short half-life of just 5–20 min, while p53 missense mutants might possess an increased half-life by escaping the ubiquitin-mediated degradation pathway [103]. Post-translational modifications (PTMs) such as acetylation are known to activate p53 transcription activity. These acetylation could occur at Lysine residues (K120, 164, 370, 372, 373, 381, 382, and 386) which (especially the C-terminal domain Lysine acetylation at 370, 372, 373, 381, 382, and 386 lysine residues) in turn trigger inhibition of ubiquitination followed by Mdm-2 degradation and rescues p53 transcription activity from repression [111–114].

In 2023, Xu et al. demonstrated that the mutation(s) at these six lysine residues are very crucial for aggregation and ubiquitin-mediated degradation [103]. They have studied p53R175H along with wt/mutant construct of CTD constitutive acetylation p53 "3KQ" (*i.e.* a triple mutant of K373Q, K381Q and K382Q) and using non-small cell lung carcinoma cells (NSCLCs). Cycloheximide (CHX) chase analysis of the wtp53 showed a half-life of just 1 h, while the WT^{3KQ} showed a half-life of 2 h, highlighting that acetylation enhances protein stability. On the other hand, p53R175H showed the maximum half-life with no degradation observed. When the constitutive acetylation mutation 3KQ was incorporated, the $p53R175H^{3KQ}$ exhibited faster degradation as compared to p53R175 alone, depicting that acetylation results in decreased stability in mutp53 (Fig. 6b).

Thus, acetylation significantly decreases the ubiquitination of wtp53, but in the case of mutp5R175H, acetylation results in instability and might lead to ubiquitin-mediated degradation and aggregation disruption. Further, they observed that acetylation inhibits aggregation and might reactivate missense mutp53 and p53R175H lack transcription activity. By incorporating acetylation by inserting a constitutive acetylation mutation 3KQ, the $p53R175H^{3KQ}$ exhibited some wtp53-mediated transcriptional activity. Also, the $p53R175H^{3KQ}$ showed decreased cell growth, Xenograft growth, and tumor weight. They used ReACp53, a designer peptide for increasing the acetylation of p53R175H-producing cells. This results in the elevation of p53 acetylation and increased solubility of mutp53 [103]. Hence, acetylation can reverse the oncogenic effects of the p53 mutation and could rescue tumor suppressor activity (Fig. 6b).

3. Small stress molecules such as polyarginines & their derivatives, and acetylcholine chloride

Small stress molecules like hydroxy-ectoine, ectoine, arginine, and mannosylglycerate are known as protein stabilizers and possess a proven intervention for treating various neurodegenerative diseases caused by the aggregation of proteins [73,74,104,115–119].

Since mutp53 possesses aggresomal properties similar to proteins responsible for Protein Misfolding Diseases (PMDs), Kanapathipillai et al. investigated the potential of arginine in modulating mutp53R248Q aggregation [73,74]. They examined the effects of polyarginine and its analogs on the stabilization and disaggregation of the mutp53R248Q mimetic peptide 'QRPILTIITL' (residues 248–257), exploring their role in mitigating the aggregation process (Fig. 6c). They found that polyarginine and its derivative polyornithine not only inhibit the aggregation of mutp53 peptide but also inhibit the growth of lung cancer H717 cells (harboring p53R248Q mutation) and breast cancer SK-BR-3 cells (harboring p53R175H). Additionally, the treatment of these small stress molecules imparts lost transcriptional function as the p21 expression was found elevated. Thus, signifying that these molecules not only stabilize and prevent aggregation of mutp53 but also endow the lost transcription activity. In another study, the Kanapathipillai group used acetylcholine chloride (cationic small stress molecule), which was also found to inhibit mutp53R248W mimic peptide "WRPILTIITL" *in-vitro* [73,104]. Thus, highlighting that the small stress molecules are capable of preventing mutp53 aggregation (Fig. 6c).

4. Designer peptide "ReACp53"

In 2016, Soragni et al. constructed an artificial peptide designed to inhibit mutp53 aggregation as a tumoricidal intervention. This peptide "LTRITLE" was engineered to act against the aggregating mutp53 residues 252–258, which were identified using ZipperDB software [72,120]. To enhance the penetration, the peptide was fused with arginines with the p53 residue "RPI", *i.e.* from residues 249–251, resulting in the creation of ReACp53. This peptide not only penetrated inside the HGSOC OVCAR3 cells harboring mutp53R248Q mutation but was also found effective in converting the mutp53 punctate complexes into wild-type-like soluble p53 (Fig. 6d). Thus, causing cell cycle arrest and apoptosis. However, treatment of this peptide also leads to activation of Mdm-2-mediated p53 decay activity.

Additionally, the ReACp53 peptide was found to be effective on cancerous cells harboring transient p53R175H. *In-vivo* administration of ReACp53 leads to suppression of tumor progression and proliferation, as well as shrinkage of Xenografts created using HGSOC (high-grade serous ovarian carcinomas). Thus, this peptide displayed immense potential in inhibiting ovarian cancer cells both *in-vitro* and *in-vivo* by preventing/inhibiting mutp53 aggregation and promoting restoration of lost wild-type-like transcriptional activity and tumor inhibitory function(s). Furthermore, this designer artificial peptide was found effective against other mutp53 aggregates as well [72].

