Targeting mutant p53 in cancer: a long road to precision therapy

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The *TP53* tumor suppressor is the most frequently mutated gene in human cancers. In recent years, a blooming of research efforts based on both cell lines and mouse models have highlighted how deeply mutant p53 proteins affect fundamental cellular pathways with cancer-promoting outcomes. Neomorphic mutant p53 activities spread over multiple levels, impinging on chromatin structure, transcriptional regulation and microRNA maturation, shaping the proteome and the cell's metabolic pathways, and also exerting cytoplasmic functions and displaying cell-extrinsic effects. These tumorigenic activities are inextricably linked with the blend of highly corrupted processes that characterize the tumor context. Recent studies indicate that successful strategies to extract core aspects of mutant p53 oncogenic potential and to identify unique tumor dependencies entail the superimposition of large-scale analyses performed in multiple experimental systems, together with a mindful use of animal models. This will hopefully soon lead to the long-awaited inclusion of mutant p53 as an actionable target of clinical antitumor therapies.

Introduction

Extensive characterization of somatic mutations in cancer has drawn a comprehensive repertoire of cancer genes, indicating potential targets for rational therapy. From these studies, *TP53* emerges as the most frequently altered gene in human tumors, and the presence of mutations in this gene are associated with adverse prognosis in various cancer types [1]. In contrast with other tumor suppressors, *TP53* is mostly (70–80%) hit by missense mutations that impact single residues in the protein's core domain, resulting in the inability to bind p53-consensus sites on DNA and to execute normal checkpoint functions. Hereafter, we will refer to missense p53 mutants as 'mutant p53'. Besides loss of function, mutant p53 proteins also exert transdominant repression over their wild-type counterpart, where present, by means of hetero-tetramerization. These effects are clearly tumorigenic, as cells lacking a functional p53 pathway are destitute of first-line responses to protect their genome from virtually all types of cancer-related insults. Beyond this, however, tumor cells acquire a selective advantage by retaining only the mutant form of the protein that is

Abbreviations
AMPK, AMP-activated protein kinase; ATRA, all-trans retinoic acid; FDA, Food and Drug Administration; GOF, gain-of-function; PLK2, Polo-like kinase-2; PML, promyelocytic leukemia; PTMs, post-translational modifications; SREBPs, sterol regulatory element-binding proteins; TCGA, the cancer genome atlas; TNBC, triple negative breast cancer; VDR, vitamin D receptor.

endowed with neomorphic features (gain-of-function, GOF). Thus, the effect of missense *TP53* mutations is most dramatic in that it radically overturns the biological meaning of the p53 tumor suppressor pathway, rendering it oncogenic.

Relying on an expanded network of protein interactions that tailor the cancer cell's transcriptome and proteome, p53 mutant proteins succeed in subverting a remarkable variety of pathways to promote cancer cell survival, proliferation, invasion, migration, stem cell expansion, chemoresistance, tissue remodeling, and chronic inflammation (Fig. 1) (reviewed in [2]). These outcomes induce addiction of cancer cells to the presence of mutant p53 [3]. On this premise, targeting cancer-associated p53 variants appears suitable for designing rational therapeutic approaches that may strike tumor cells selectively, with minimal impact on healthy tissues [4,5]. Inhibiting mutant p53 with different strategies has actually proved to effectively blunt malignant phenotypes *in vitro* and *in vivo* [3,6–9]. This sounds highly relevant for a wide population of cancer patients worldwide, considering the exceptionally high frequency of *TP53* mutation across all cancer types. A number of compounds that elicit mutant p53 destabilization, inactivation, or reactivation of wild-type functions have been described (reviewed in [5]). Among p53 reactivators, the small molecule APR-246 (PRIMA-1MET) that induces a conformational change toward wild-type-like structure [10] has successfully completed a Phase I clinical trial (ClinicalTrials.gov identifier: NCT00900614). Although some compounds are still in early stages of development, to date anticancer therapies based on single agents targeting mutant p53 have rarely reached clinical trials, due to either poor efficiency or low specificity of several compounds in preclinical phases. In facing this evidence, several causes for reflection emerge: does mutant p53 indeed represent a valuable target for anticancer therapy? What aspects of its multifaceted regulation and activities should be considered to design effective therapeutic strategies intercepting upstream and downstream pathways of mutant p53 GOF? Can we identify 'core' and mutant-specific GOF properties among the multiplicity of tumor-associated p53 mutants? A global perspective on the current state of research highlights major unresolved questions and a number of emerging issues. Among these, here we wish to focus on the complex crosstalk between mutant p53 and the tumor context, as well as on the promise of large-scale studies for identifying core GOF activities. As will be discussed throughout the text, these areas of research can be expected to provide actionable targets for developing rational combination therapies that may ultimately come to inclusion of the mutant p53 network in clinical practice.

Mutant p53 oncogenic loops: from chromatin to cell metabolism and back

Studies of the mechanisms underlying mutant p53 oncogenic functions (extensively reviewed elsewhere [2,11]) have disclosed a plethora of aberrant interactions with cellular pathways to reprogram cell behavior into an aggressive mode. Many of these activities occur in the nucleus, where mutant p53 is stably associated with chromatin [12]. Expression of mutant p53 alters chromatin structure, fostering genomic instability. Examples of GOF activities include direct stimulation of topoisomerase 1 (Top1) and consequent hyper-recombination [13], disruption of MRE11–RAD50–NSB complex thus inhibiting ATM and DNA repair [14,15], and increased nuclear recruitment of DNA replication (PCNA, MCM4) and repair (PARP) factors [12]. These properties may be exploited for therapeutic purposes, as suggested by the reported synthetic lethality of PARP inhibition by rucaparib administration in cancers expressing mutant p53 [12].

