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Perspective

Disarming mutant p53 oncogenic function☆

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ABSTRACT

In the last decade intensive research has confirmed the long standing hypothesis that some p53 point mutants acquire novel activities able to cooperate with oncogenic mechanisms. Particular attention has attracted the ability of several such mutants to actively promote the development of aggressive and metastatic tumors *in vivo*. This knowledge opens a new dimension on rational therapy design, suggesting novel strategies based on pharmacological manipulation of those neomorphic activities. P53 point mutants have several characteristics that make them attractive targets for anti-cancer therapies. Remarkably, mutant p53 has been found predominantly in tumor cells and may act pleiotropically by interfering with a variety of cellular processes. Therefore, drugs targeting mutant p53 may selectively affect tumor cells, inactivating simultaneously several mechanisms of tumor promotion. Moreover, the high frequency of missense mutations on the p53 gene suggests that interfering with mutant p53 function may provide a valuable approach for the development of efficient therapies able to target a wide range of tumor types.

1. Introduction

Remarkable advances in understanding tumor cell biology have opened up a new dimension for cancer therapies. In the last decades, new rational approaches aiming to disarm the specific mechanisms responsible for the pathology have begun to emerge. Those approaches are intended to overcome the initial strategies designed to attack actively proliferating cells, which showed limited success and scarce specificity. Although promising, the search for novel cancer therapies is not devoid of obstacles, the major of which is posed by the overwhelming molecular heterogeneity found in human cancer. In fact, even if it seems clear that tumorigenesis involves the acquisition of several common traits, including uncontrolled proliferation, resistance to cell death and eventually invasive capabilities, the alterations that may lead to a neoplastic phenotype are myriad [1].

Nevertheless, some molecular alterations, as mutations in the TP53 gene, are frequently found in human tumors, even exceeding 50% of cases depending on tumor type (COSMIC database http://www.sanger.ac.uk/cosmic), suggesting that pharmacological manipulation of the p53 pathway may provide valuable therapeutic tools for a wide range of cancers. Moreover, mounting evidences have implicated p53 point mutants as promoters of aggressive and metastatic tumor phenotypes. Taking into consideration that metastatic spread is the cause of death in more than 90% of solid tumors, the ability of p53 point mutants to promote metastasis has attracted enormous interest as a pharmacological target.

2. Alterations on the p53 pathway: new paradigm, novel targets

Placed at the center of a highly interconnected pathway, p53 regulates cell fate in response to a wide array of external and internal signals [2]. The p53 pathway has a prominent role in tumor suppression by preventing tumor development from cells undergoing oncogenic stress. This function is operated by different mechanisms that ensure DNA integrity and regulate proliferation, metabolism and Reactive Oxygen Species (ROS) production [3], but may also induce irreversible responses like apoptosis or senescence [4]. Protein activation is finely controlled by complex combinations of posttranslational modifications and regulators that lead to the activation of different transcriptional programs and/or direct interaction with pro-apoptotic partners [5]. Remarkably, mutations in *TP53* allow tumor cells to subvert the biological meaning of the p53 pathway turning it into a tumor promoting network. These mutations usually eliminate the tumor suppressor function, however, the way in which this occurs is different from inactivation of

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other tumor suppressors where protein expression is avoided. In contrast, in the case of p53, more than 70% of the mutations are missense, leading to the expression of full-length point mutants which accumulate in human tumors [6,7]. The presence of point mutations radically alters p53 function causing much more than simple loss of wt function. On one hand, mutant proteins may exert a dominant negative effect by binding and inhibiting wt p53, while on the other, point mutants acquire novel functions that actively promote the development of an aggressive and metastatic phenotype, collectively known as Gain of Function (GOF) activities [4,8–10]. Furthermore, elegant *in vivo* models for *bonafide* GOF, lacking wt p53, demonstrated that neomorphic mutant p53 functions are able to promote metastasis [11–15]. Compelling evidences from animal and *in vitro* models further confirmed that the expression of p53 point mutants is a crucial event in tumorigenesis that tips the balance toward overt malignant progression [16]. Data from clinical studies support this notion, showing that the presence of p53 missense mutations correlates with poor clinical outcome in several human cancers [8]. Thus, in the last decade, while from the wild type side of the pathway we changed our view reinforcing the relevance of its tumor suppressor role, the deleterious consequences that p53 point mutants may have on tumor progression have been more and more unveiled. This knowledge is of great interest for the field of rational therapy design since mutant p53 has several characteristics that make it an attractive target for anti-cancer therapies.

First, it is expected to accumulate almost exclusively in transformed cells or at least under pathological conditions, which would allow to selectively affect tumor cells and spare normal cells expressing wt p53, providing an exquisite specificity. Second, it promotes proliferation and resistance to cell death, consequently blocking its activity may inhibit the development of tumor masses and may even cooperate to achieve complete tumor clearance. Third, it activates a biological program leading to tumor aggressiveness, implying that blocking its function would restrain the development of metastasis. Fourth, the similarities with wt p53, give the opportunity to recover tumor suppression function.