However, this peptide exhibited off-target effects, as it also affects the wtp53, leading to partial unfolding of the wtp53. Hence, leading to systematic toxicity in normal cells. Furthermore, the short *in-vivo* half-

life and poor stability of this peptide posed challenges for artificial peptide therapy [73].

5. PRIMA-a quinuclidinone family analog

Jerson L. Silva's group identified that PRIMA-1 (p53 reactivation with induction of massive apoptosis-1) could inhibit mutp53 aggregation and potentially reactivate its lost tumor suppressor function [79]. PRIMA-1 (2,2-bis(hydroxymethyl)-1-azabicyclo[2,2,2]octan-3-one) has the ability to induce apoptosis in cells harboring mutp53 by converting them into a wild-type-like conformation through its primary nucleophile acceptor metabolite, 2-methylene-3-quinuclidinone (MQ) (Fig. 6d).

In-vitro experiments demonstrated that the aggregated p53DBDR248Q disaggregates following PRIMA-1 treatment. To further validate their finding, the Silva group evaluated the PRIMA-1 mediated mutp53 clearance of aggregation in OVCAR-3 harboring p53R248Q and MD-MB-231 cells containing p53R280K. They used anti-amyloid A11 antibody and ThT staining, confirming their observations with SEC (size exclusion chromatography) of the cell (harboring mutp53) lysate presence and absence of PRIMA-1 treatment.

6. Zinc metallochaperone (ZMC): bifunctional ligands L^1 and semi-carbazone ZMC1

Zinc metal is essential for the proper folding and conformation of p53. This Zn^{2+} atom is responsible for maintaining the architecture of the DBD domain. The absence of Zn^{2+} promotes spontaneous aggregation [68–70]. For instance, the zinc-binding site in p53Y220C is often disrupted, resulting in mutp53Y220C aggregation. Miller et al. designed the novel bifunctional ligand L^1, which interacts with the hydrophobic core *via* Iodine at one end and possesses the metal chaperone activity at the other, assisting in restoring the Zinc binding to mutp53 [109]. The bifunctional ligand L^1 was shown to be highly efficient in restoring the lost function of mutp53Y220C by disaggregation and interacting with the Zn-binding segment, thereby reinstating the zinc-binding ability of the mutp53Y220C (Fig. 7a).

Similarly, the metallochaperone ZMC1 (semi-carbazone such as NSC319726) can restore both the structure as well as the function of mutp53R175H. ZMC1 acts as an ionophore metallochaperone, effectively reestablishing the proper conformation and activity of the mutp53 (Fig. 7a). [106–108].

7. HSP90 inhibitor

Recently, Li et al., established that mutp53R280T interacts abnormally with Hsp90, resulting in increased aggregation and decreased cell cycle arrest and apoptosis in Nasopharyngeal cancer HNE1 cells [80]. Interestingly, they showed that treatment of HSP90 inhibitor Tanerpimycin/17-AAG not only inhibits Hsp90 but also prevents the Hsp90-induced aggregation of mutp53. These findings led to a disintegration of p53 fibrils into smaller aggregates in the cytoplasm (Fig. 7b).

The synergistic treatment of 17-AGG and ReACp53 not only prevents the formation of large amyloid-like fibers, but also disintegrates mutp53 aggregates into a functional tetrameric state which can perform wild-type-like functions. This combined approach could serve as a promising therapeutic strategy in cancers harboring p53R280T mutation (Fig. 7b).

8. Tripyridylamide ADH-6

ADH-6, a tripyridylamide identified from oligopyramide library screening, was proposed to interact *via* cation-π interactions with the cationic side chains of the mutp53R248W mimetic peptide composed of residues 248–273, exhibiting an *in-vitro* binding affinity of 366 nM [110]. Thioflavin-T staining confirmed a significant decrease in aggregation of mutp53R248W in the presence of ADH6. Subsequently, similar findings were observed in MIA PaCa-2 pancreatic cancer cells harboring p53R248W mutation, as evidenced by ThS and PAb240 staining, which showed a reduction in the puncta. Treatment of ADH6 resulted in p53-mediated cytotoxicity, with the IC50 of 2.7 μM and 2.5 μM at 24 and 48 h, respectively, in MIA PaCa-2 cells, and 2.6 μM and 2.5 μM at 24 and 48 h, respectively, in p53R175H bearing SK-BR-3 cells. Additionally, mutp53R248W was found to transcribe PIG3, p21 and NOXA after ADH6 treatment in MIA PaCa-2 cells, indicating that ADH6 induces mutp53-mediated cell cycle arrest and apoptosis (Fig. 7c). Finally, the *in-vivo* administration of ADH-6 in MIA PaCa-2 cells-bearing mice induced regression of tumors harboring mutp53, demonstrating its potential as an effective anti-tumor agent against the mut-p53-bearing tumors.