A vast number of studies highlight multifaceted activities of mutant p53 at the chromatin level, with a profound impact on transcriptional regulation of gene programs that fuel tumor progression and metastatic spread (Fig. 2). Although not endowed with DNA-binding sequence specificity, mutant p53 proteins establish a compound set of interactions with several transcription factors and regulators, further shaped by cancer-related stimuli (Fig. 1). Some of these partnerships mirror physiological interactions of wild-type p53 with tumor suppressors that support its checkpoint functions; however, p53 mutants blunt or even divert these factors to cancer-promoting outputs. Examples include the promyelocytic leukemia (PML) protein and the NFY transcription factor. PML is known to facilitate stabilization and activation of wild-type p53 in response to stress ([16] for review), while NFY was shown to assist wild-type p53 in transcriptional repression of key regulators of the G2/M cell cycle transition [17]. In complex with NFY, mutant p53 instead hijacks PML to the execution of a pro-oncogenic transcriptional program [18].

p53 mutants can also establish neomorphic interactions with transcriptional regulators that are not bound by wild-type p53. For instance, the altered DNA-binding domain of mutant p53 mediates interaction with the p53 homologs p63 and p73 [19–21], resulting in inhibition of p73-dependent apoptosis and

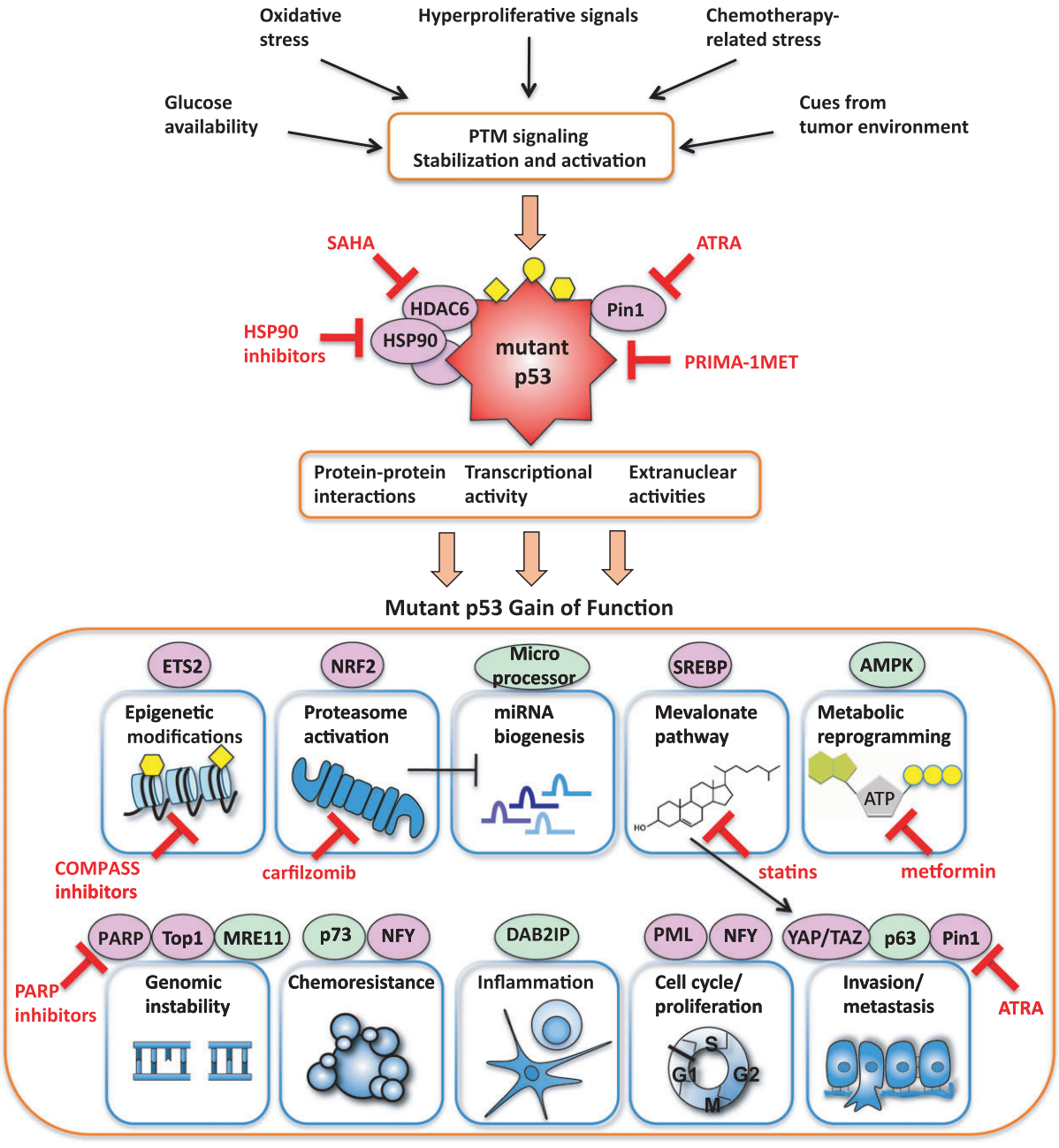

Fig. 1. The mutant p53 oncogenic network. Several cancer-related conditions concur to stabilization and activation of mutant p53 in tumor cells, including hyper-proliferative signals (also promoting chronic DNA damage and oxidative stress), glucose availability, and chemotherapy-related stress. Signal transduction cascades lead to post-translational modifications (PTM) of mutant p53 and enable its gain-of-function activities (GOF). The Hsp90 chaperone complex promotes mutant p53 protein