Here we will discuss some aspects of the mechanisms underlying mutant p53 function that may reveal potential strategies to develop novel anti-cancer therapies. As the initial evidences showing elevated mutant p53 levels in tumors suggested, protein stabilization stands out as a central aspect its oncogenic function, therefore, inducing mutant p53 degradation stands out as a potential strategy. As we gain insight into the molecular bases of mutant p53 function it becomes clear that its regulation involves complex mechanisms of posttranslational modifications as well as the interaction with other partners (Fig. 1). Therefore, another strategy to target mutant p53 oncogenic function may be to avoid proper activation. Resembling the wt counterpart, mutant p53 function is most likely regulated by a barcode of post-translational modifications [17]. Moreover, missense mutations are more frequent in the DNA Binding Domain (DBD), consequently most tumor associated p53 point mutants have N- and C-termini that are virtually identical to their wt counterpart. Considering that those regions harbor domains that receive key signals for protein regulation it may be envisioned that several signaling pathways that impinge on wt p53 may also regulate mutant p53 function.

In addition, blocking mutant p53 downstream activities may be a valid strategy. Our current understanding of mutant p53 GOF describes mutant p53 as a protein that affects different aspects of cell behavior by physically interacting with protein partners, thereby altering their normal function [4,8,9]. Through these alterations, mutant p53 may enhance proliferation, genomic instability, resistance to cell death and invasive capabilities. Even if not completely understood, recent evidences have provided valuable insights on the molecular mechanisms involved. Some aspects of mutant p53 function were explained by direct interference of protein function, independently of changes in gene expression, as is the case for MRE11 [18] or BTG2 [19]. However, an unexpected role for mutant p53 as a regulator of gene expression was described. Even though most cancer-related p53 mutants lose the ability to bind p53 Response Elements (p53RE) on gene promoters, experimental evidences have shown they are still able to significantly alter transcription, albeit of different gene repertoires comparing with its wt counterpart [10]. Several mechanisms were proposed to explain this activity. Mutant p53 was shown to bind to transcription factors and modify their activity. In some cases like p63 and p73 this interaction is inhibitory but in others, mutant p53 enhances the transcription of pro-oncogenic genes [4]. Other evidences have shown that mutant p53 interacts with non coding sequences on DNA and suggested that it may act as an epigenetic regulator, and may bind directly to DNA in a non-sequence-specific manner [20,21].

3. Potential mutant p53-based strategies

3.1. Inducing mutant p53 degradation

Mutant p53 is highly expressed in human tumors and cancer-derived cell lines [7], in fair contrast to wt p53 which is hardly detectable in unstressed cells and whose expression becomes transiently elevated only in response to distinct signals [5]. These high protein levels are thought to be responsible for large part of mutant p53 oncogenic function, since depletion of mutant p53 reduces the aggressive features associated to GOF [4,10], and precocious stabilization of mutant p53 in knock-in mice is associated with enhanced aggressiveness [14,22]. Therefore, therapies able to induce its degradation would be extremely valuable (Fig. 2). As a proof of principle, experimental evidences have shown that mutant p53 levels may be reduced in culture under defined conditions, including pharmacological treatments [23–25].

Mutant protein stability is dramatically increased in non-stimulated cell lines, approaching a half life of several hours, comparing with approximately 30 min for wt p53. Initially, it was suggested that point mutations may alter protein structure in a way that conferred enhanced resistance to degradation or that, having lost the ability to induce transcription of MDM2, p53 mutants may be more stable as a consequence of reduced expression of its main E3 ubiquitin ligase. Even if both mechanisms may contribute, evidences from knock-in mice showed that mutant p53 protein levels are barely detectable in normal tissues but are elevated exclusively in tumors [11,12,14], implying that the inability to induce MDM2 transcription or the mutation *per se* were not responsible for its stabilization.

Instead, a growing body of evidences has proposed that most p53 mutants are inherently unstable proteins in non-transformed cells, which however become extremely long-lived in tumor cells as a consequence of other accumulating alterations during malignant transformation [14,26,27]. Moreover, mutant p53 levels in normal tissues of knock-in mice are increased upon stimuli that stabilize wt p53, such as ionizing radiation and genotoxic insult [14,22], suggesting that both wt and mutant p53 protein stability may be regulated by similar mechanisms. Our knowledge on the mechanisms that regulate mutant p53 degradation is still fragmentary. Nevertheless, intense research on this field has provided valuable clues by showing that mutant p53 may be targeted for ubiquitin-dependent degradation by MDM2 and CHIP E3 ubiquitin ligases [26,27].