6. Conclusion and future perspective

It is well understood that the master regulator, aka p53, is frequently mutated in human cancers [9,16]. However, the amyloid-like aggregation of this cellular guardian was not well known until recently. Over the last decade, the exploration of the aggregating behavior of mutp53 has drawn significant attention from many research groups, resulting in substantial evidence regarding the nature of these aggregating p53 mutations. From a pool of 1297 missense unique mutations, 26 have been reported to promote aggregation of p53 in diverse human cancers (Table 1, Fig. S1). Most of these mutations are confined to DBD, and to a lesser extent, the OD of p53.

These mutations confer mutp53 with unusual properties, including amyloid-like aggregation, co-aggregation of mutp53 with wtp53; co-aggregation and bio-isolation with other p53 family members, Hsp(s) and other tumor suppressors; granting the potential of a prion which causes chemoresistance. A wide variety of tools, techniques and strategies, including in-silico methods, *in-vitro* staining methods, anti-p53 antibodies, organic solvents and biophysical tools (such as DLS, OIM, XRD and EM) have been employed enormously to study these mutp53 aggregates *in-vitro* as well as *in-vivo* (Table 3). This review not only describes the unusual properties of different aggregating p53 mutations but also emphasizes the various therapeutic strategies that work by disaggregating mutp53 (Table 2). These therapeutic strategies hold immense potential as anti-cancerous drugs or as synergistic therapy alongside conventional anti-cancerous treatments. However, there is a strong need to evaluate the efficacy and sensitivity of these molecules across all types of aggregating mutp53 to discover a common drug that can effectively target all types of aggregating p53 mutations.

Abbreviations

Mutant p53 Mutp53
DNA Binding Domain DBD
Oligomerization Domain OD
trans-Activation Domain TAD
Proline-Rich Region PRR
Regulatory Domain RD
Prion Protein PrP
Cellular form of PrP PrPc
Scrapie isoform of the prion protein PrPSc
Loss-of-Function LOF
Gain-of-Function GOF
Dominant Negative DN
Wild type wt
sulfated-glycosaminoglycans GAGs
Protein Misfolding Diseases PMDs
Post-Translational modifications PTMs

7. Materials and methods

All figures included in the manuscript are original and have been

Table 3
Different tools for predicting the aggregating nature of different mutp53.

Type of tool	Name	Use	Reference
In-silico methods	Tango	Predicts hot spot (β-aggregation nucleating region in p53DBD.	[121]
	ZipperDB algorithm	Identifies the amyloid adhesive segments aka Steric zipper of p53DBD based on the neural networking.	[120]
	Aggrescan	Predicts the propensities of β-peptides that are prone to aggregation and also helps in drawing comparisons between different protein sequences based on the effects of mutations.	[122]
	FASTA	Predicts the amyloidogenic segments in the p53DBD based on the intermolecular pairing of β strands. It utilizes energy function to predict the interacting portions resulting in stabilizing the corresponding β structure.	[123]
	Waltz	Relies on the experimental data to predict the amylogenic regions in a protein sequence.	[124]
	Zyggregator	Utilizes the physio-chemical properties such as charge, hydrophobicity and its propensity to acquire secondary structures, of a protein to predict the aggregation ability.	[125]
In-vivo and in-vitro staining methods	Congo Red	Organic azo dye which stains β-amyloid proteins.	[22,126]
	Thioflavin T	Benzothiazole dye which stains misfolded β-amyloid proteins or aggregates.	[24]
	Thioflavin S	Binds to amyloid proteins like thioflavin T.	[24]
	ANS	8-Anilinonaphthalene-1-sulfonic acid (ANS) binds to hydrophobic sites of the protein.	[127]
Anti-p53 antibodies	pAb240	Recognizes misfolded mutp53.	[84]
	DO1	Recognizes 20–23 regions of the wtp53.	[128]
	DO7	Recognizes 20–23 regions of the wtp53.	[128]
Organic solvents	1,6 hexanediol	Used to dissolve dense liquid condensates.	[129]
Biophysical tools	Dynamic light scattering (DLS)	Estimate the size of the protein or aggregates.	[76]
	Oblique illumination microscopy (OIM)	Estimate the size of the protein or aggregates.	[76]
	Size exclusion chromatography (SEC)	Estimate the size of the protein or aggregates.	[130]
	Electron microscope (EM)	Visualize and analyze the size of the protein or aggregates.	[76]
	XRD	Visualize and analyze the size of the protein or aggregates.	[13]

created using Microsoft PowerPoint 365. The protein structure models (including cartoon, surface, and ball-and-stick representations) were generated using Pymol 4.6, based on the following PDB IDs: 6SHZ, 3KMD, and 1OLG [131–133]. The site-directed mutagenesis was conducted at the desired residues using Pymol. Additionally, illustrations of tissues such as the breast, lung, and brain were adapted from Smart Servier Medical Art (www.servier.com) and subsequently edited using Microsoft PowerPoint.

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CRediT authorship contribution statement

Nisha Manav: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Pratibha Sharma:** Writing – review & editing, Visualization, Formal analysis, Data curation. **Sankat Mochan:** Writing – review & editing, Formal analysis. **Lakshay Malhotra:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Methodology, Investigation, Formal analysis, Conceptualization, Data curation, Graphics generation.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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