stability in tumor cells, while the phosphorylation-dependent prolyl-isomerase Pin1 enhances its transcriptional activity. Oncogenic functions of p53 missense mutants are highly pleiotropic (square boxes) and rely on partnerships with multiple cellular factors (pink circles indicate active partners of mutant p53, while green circles indicate proteins inhibited by mutant p53). Regulation of cellular processes by p53 missense mutants may directly promote tumorigenesis (e.g., transcription-mediated increase of proliferation, metastatic spread, and chemoresistance) or may indirectly impinge on oncogenic pathways through rewiring energy metabolism, mevalonate pathway, affecting proteasome activity and miRNA biogenesis, and impacting the tumor stroma (see text for details). Integration of multiple GOF activities determines mutant p53-dependent tumorigenicity in a given tumor context. Importantly, mutant p53 GOF mechanisms disclose actionable targets and molecules for precision therapies: some recently published examples are indicated (in red).

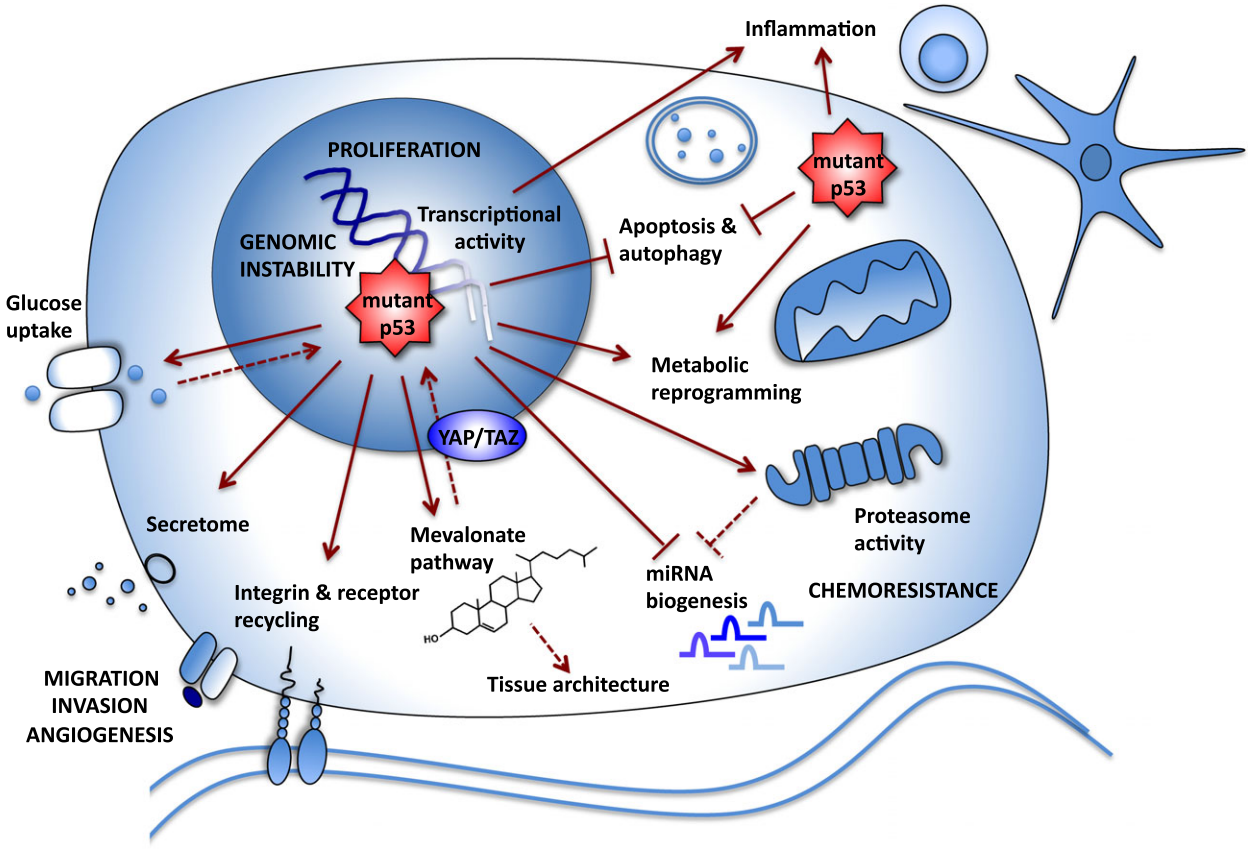

Fig. 2. Mutant p53 GOF activities impact multiple aspects of cellular biology. In the nucleus, p53 missense mutants affect chromatin structure, genomic stability, and activate transcription programs impacting cancer cell metabolism, proteasome activity, and microRNA biogenesis, among others. These activities may in turn sustain mutant p53 stability (e.g., by increasing glucose levels) and crosstalk with other oncogenic pathways (e.g., the mevalonate–YAP/TAZ axis), thus conferring selective advantages for tumor growth and aggressiveness. Extranuclear functions of mutant p53 also contribute to its oncogenic potential: these include metabolic rewiring, suppression of apoptosis and autophagy, as well as cell-extrinsic effects impacting the tumor–stroma crosstalk.

chemosensitivity [22], as well as suppression of TA-p63 antimetastatic target genes [23,24].