As for wt p53, MDM2 seems to be a major determinant of mutant p53 levels. Indeed, compelling evidences from knock-in mice where expression of *p53R172H* was combined with *Mdm2*

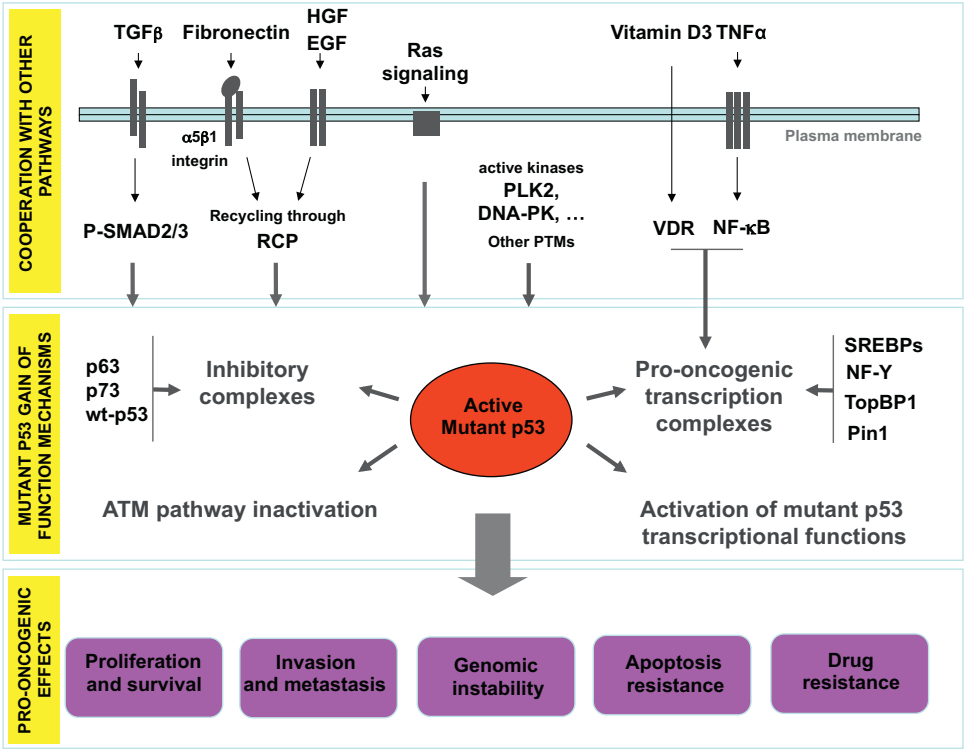

Fig. 1. Summary of the proposed mechanisms for mutant p53 oncogenic function. The upper panel shows the pathways that cooperate with mutant p53 pro-oncogenic mechanisms and signals or interactors that are required for its activation. The middle panel shows a schematic representation of the proposed molecular mechanisms of mutant p53 gain of function (GOF). In the bottom panel the biological effects of mutant p53 function are indicated (see text for details). Membrane receptors for the indicated signaling molecules are depicted in dark gray; a double line represents the plasma membrane. PTMs: posttranslational modifications; P-SMAD2/3: phosphorylated SMAD2 o SMAD3.

deletion showed early stabilization of mutant p53 in normal tissues, confirming that this E3 ubiquitin ligase is a physiologic regulator of mutant p53 levels [14]. Consequently, early mutant p53 stabilization in double mutant mice was associated with a strikingly elevated metastasis frequency and reduced survival. Moreover, even in conditions were p53 point mutants are hyperstable, they may still be susceptible to degradation provided that the whole mechanism is reactivated, as suggested by experiments in cell lines, were supraphysiologic levels of ectopic MDM2 readily induced ubiquitin-dependent mutant p53 degradation [26,28,29]. Therefore activating MDM2-induced degradation may be a valid strategy to eliminate mutant p53. However, considering that other p53-independent tumor promoting functions of MDM2 were also reported [30–32], it would be advisable to target exclusively the mutant p53/MDM2 interaction. Besides, these findings also implicate that the use of therapies based on MDM2 inhibitors, would be conditioned by p53 status. This is the case of Nutlin-3a and related imidazoline compounds, which are small molecules that block the interaction between MDM2 and p53 by binding to the p53-binding pocket in the N-terminal domain of MDM2 [33]. Nultlin-3a efficiently prevents wt p53 degradation and promotes tumor suppressor activities both *in vitro* and *in vivo*. At present, Nutlins are being tested in clinical trials for treatment of tumors with wt p53 status, including retinoblastoma, blood malignancies and liposarcoma [34–36]. In contrast, treatment of tumors harboring mutant p53 with Nutlin-3a could be expected to be detrimental, since it would help to stabilize the mutant protein. Evidences from cell lines showed that this effect may depend on the cellular context. Mutant p53 expressing cell lines such as PANC1, T47D or MDA-MB-231 treated with Nutlin-3a showed increased mutant p53 levels [25,26], while treatment of Malignant Peripheral Nerve Sheath Tumor (MPNST) cells showed no effect [37]. Noteworthy, treatment of mutant p53 or p53 null cells with Nutlin-3a in combination with genotoxic agents, may activate apoptosis by releasing E2F1 from MDM2-induced degradation [37], suggesting a potential use of Nutlins for treatment of mutant p53 bearing tumors. Therefore, depending on the balance between the pro-oncogenic effects of mutant p53 stabilization and the p53-independent apoptotic pathways activated by Nutlins, different biological outcomes may be expected.