Cooperation with other oncogenic pathways frequently underpins transcription-based GOF of mutant p53, and may also ensue from it. An explanatory example in the context of breast cancer is offered by the interaction of mutant p53 with the sterol regulatory element-binding proteins (SREBPs), master regulators of fatty-acid and cholesterol biosynthesis [6]. Synergistic activation of SREBP target genes by mutant p53 then leads to reprogramming of cancer cell metabolism via induction of the mevalonate pathway. A consequence of this effect is the dismantling of normal mammary tissue architecture. Remarkably, another major oncogenic output of boosting sterol biosynthesis in breast cancer cells is the induction of Rho GTPase-dependent nuclear accumulation and activation of two master regulators of organ size control and tissue regeneration, the YAP/TAZ transcription cofactors [25]. Unscheduled induction of YAP/TAZ is widespread in human cancers, promoting tumor cell proliferation, disorganized polarity, and cancer stem cell attributes including chemoresistance [26]. Thus, similar to many oncogenic pathways and cancer-related signaling cues, mutant p53 contributes to unleashing YAP/TAZ activity. In addition, YAP was reported to interact with mutant p53 and NFY onto the regulatory regions of proliferation-related genes (cyclin A, cyclin B, and CDK1), thus synergizing toward hyper-activation of a common gene program that fosters cancer growth [27].

Mutant p53 networks reveal specific tumor weaknesses

The partnerships established by mutant p53 with oncogenes and corrupted tumor suppressors endow transformed cells with aggressive phenotypes that accelerate cancer

progression and determine therapy resistance. Nonetheless, these vicious interplays disclose tumor-specific dependencies that may offer the rationale for precision therapeutic strategies on a poly-pharmacological base. In this respect, YAP/TAZ represent attractive targets for cancer therapy, being dispensable for normal homeostasis of many adult tissues, but essential for cancer emergence in the same context. Among the pharmacological strategies that may be pursued for targeting YAP/TAZ, their control by the mevalonate pathway may represent an exciting possible route. Indeed, the cholesterol-lowering drug statins, which act by inhibiting the key enzyme of this cascade, namely HMG-CoA reductase, were shown to be effective in forcing cytoplasmic relocalization of YAP/TAZ in breast cancer cells bearing mutant p53 [25], and in down-tuning the expression of a mutant p53-dependent gene program [27]. Importantly, statins are FDA-approved drugs widely used for prevention of cardiovascular diseases, and their use has been associated with reduced cancer risk in epidemiological studies. Consistently, statins were shown to blunt YAP/TAZ- [28,29] and mutant p53-dependent growth of tumor cells *in vitro* and *in vivo* [6,25], and their effectiveness was further increased by combination with Src inhibitors [30]. Other FDA-approved mevalonate inhibitors, such as bisphosphonates, also inhibit YAP/TAZ [25]. Although not yet explored, combinational strategies targeting both mutant p53 and YAP/TAZ can be expected to uncouple their crosstalk in tumor cells and to be most effective in curbing growth and metastatic spread of tumors bearing mutant p53.

Metabolic reprogramming is induced by multiple oncogenic pathways during tumor progression to sustain energy production, anabolic growth, proliferation, motility, and cancer stem cell identity, and encompasses both energy-related arms (Warburg effect, glucose metabolism, and glutaminolysis) and lipid metabolism. In addition to stimulating the mevalonate pathway, mutant p53 drives also the Warburg effect in tumor cells. In head and neck cancer cells, this entails inhibition of AMP-activated protein kinase (AMPK) signaling, thus fostering aerobic glycolysis under energy stress [31]. Notably, AMPK inhibition is also expected to enhance YAP/TAZ activation [32]. At the same time, mutant p53 also increases glucose uptake, by promoting translocation of the glucose transporter GLUT1 to the cell surface [33]. Glucose dependency identifies a unique point of vulnerability of cancer cells that is being investigated as a potential setting for therapeutic intervention. Interestingly, mutant p53 protein stability appears to be influenced by dietary supplies. In particular, glucose deprivation has been shown to trigger mutant p53 deacetylation, thus marking it for autophagy-mediated proteolysis [34]. By its ability to stimulate glucose uptake in cancer cells, mutant p53 may thus oppose its own degradation. Remarkably, glucose restriction-induced degradation of mutant p53 in turn causes further activation of the autophagic process, leading to cancer cell death. Accordingly, in mouse models, glucose restriction regimen dampens mutant p53 accumulation and blunts tumor growth [34]. This mutually inhibitory relationship of mutant p53 with the autophagic process might be therapeutically manipulated toward anticancer outcomes. For instance, the antidiabetic drug Metformin leads to reduction of circulating glucose and insulin levels by causing mitochondrial energy stress in the liver. Given its widely reported inhibitory effects on cancer cell growth [35], repurposing of Metformin for both treatment (www.ClinicalTrials.gov) and primary prevention of breast cancer [36] is currently being investigated in large clinical trials.

Reciprocal control with cellular glucose levels may reflect a wider ability of mutant p53 to respond to fluctuations in the metabolic state of cancer cells, mirroring the property of wild-type p53 to sense metabolic stress and control multiple metabolic axes. Although this topic has not been fully explored yet, further examples of intimate connections between mutant p53 and the pathways integrating energy demand and anabolic processes may emerge, revealing tumor dependencies that could be exploited for therapeutic protocols or even chemo-preventive strategies.

Regulation of mutant p53 by the tumor context

Clearly established through the study of mutant p53 knock-in mouse models [37,38], a remarkable property of p53 mutants is the dependency on a transformed cell context for full activation of their malignant potential [39]. A paradigmatic aspect is the selective accumulation of p53 mutant proteins in tumors, as opposed to their inherent instability in normal tissues [39]. Constitutive inhibition of mutant p53 degradation occurs exclusively in transformed cells, and is critical for reaching the high protein amounts required for GOF manifestation.