In line with the idea that similar mechanisms may induce wt and mutant p53 stabilization it has been shown that doxorubicin and γ-radiation treatment results in increased mutant p53 levels in normal tissues of homozygous *p53R172H* knock-in mice [14,22]. For mutant p53 mice treated with γ-radiation a significant decrease in survival was also observed comparing with treated $p53^{-/-}$ mice or untreated mutant p53 mice [22]. These observations claim that depending on the presence of mutations on *TP53* different clinical outcomes may be expected in patients treated with therapies based on genotoxic insult, with deleterious consequences in cases expressing p53 point mutants. Notably, treatment with the ROS scavenger N-acetyl-L-cysteine (NAC) was able to significantly reduce mutant p53 stabilization upon γ-radiation treatment in *p53R172H* knock-in mice [22], implying that oxidative stress generated by radiation may act a stimulus for mutant p53 stabilization. Therefore, the pharmacological use of drugs able to reduce oxidative stress may also be considered in therapies aimed at inducing mutant p53 degradation.

Although p53 mutants may retain some characteristics of the wt counterpart it is clear that profound differences in the mechanisms that regulate protein stability should exist in order to achieve such opposite outcomes. A clue to understand the extraordinary stabilization of mutant p53 in tumor cells came from the observation that mutant p53 engages in stable complexes with the HSP90 machinery, which includes HSP90, HSP70 and co-chaperones [24,26,38–40]. Surprisingly, MDM2 and CHIP were