The molecular mechanisms acting in tumor cells to shelter mutant p53 from ubiquitin-mediated degradation are only partially understood. Different groups have indicated the Hsp90 chaperone machinery as a major player [40]. This system includes Hsp90, Hsp70, and other cochaperones, and is aberrantly activated with high frequency during oncogenic transformation. Hsp90 causes the functional inactivation of the ubiquitin ligases

MDM2 and CHIP bound to mutant p53, thus sustaining its stability [41,42]. Based on this knowledge, strategies aimed either at disrupting the p53–chaperone complex or at chaperone inhibition have been proposed to trigger mutant p53 degradation in cancer cells. Following on proof-of-principle studies *in vitro*, a recent work has demonstrated that long-term pharmacological blockade of Hsp90 with new generation inhibitors (the geldanamycin derivative 17-DMAG and ganetespib) and of its positive regulator HDAC6 with the deacetylase inhibitor SAHA (Vorinostat) significantly increased survival of mutant p53 knock-in mice, and this effect was associated with mutant p53 degradation *in vivo* [3]. HDAC inhibitors are a promising class of anticancer chemotherapeutic drugs, some of which are undergoing clinical trials. However, the search for highly efficient and well-tolerated drugs that specifically target mutant p53 stability with no effect on wild-type p53 is still open. Arguably, a more comprehensive view of the array of cancer-related pathways and conditions leading to stabilization and hyper-activation of mutant p53 in tumor cells may reveal critical hubs to guide the identification of compounds and of their combinations, suitable for implementation of therapeutic strategies. In this respect, radiation and chemotherapy-related genotoxic stress, as well as high levels of reactive oxygen species (ROS)—frequently associated with tumor growth—have been shown to cause mutant p53 protein stabilization *in vivo* [43].

A field of intense research concerns the pathways that transduce the signaling *milieu* generated within the tumor context into cues that unleash mutant p53 oncogenic potential. Notably, many of the signal transduction pathways that induce post-translational modifications (PTMs) on wild-type p53 are altered in cancers. There is evidence that in tumor cells, mutant p53 proteins receive PTMs on the same residues as in the wild-type counterpart, and that some of these events alter the stability, protein interactome, and activity of mutant p53, contributing to oncogenic GOF properties (reviewed in [44,45]). For example, Ras-dependent phosphorylation of mutant p53 on Ser6 and Ser9 promotes the formation of a mutant p53/SMAD complex, which in turn inhibits p63 anti-metastatic activities [23]. N-terminal phosphorylation catalyzed by JNK enhances mutant p53 GOF [46]. In conditions of chemotherapy-related stress, mutant p53 was shown to induce Polo-like kinase-2 (PLK2) gene expression. In turn, PLK2-dependent phosphorylation promotes mutant p53 acetylation and stimulates its interaction with p300 and NFY, thereby enhancing transactivation of a gene set that sustains cell proliferation and chemoresistance [47]. Our group has shown that the phosphorylation-dependent prolyl-isomerase Pin1 is crucial for manifestation of mutant p53 GOF activities in breast cancer cells, and for tumorigenesis in a mutant p53-KI mouse model of Li–Fraumeni Syndrome [24]. Our results suggest that oncogenic signaling cues leading to mutant p53 phosphorylation are transduced into structural changes by Pin1, shaping mutant p53 interactome and transcriptional outcomes. Specifically, Pin1 was shown to cooperate with mutant p53 to the execution of a gene expression program that confers aggressive features to breast cancer cells and is predictive of negative outcome in breast cancers with *TP53* mutations [24]. Some of the genes induced by mutant p53 and Pin1, such as the transcriptional cofactor DEPDC1, appear to be critical for mutant p53-dependent migration and invasion of breast cancer cells. DEPDC1 is normally absent from most adult tissues, and analysis of TCGA cancer database indicates that its expression is highly induced in several cancer types, correlating with tumor grade. Thus, druggable components of the Pin1/mutant p53 axis might provide actionable targets for therapy of tumors with high *TP53* mutation rate, such as triple negative breast cancer (TNBC) and ovarian cancer that still lack targeted therapeutic options. Pin1 appears particularly attractive for the design of small molecule inhibitors: it is highly specific, overexpressed in cancers, and essential for tumor growth and progression, while being largely dispensable for normal tissue homeostasis [48]. Importantly, Pin1 depletion blunts tumor growth and metastatic spread and restores chemosensitivity by impinging on cancer stem cell expansion [49]. Unfortunately, none of the available Pin1 inhibitors has reached clinical trials so far. An exception is the recent discovery that all-trans retinoic acid (ATRA), used for treatment of acute promyelocytic leukemia, directly interacts with the substrate-binding pockets in the Pin1 active site. It has been shown that ATRA exerts inhibition and degradation of Pin1 in tumor cells, blunting Pin1-dependent oncogenic mechanisms and breast cancer growth *in vivo* [50]. However, possibly due to its low potency as Pin1 inhibitor, ATRA showed moderate efficacy against advanced breast cancer in clinical trials [51], leaving open the search for more effective molecules.

Mutant p53-dependent alterations of cellular physiology and tumor microenvironment