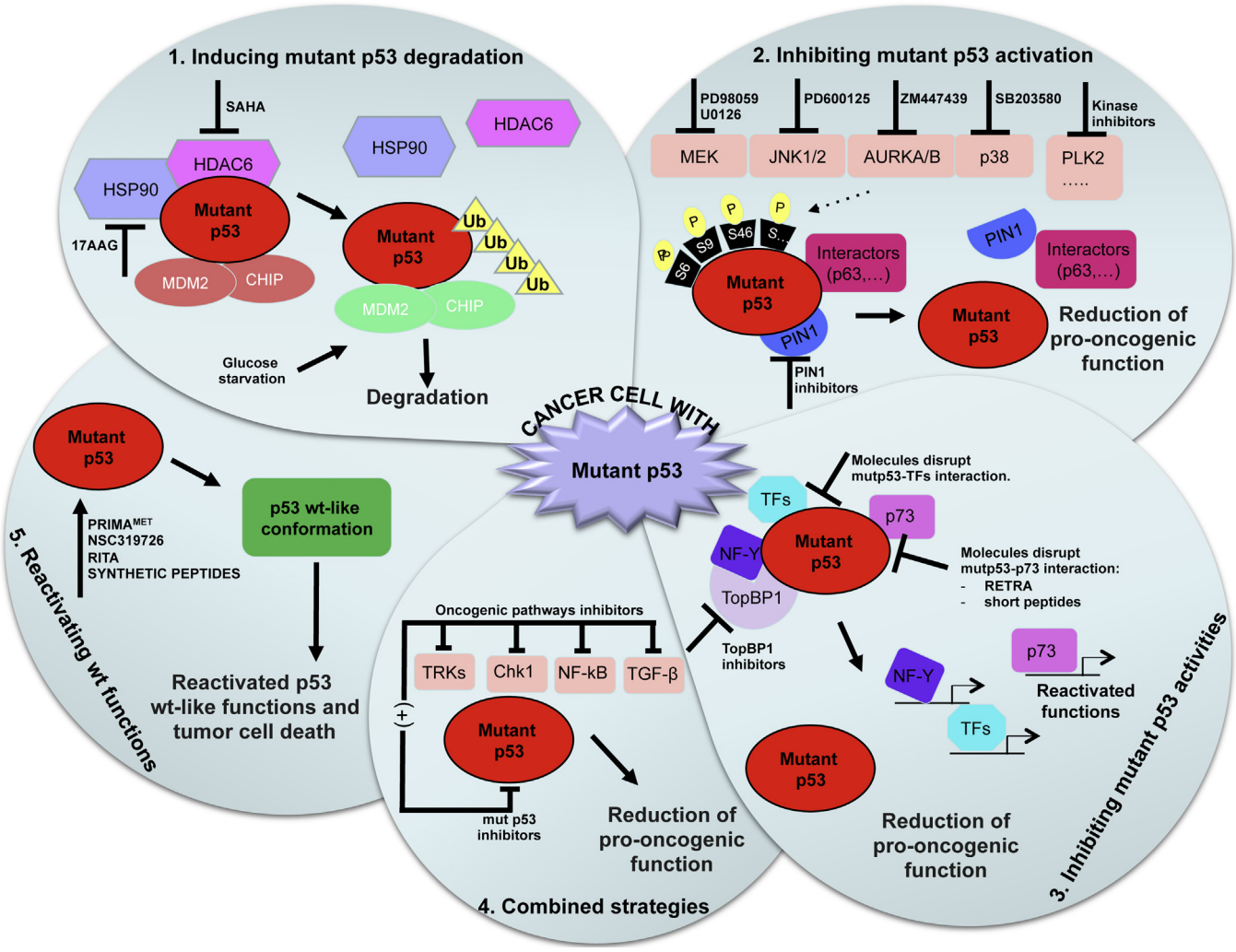

Fig. 2. Potential mutant p53-based therapeutic strategies. (1) Inducing mutant p53 degradation: disruption of stable complexes between mutant p53 and the HSP90 machinery by 17AAG (HSP90 inhibitor) or SAHA (HDAC6 inhibitor) may release MDM2 and CHIP E3 ubiquitin ligases from inhibition. Mutant p53 degradation may also be enhanced by glucose restriction; (2) Inhibiting mutant p53 activation: pharmacological inhibition of kinases or mutant p53 activators as Pin1 may restrain its pro-oncogenic activities by avoiding proper activation; (3) Inhibiting mutant p53 activities: mutant p53 functions may be restrained by avoiding the interaction with partners or interfering with the functional significance of mutant p53 complexes; (4) Combined strategies: using simultaneously drugs that target mutant p53 and inhibitors of pathways that cooperate with its malignant functions may synergize to hinder tumor progression; (5) Reactivating wt (wild type) functions: pharmacological reactivation of wt-like functions in p53 point mutants may recover tumor suppressor capabilities specifically in tumor cells. TFs (transcription factors); TRKs (tyrosine kinase receptors).

shown to be inactivated by recruitment into the same complexes [26]. Disruption of this complex or inhibition of chaperone activity in cultured cells released both E3 ubiquitin ligases from inhibition and triggered mutant p53 degradation [26,41]. On this basis the design of pharmacological strategies aimed at inducing selective degradation of mutant p53 through inhibition of HSP90 were suggested. Pharmacological inhibition of HSP90 ATP-dependent chaperone activity by Geldanamycin or 17AAG is able to reduce mutant p53 stability [24,26] (Fig. 2). Several HSP90 inhibitors were shown to inhibit tumor growth in pre-clinical models and in patients, and some of them are in phase II clinical trials [42]. However for most of them the effect usually disappears when treatment is stopped, in line with the idea that HSP90 should exert a general effect in alleviating proteotoxic stress. It would be interesting to analyze whether if this inhibitors may have more strong and permanent effects in mutant p53 bearing tumors considering the specific effect of HSP90 on mutant p53 stability.

Histone deacetylase inhibitors (HDACi) provided another example on how to overcome the hyperstability of mutant p53 in tumor cells. HDACi are being intensively studied as promising chemotherapy drugs since they elicit different anticancer responses with a remarkable specificity for tumor cells, and some of them are undergoing clinical trials [43]. At least three HDACi have been reported to reduce mutant p53 levels in cell lines, FR901228, Trichostatin A (TSA) [23] and suberoylanilide hydroxamic acid (SAHA) [25], which however were proposed to act through different mechanisms. SAHA inhibits HDAC6, which normally activates HSP90 by promoting deacetylation of K294 (Fig. 2). Upon SAHA treatment, MDM2 and CHIP may be released from inhibition by HSP90 complex and induce mutant p53 degradation [25]. Nevertheless, an inhibitory effect of HDAC inhibitors on transcription of TP53 was described, that may also cooperate with the observed effects [44]. In the case of FR901228 and TSA, even if they may counteract HSP90 activity at rather high concentrations, the effect on mutant p53 levels was observed at lower concentrations and was accompanied by transcriptional induction of p21 and MDM2 and conformational changes in mutant p53, suggestive of reactivation of wt-like conformation [23].

Further contributing to delineate the mechanisms of mutant p53 regulation, it was shown that phosphorylation of mutant p53 on S15 and S37 contributes to protein stabilization [45]. The kinase responsible for these modifications is DNA-PK, which is also able

to phosphorylate wt p53, but with no significant effect on stability [46,47]. Stathmin (Stathmin 1, OP18) contributes with mutant p53 stabilization by enhancing DNA-PK expression [45]. Accordingly, stathmin downregulation reduced the expression of mutant p53 target genes and enhanced cell death in ovarian cancer cell lines expressing mutant but not wt p53 [45]. Intriguingly, stathmin expression may be repressed by wt p53 [48] but may be positively regulated by some p53 point mutants [49], suggesting that a positive oncogenic loop may be established. Clinical data supported this connection showing that high stathmin levels were correlated with p53 overexpression in samples from high-grade serous epithelial ovarian carcinomas (EOC) [45]. High levels of stathmin in human tumors were associated with the development of an aggressive disease [50]. In contrast, stathmin overexpression is an early event in EOC, already detectable in premalignant lesions [51]. Type II EOCs include undifferentiated High-Grade tumors (HG-EOC), characterized by mutations on TP53 in more than 75% of cases and represent the most aggressive cases [52,53]. Interestingly, it was recently proposed that mutant p53 accumulation may represent an initiating event in type II HG-EOC [54]. Treatment of ovarian cancer cell lines with the DNA-PK inhibitor NU7441 reduced mutant p53 phosphorylation on S15 and S37, suggesting a potential use in strategies aiming at inducing mutant p53 degradation [45]. An interesting candidate could be the small molecule CC-115, which acts as a double inhibitor of mTOR and DNA-PK and is being tested in Phase I clinical trials, including hematological malignancies and solid tumors.