While a growing number of reports points toward transcription control as an important mechanism of mutant p53 oncogenicity, there is also substantial evidence that

mutant p53 proteins can alter several cellular processes through multifaceted activities that occur at different levels within the cell (Fig. 2). Although comparatively less explored, extranuclear functions of mutant p53 concur to its oncogenic potential, determining the ability to sense and modify the tumor context. For instance, alteration of cancer cell metabolism by mutant p53 entails direct inhibition of AMPK in the cytoplasm [31]. Cytoplasmic localization of mutant p53 was identified as an important feature for its ability to suppress autophagy [52], thus supporting tumor cell survival. In addition, mutant p53 has also been reported to inhibit apoptosis through cytoplasmic activities [53,54]. Mutant p53 may also impact cancer traits by acting on mitochondria, and some p53 mutants have in fact been shown to localize in these organelles [55,56]. In Li–Fraumeni patients and mouse models bearing germline *TP53* mutation, increased mitochondrial respiration was reported, and this was associated with mutant p53-dependent increase in mitochondrial regulators and metabolic enzymes [57]. It is tempting to speculate that long-term modification of metabolism caused by mutant p53 expression in normal tissues might support subsequent transformation. It is unclear, however, if mutant p53 has the same impact on mitochondrial physiology in tumor cells. In fact, a functional protein cluster related to mitochondrial biogenesis appeared to be downregulated by p53 missense mutants in a transcription-independent fashion [9]. In light of the expanding role of mitochondrial biology in tumorigenesis [58], investigating the mechanisms of mitochondrial GOF of mutant p53 and their significance for tumor progression may provide the rationale supporting anticancer metabolic interventions, e.g., repurposing the above-cited antidiabetic drug metformin that also inhibits mitochondrial respiration [35].

The ability of cancer cells to establish proficient interactions with tumor stroma and to actively shape a permissive microenvironment is crucial for cancer progression. Mutant p53 expression impacts the tumor–stroma crosstalk at several levels (Fig. 2). Treatment with vitamin D3 has been reported to exert proapoptotic and anticancer effects in several experimental models [59]; however, vitamin D3/vitamin D receptor (VDR) signaling may show antiapoptotic effects as well [60]. These apparently opposing outcomes might be at least partly explained by the finding that mutant p53 can interact with VDR, enhance its nuclear import and alter the transcriptional outcome of vitamin D3 signaling toward a prosurvival program. Accordingly, vitamin D3 was found to protect against chemotherapy-induced cytotoxicity in tumor cells harboring mutant p53 [61]. Mutant p53 can also affect cell surface receptor signaling to promote invasive and metastatic properties of cancer cells. Examples include enhanced recycling of integrins and growth factor receptors such as EGFR [62] and MET [63]. Mutant p53 proteins were shown to increase cancer cell angiogenic potential through induction of ID4, a member of ID family proteins involved in promoting neovascularization through post-transcriptional stabilization of the proangiogenic cytokines IL8 and GRO-α [64]. Recent reports have highlighted additional cell-extrinsic effects of mutant p53 proteins in sustaining invasive cancer phenotypes. Using p63 as a chaperone to bind its target promoters, mutant p53 directs the expression of a proinvasive cluster of secreted factors [65]. In addition, mutant p53 has been shown to upregulate the secretion of CXC chemokines through the NF-κB pathway, thus contributing to enhance cell migration [66].

A recently emerged property of mutant p53 concerns the ability to stoke tumor-promoting inflammation, by enhancing and prolonging NF-κB activation in response to TNF-α stimulation [67,68]. In the context of colitis-associated colorectal cancer, where *TP53* mutations represent initiating events, mutant p53 was shown to intensify chronic colitis in mice, increasing the risk for developing invasive colon carcinoma [67]. In the nucleus, mutant p53 binds the promoters of inflammatory genes [67,69] and amplifies TNF-induced expression of NF-κB targets [67]. In the cytoplasm, mutant p53 acts directly downstream of the TNF receptor to intercept the cytoplasmic signal transducer DAB2IP. This favors TNF-α-dependent activation of NF-κB and also reduces the activation of the ASK1/JNK kinases, inducing a gene expression program that stimulates tumor cell survival and invasion [68]. Somewhat paradoxically, the resulting gene signature also includes powerful immune-stimulatory chemokines, whose expression predicts better prognosis in breast cancer patients. This knowledge may be potentially exploited for directing therapeutic options.

The discovery of cell-extrinsic aspects of mutant p53 GOF brings into discussion the limitations inherent to the widespread use of xenograft assays in immunocompromised mice for the investigation of mutant p53 oncogenic properties in cancer aggressiveness. Experimental models with intact host immune system that may represent an active component for tumor development and therapy response are expected to provide a more informative and realistic picture.

Large-scale multi-omic approaches: a twist in mutant p53 research

The definition of mutant p53 oncogenic networks has been recently propelled by global approaches based on

the use of large-scale 'omic' techniques. Extensive comparison of DNA interactome (ChIP-seq), transcriptome (RNA-seq, microarrays, etc.), and proteome analyses of different p53 mutants from various cancer models is expected to pinpoint essential pathways of mutant p53-induced oncogenicity. Recent multi-mutant p53 studies have also attempted to overcome the limitations caused by focusing on single hot-spot p53 mutations, to uncover general, genome- and proteome-wide influences of missense p53 mutant variants in cancer cells. Efforts to define oncogenic properties shared by multiple p53 variants are instrumental to design *bona fide* universal antimutant p53 therapies.

One of these studies has pointed out that multiple p53 mutants share the ability to drive global epigenetic alterations in breast cancer [8]. By comparing wild-type p53 DNA interaction patterns to those observed for three distinct p53 mutants in their respective breast cancer cell lines, this study highlighted the synergistic induction, by mutant p53 and the transcription factor ETS2, of a gene set comprising the histone methyl transferases, MLL1 and MLL2, and the acetyltransferase, MOZ. Thus, a conserved GOF activity of mutant p53 is based on co-opting chromatin modifiers to guide epigenetic modifications in support of tumor cell proliferation. Interference with MLL1/2 expression was shown to reduce mutant p53-dependent tumor growth in xenograft assays, and treatment with inhibitors of the COMPASS scaffold complex proved effective in reducing cell growth associated with mutant p53 *in vitro*. Although further *in vivo* proof is required, the promise of small molecule compounds to target chromatin regulators may be projected for wide-range application in treating cancers dependent on mutant p53.