Evidences showing that glucose restriction affects mutant p53 stabilization opened a new perspective on our understanding of the biological relevance of p53 proteins (Fig. 2). Incubation of different cell lines in conditions of glucose starvation induced a marked reduction on endogenous mutant p53 [27]. This was accompanied by deacetylation and ubiquitination of the mutant protein. On the contrary, glucose restriction promotes stabilization of wt p53 [27]. Also in this case MDM2 is involved in mutant p53 depletion, however, the process is independent from the proteasome machinery. Instead, degradation is achieved through autophagy [27,55]. Through activation of autophagy, glucose restriction ultimately leads to cell death, and mutant p53 depletion was found to enhance this citotoxic effect. Therefore, in cells harboring wt p53 metabolic stress may help to stabilize wt p53, which contributes to trigger autophagy and eventually cell death. Chronically stabilized mutant p53, on the contrary, may prevent cell death by inhibiting autophagy. These findings may impact on clinical management of cancer in different ways. On one hand, pharmacological manipulation of glucose metabolism may provide novel tools to induce mutant p53 degradation. On the other, they suggest the exciting possibility that dietary regulation may help to restrain tumor progression in cases bearing mutant p53. Supporting the clinical use of glucose restriction in anti-cancer therapies, evidences from mouse models showed that a carbohydrate-restricted diet reduced mutant p53 levels *in vivo* and the tumorigenic potential of human tumor cell lines expressing mutant p53 in nude mice.

3.2. Inhibiting mutant p53 activation

Wt p53 function is regulated by complex combinations of post-translational modifications including phosphorylation, acetylation, ubiquitination, methylation, sumoylation and neddylation [5]. On the contrary, even if some modifications on mutant p53 were described both in cultured cells and in human tumors, particularly phosphorylation [56,57] ubiquitination [26,29] and acetylation [27,56,58], we still lack a complete knowledge on how posttranslational modification codes affect protein function. Several residues on mutant p53 were shown to be phosphorylated and for some of them the modification affected protein function. Some of those residues were reported to be phosphorylated also on wt p53, however the possibility that different kinases may act on different p53 forms should be considered. Interestingly, phosphorylation of some residues on mutant p53 is readily detected even in the absence of stimuli that are normally required for wt p53 phosphorylation [59]. These modifications are attenuated upon downregulation or pharmacological inhibition of kinases, suggesting that in tumor cells some signaling pathways are chronically activated, resulting in persistent protein modification. Treatment with PD98059 (MEK inhibitor) reduced phosphorylation on S6 and S9 [60], while treatment with inhibitors SP600125 (JNK1-2), U0126 (MEK), SB203580 (p38), ZM447439 (Aurora A, B) reduced phosphorylation on S46 [59]. Therefore, it seems logical to speculate that inhibiting the kinases acting on those sites may represent a potential therapeutic strategy (Fig. 2). For example, mutations avoiding phosphorylation on S6, S9, S46 and S315 reduced pro-migratory activity and invasiveness [59,60]. Nevertheless, evidences for the ability of most proposed kinases to directly phosphorylate mutant p53 is still lacking. Instead, PLK2 was shown to phosphorylate mutant p53 on several sites including T377 [61]. Phosphorylation at this particular site was not described before neither in wild-type nor mutant p53. Through these modifications PLK2 enhances the transcriptional activity of the mutant p53/NF-Y complex, leading to aberrant expression of target genes increasing cell proliferation and drug resistance. Therefore, inhibition of PLK2 may provide a way to reduce mutant p53 oncogenic function. Interestingly, PLK2 itself was identified as a mutant p53 target gene induced upon treatment with chemotherapeutic drugs [61]. Nevertheless, the consequences of PLK2 inhibition deserve more investigation since its role on tumorigenesis is not clear. Some reports support a PLK2 pro-oncogenic function [62,63], however PLK2 promoter is silenced by hypermethylation in B-cell malignancies and ovarian cancer [62,64]. Phosphorylation on S392, was also reported, nevertheless, its consequences on mutant p53 function are not well understood. This modification was proposed to reduce transformation potential and chemoresistance *in vitro* [65]. However, S392 was shown to be frequently hyperphosphorylated in tumor-derived cell lines [56] as well as in squamous cell carcinoma (SCC) [66], actinic keratosis, Bowen's disease, basal cell carcinoma [67] and transitional cell carcinoma (TCC). Moreover, a correlation between S392 phosphorylation and poor clinical outcome was observed for SCC [66] and in the case of TCC an association between S392 phosphorylation and histological tumor grade and tumor stage was found [68]. Interestingly, acetylation of the C-terminus of mutant p53 by PCAF leads to p53 wt function reactivation [69].

Supporting a promising role for kinase inhibitors, it is interesting to note that synthetic lethality between CHK1 and p53 mutation during DNA damage response was described in human-in-mouse models of Triple Negative Breast Cancer (TNBC) [70,71]. Moreover, basing on the identification of differentially expressed genes between wt and mutant p53 cell lines, candidate synthetic lethal genes to mutant p53 were identified. These candidate druggable targets are mostly kinase-encoding genes involved in regulation of the cell cycle such as PLK1, PLK4, CDK1, AURKA, and NEK2 for which inhibitors exist that may be used as potential anticancer agents [72].

In addition to enzymes directly responsible for protein modification, alteration of signaling pathways at other levels may also affect mutant p53 function (Fig. 2). The cooperation between hyperactivated Ras signaling and mutant p53 in tumor progression has been supported by persuasive *in vitro* and *in vivo* evidences [11,13,73]. The Ras family includes several small GTPases that have a complex role as upstream modulators of signaling pathways [74]. Elegant mouse models that combined tissue specific expression of activated Ras with p53 point mutants, revealed the deleterious effect of their cooperation. For instance, in a model for pancreatic ductal carcinoma (PDA), where expression of knocked-in mouse *p53R172H* and

KRasG12D was restricted to pancreas progenitor cells, Ras activation was enough to promote tumorigenesis, however, concomitant expression of mutant p53 dramatically accelerated tumor onset and reduced median survival. Notably, mice carrying activated Ras and a mutant p53 developed aggressive and metastatic disease at high frequency [73]. Even if those mice harbored wt p53, the observation of consistent loss of heterozygosity that eliminated the wt allele gave strong support to the notion of mutant p53 GOF. Despite the high frequency of *KRAS* mutation in human PDAs, mice expressing only activated Ras failed to recapitulate the aggressive characteristics of the human disease which are instead readily observed in tumors from mice carrying both activated Ras and mutant p53 more.

A model for Non Melanoma Skin Cancer (NMSC) provided unambiguous evidences that the observed phenotypes depended upon neomorphic mutant p53 activities. A model based on inducible expression of knocked-in *p53R172H* and *KRasG12D* in the skin was used, where exclusive expression of mutant p53 was achieved by simultaneous deletion of the remaining wt allele [13]. Again, simultaneous expression of activated Ras and mutant p53 was followed by enhanced tumor development and increased metastasis frequency comparing with expression of activated Ras or mutant p53 alone. Noteworthy, in both the PDA and MNSC models, tumors bearing activated Ras and mutant p53 showed elevated levels of genomic instability. These models encourage the idea of disarming mutant p53 function as a way to restrain the development of aggressive tumors. Possibly, Ras activation may contribute to mutant p53 function by enhancing phosphorylation. As discussed above, several kinases downstream of Ras were implicated in mutant p53 phosphorylation. Other evidences reinforcing this notion have shown that activated Ras induces phosphorylation of ectopically expressed p53R280K on Ser33 and Ser46 [59]. Similarly, activated Ras induces phosphorylation of Ser/Thr-Pro sites on murine p53R172H.

Alternatively, mutant p53 activation may be avoided by preventing the action of Pin1, a peptidyl–prolyl isomerase belonging to the parvulin family. Pin1 catalyzes the isomerization of the intervening peptide bond in phosphorylated S-P or T-P motifs (S/T-P motifs) that is otherwise restrained by the cyclic structure of P residue [75,76]. Upon isomerization local conformational changes are induced that may have different consequences depending on the substrate, including altered function, stability or subcellular localization [77]. Pin1 is the only prolil isomerase able to bind its substrates upon phosphorylation of S or T, which renders S/T-P motifs unavailable for other prolil isomerases and further blocks spontaneous isomerization, providing a versatile mechanism to link phosphorylation signaling with protein function. Despite being involved in several aspects of normal cell physiology, in the last decade Pin1 has emerged as a critical factor in tumorigenesis [78]. Pin1 is frequently overexpressed in human tumors [79] and mounting evidences have demonstrated its ability to amplify oncogenic mechanisms [78,80], drawing the attention itself as a potential pharmacological target.

The simultaneous presence of mutant p53 and abnormally elevated levels of Pin1 endows tumor cells with the unique opportunity to establish a molecular axis that exploits deregulated phosphorylation signaling to activate downstream mechanisms of aggressiveness [59]. Upon phosphorylation on S/T-P motifs, Pin1 binds mutant p53 and amplifies several aspects of its oncogenic function *in vitro* and *in vivo*, including, cooperation with oncogenic Ras in cell transformation in mouse embryo fibroblasts and enhancement of migration and invasion in breast cancer cell lines. Noteworthy, Pin1 also binds wt p53 at the same S/T-P motifs but promoting in that case protein stabilization and apoptotic response, further underlying that wt and mutant p53 may share similar regulatory mechanisms [81–83]. The cooperation between Pin1 and mutant p53 amplifies al least two independent but complementary mechanisms [59]. On one hand Pin1 enhances complex formation between p63 and mutant p53, blocking transcriptional activation of the anti-metastatic p63 targets *CCNG2* and *SHARP1* [60]. On the other, Pin1 promotes the ability of mutant p53 to activate a transcriptional program that promotes aggressiveness. This program includes 10 genes (Pin1/mutant p53 signature) whose expression in primary human breast tumors is correlated with reduced survival and metastasis development and some of them are directly involved in the pro-migratory function of mutant p53 [59]. Arguing for a role of the Pin1/mutant p53 axis as a driver of aggressiveness in human tumors, analysis of a breast cancer patient cohort showed that while Pin1 overexpression had no prognostic value, the combination of elevated Pin1 levels with the presence of p53 missense mutations correlated with poor clinical outcome and behaved as an independent prognostic factor [59]. Further supporting the notion that mutant p53 depends on Pin1 to fully unleash its oncogenic potential *in vivo*, in *p53R172H* knockin mice, lack of Pin1 was correlated with an increase in survival and a marked alteration in tumor spectrum characterized by absence of carcinomas.