A vast body of evidence points toward transcription control as a major route of mutant p53 GOF. It is, however, arguable that the approaches described above may reveal insufficient to return a comprehensive picture of mutant p53 signaling. Indeed, recent large-scale proteo-genomic analyses of tumor tissues have disclosed that the transcriptional effects of genetic alterations fail to reliably predict the ultimate outcome at the protein level [70,71]. Activation of oncogenic circuits may impact the cell's proteome through transcription-independent effects, namely selective regulation of protein production and disposal by non-coding RNAs and protein degradation pathways. There is increasing awareness that complex processes such as tumor relapse, metastasis, and chemoresistance cannot be truly understood without superimposing to the genomic and transcriptomic characterization of tumors, a parallel picture of their proteomic landscape. On this reflection, a step forward comes from studies integrating mutant p53-related molecular profiling of cancer cells at the DNA, RNA, and protein level, by use of large-scale multi-omic approaches.

A recent work by Polotskaia and colleagues combined subcellular fractionation and SILAC approach in breast cancer cells, thereby unveiling the ability of mutant p53 to drive proteome modulation without corresponding changes in transcription [12]. This study highlighted that endogenous mutant p53 governs chromatin association and activity of poly(ADP ribose) polymerase 1 (PARP1) and of the nuclear replication proteins MCM4 and PCNA. These findings disclose a probable impact of mutant p53 on DNA replication and repair, and support the use of PARP inhibitors in synthetic lethal approaches for targeting breast cancers expressing mutant p53.

A multi-omic approach proposed by our group combined DNA interactome, transcriptome, and proteome analysis of different mutant p53 variants to highlight 'core' and mutant-specific GOF properties [9] (Fig. 3). This study indicated that a broad influence of mutant p53 on cancer cells' protein content extends beyond the control of chromatin and transcriptional processes, to the level of proteasome-mediated proteome alteration. Indeed, upregulation of proteasome expression and activity was identified as a common program of multiple missense mutant p53 variants in cell lines derived from several solid tumor types and in a mutant p53 knock-in mouse lymphoma model. This activity represents a bona fide 'core' GOF process, as supported by its strong association with poor prognosis and mutant status of *TP53* in patients of different cancer types [9].

Mechanistically, in breast cancer cells, mutant p53 cooperates with the oxidative stress response transcription factor Nrf2, often constitutively activated in tumors, to boost transcriptional activation of proteasome genes. Interestingly, in the same cells, mutant p53 can downregulate another set of Nrf2-dependent genes, such as HMOX-1 or NQO1, belonging to acute, canonical oxidative stress response [9,72]. It is tempting to speculate that mutant p53 acts as a molecular switch of Nrf2 transcriptional program, and this effect could help to clarify the dual role of Nrf2 as a context-dependent oncogene or oncosuppressor [73].

Alteration of protein homeostasis by mutant p53-dependent proteome activation downregulates multiple tumor suppressors, including regulators of cell cycle (p21, p27), apoptosis (NOXA), mitochondrial homeostasis (TSFM, SUCLA2), and RNA processing (KSRP). The proteasome-mediated degradation of KSRP—an mRNA and miRNA maturation factor [74]—enables mutant p53 to counteract the maturation of tumor-suppressive miRNAs, including let-7a and miR-

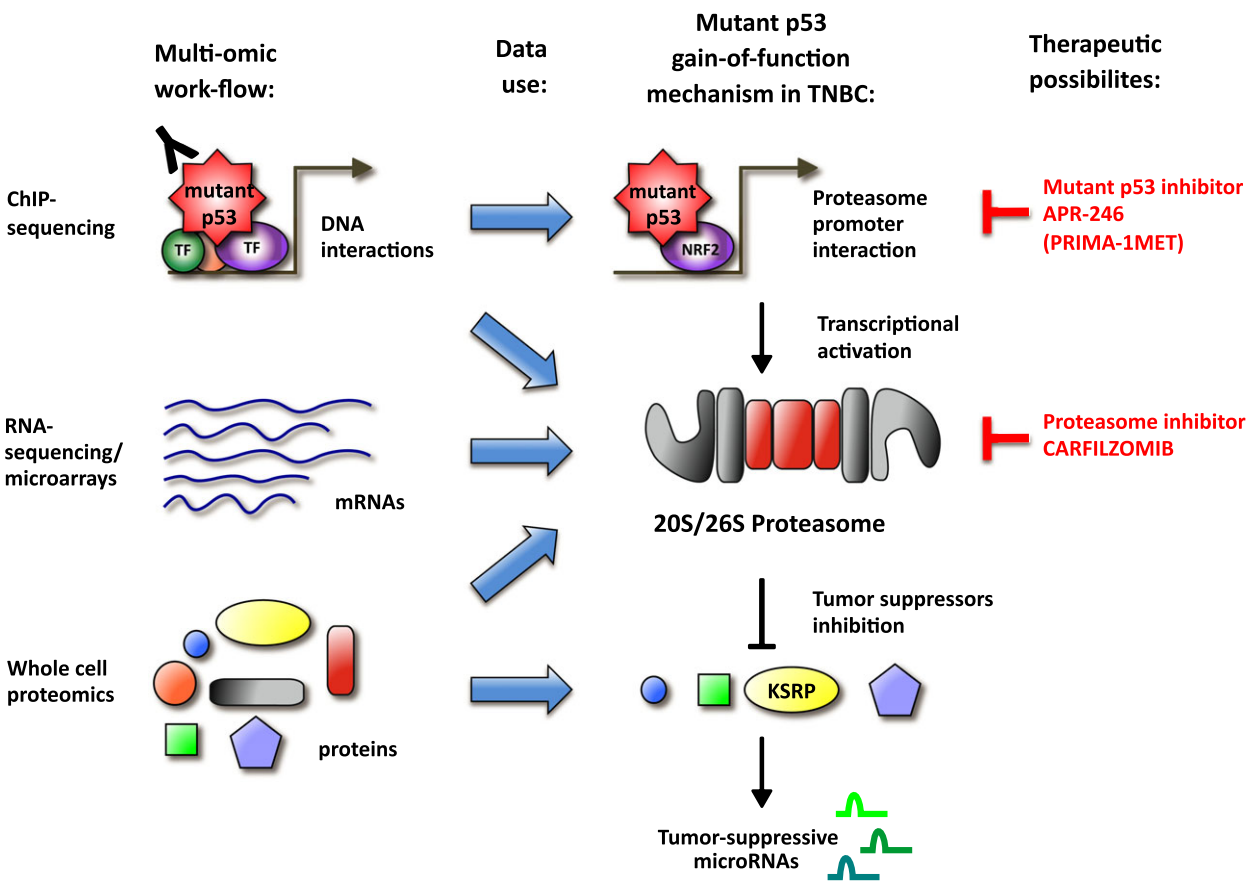

Fig. 3. Multi-omics analysis reveals global effects of mutant p53 on proteome and miRNA biogenesis. Schematic work-flow of the approach for identification of 'core' mutant p53 GOF mechanisms [9]. The multi-omic data (ChIP-seq, transcriptomics, and proteomics) on multiple p53 missense mutants in TNBC cell lines was integrated, highlighting that p53 mutants activate transcriptionally several components of the 20S/26S proteasome. The ChIP-seq data also indicated that this relies on cooperation of mutant p53 with the transcription factor Nrf2. The proteomic data were used to discover downstream targets destabilized by the mutant p53–proteasome axis (including the KSRP–microRNA pathway). Determination of this 'core' mutant p53 GOF axis additionally lead to discovering of the mutant p53/Nrf2-dependent resistance to proteasome inhibitors (such as carfilzomib) in TNBC cells, which could be avoided by a concomitant targeting of the proteasome and mutant p53 by APR-246 (PRIMA-1MET).

30c. This implies that oncogenic activities of the cellular proteasome machinery include unprecedented effects on general miRNA biogenesis. miRNA downregulation is commonly observed in cancers [75,76], moreover inhibition of miRNA biogenesis has tumorigenic consequences [77], suggesting that miRNAs are primarily tumor suppressors. Consistently, several oncogenes and tumor suppressors are emerging as regulators of miRNA biogenesis. Some reports have linked the expression of p53 missense mutants with specific miRNA signatures that target oncogenic signaling pathways, predicting poor outcome in solid tumors [78,79]. These signatures share, at least in part, common miRNA members, as highlighted by analysis of TCGA datasets [80], and appear to be unrelated to wild-type p53, pointing toward mutant p53 as an active modulator of oncogenic shifts in cellular miRNA populations. Several miRNAs were described as transcriptional targets of p53 missense mutants, able to mediate various GOF aspects including migration/invasion [81,82] and chemoresistance [83]. Moreover, recent evidence highlights a role of mutant p53 also in post-transcriptional control of miRNA maturation. Besides regulating KSRP [9] (a Dicer complex component), mutant p53 has been shown to downregulate Dicer expression at the protein level [84]. Moreover, it has been recently shown that different p53 missense mutants sequester the p72/p82 subunits of the Drosha/Microprocessor complex, negatively affecting a population of tumor-suppressive miRNAs [80].

The discovery of the proteasomal core oncogenic program of mutant p53 holds therapeutic implications.

Knockdown or inhibition of mutant p53 in TNBC cell lines abolished the 'bounce-back response' to proteasome inhibition, namely the mechanism of resistance to proteasome inhibitors mediated by Nrf2-dependent increase of proteasome subunits' expression. Consistently, direct targeting of mutant p53 with the small molecule APR-246 in combination with the proteasome inhibitor carfilzomib (Fig. 3) proved effective in reducing mutant p53-dependent primary tumor growth and eradicating metastasis in mouse TNBC xenograft assays [9]. This represents an important proof of principle for effective simultaneous targeting of p53 missense mutants together with their core downstream pathways. It can be expected that strategies combining mutant p53 inhibitors [10,85,86] with blockade of proteasome activity may prove beneficial for treatment of many solid tumors.

Concluding remarks

The study of mutant p53 biology is reaching a turning point. Upon having accumulated seminal, although scattered, evidence of the profound impact of mutant p53 on fundamental cellular processes, a more inclusive picture is now emerging. The raise of multi-omic approaches that pool together multiple mutant p53 variants in their endogenous cancer cell context provide us with a clear understanding of the core oncogenic functions of p53 missense mutants. This aspect will prove crucial to finally include mutant p53 among feasible treatment targets in clinical oncology. An open question remains how mutant p53 GOF activities are enabled by the unique blend of cancer-related cues that prevail in specific tumor types. The many modalities through which oncogenic missense p53 mutants engage in crosstalk with cancer networks suggest that targeting individual pathways may not be highly effective in a therapeutic perspective. It is advisable that, in tumors where p53 missense mutants behave as active oncogenes, mutant p53 networks become targeted at the multi-pathway level. We anticipate that large-scale analysis of the processes regulated by several mutant p53 variants in their tumor context *in vivo* may indicate directions for combinatorial approaches that will improve safety and dampen resistance mechanisms, thus ameliorating therapeutic efficacy in treatment of tumors and metastasis harboring missense mutant p53.

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Author contributions

FM, DW, and GDS collected and discussed the material. FM and DW prepared the figures. FM, DW, and GDS wrote the manuscript.

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