Interfering with the Pin1/mutant p53 axis may therefore provide a useful strategy for the design of novel anti-cancer therapies, either by targeting the interaction between both proteins or by inhibiting Pin1 isomerase activity. Several Pin1 inhibitors were described that were shown to counteract Pin1 effects in cell lines [84]. Nevertheless, these small molecules have not yet find their way to clinical use, perhaps due to the lack of perception on the conditions where Pin1 actually propels oncogenic processes. Intriguingly, Pin1 overexpression did show a correlation with poor clinical outcome in other cancer types independently of p53 mutations, such us lung [85], prostate [86] and oral squamous cell carcinoma [87], however, p53 mutation was not considered in those studies. These differences in the clinical significance of Pin1 overexpression may be rationalized considering the provocative hypothesis that in breast cancer the mutant p53 network may be the major determinant of aggressiveness among the oncogenic mechanisms amplified by Pin1, while in other tumor types, other mutant p53-independent mechanisms may take over. On this basis, strategies targeting the Pin1/mutant p53 axis may be particularly relevant for breast cancer. Nevertheless, when considering Pin1 as a pharmacological target it should be kept in mind that it may also collaborate with mechanisms of tumor suppression as for example p53- or p73-induced apoptosis [81,88] as well as degradation of CMYC [89] and CCNE [90]. Therefore it is conceivable that depending on the balance between pro-oncogenic and anti-oncogenic pathways boosted by Pin1 that are active in a particular cell context, Pin1 inhibition may also promote cell proliferation or tumorigenic potential.

3.3. Inhibiting mutant p53 activities

Another way to hinder mutant p53 function may be avoiding the interaction with partners or interfering with the functional significance of mutant p53 complexes (Fig. 2). In some cases, p53 mutants alter the regulation of the partner in a way that promotes oncogenic processes. Other complexes are inhibitory and block the ability of these proteins to collaborate with tumor suppression. Several pro-oncogenic complexes involve the interaction of mutant p53 with transcription factors and subvert their activity favoring the transcription of tumor promoting genes. Some of the transcription factors described to interact with mutant p53 such as NF-Y, VDR and SREBPs, appear particularly relevant for the clinical management of cancer (Fig. 1).

Mutant p53 interacts with NF-Y in unstimulated cancer-derived cell lines and enhances transcription of NF-Y targets upon genotoxic insult induced by treatment with chemotherapeutic drugs

like doxorubicin and cisplatin [58,91]. Taking into account that doxorubicin may cooperate with mutant p53 stabilization [22] inhibition of complex formation may help to counteract mutant p53 pro-oncogenic effects in therapies based on genotoxic insult. The activity of the mutantp53/NF-Y complex may also be decreased by targeting Topoisomerase IIβ binding protein (TopBP1). TopBP1 interacts with mutant p53 and promotes its ability to complex with NF-Y, enhancing recruitment of mutant p53 and p300 on target promoters and expression of mutant p53/NF-Y targets upon genotoxic insult [91]. Accordingly, downregulation of TopBP1 counteracted several aspects of mutant p53 function including resistance to apoptosis upon DNA damage and enhanced proliferation. Moreover, TopBP1 was found to be overexpressed in breast cancer and this alteration was correlated with poor clinical outcome [91,92].

The interaction between VDR and mutant p53 may be relevant to define the therapeutic use of Vitamin D3. Upon activation by binding to Vitamin D3, VDR regulates gene expression either by transcriptional induction or repression of selected targets. Vitamin D3 has attracted the attention as a leading compound for chemotherapy because it exerts pro-apoptotic and anti-cancer effects in a number of *in vitro* [93,94] and *in vivo* [95–97] experimental models. Nevertheless, Vitamin D3/VDR signaling may show anti-apoptotic effects as well [98,99]. Moreover, VDR is upregulated in several human cancers [100,101] and elevated VDR expression was shown to correlate with tumor stage [102,103] The finding that mutant p53 alters VDR activity shed some light to understand this opposing biological outcomes [104]. Complex formation with mutant p53 enhances nuclear import and alters gene expression by favoring Vitamin D3-mediated transcriptional activation of pro-survival genes and repression of pro-apoptotic ones. Accordingly, Vitamin D3 treatment reduced cell death induced by doxorubicin or cisplatin exclusively in cells harboring either endogenous or ectopically expressed mutant p53. These evidences suggest that Vitamin D3 treatment should be avoided in mutant p53 harboring tumors. Alternatively, strategies based in releasing VDR from mutant p53 may restore its normal function and enhance Vitamin D3-induced apoptosis.

The finding that mutant p53 interacts with SREBP-1 and SREBP-2, unveiled several interesting possibilities for pharmacological treatment. Mutant p53 was shown to enhance aggressive characteristics of breast cancer cells in 3D cultures by promoting the transcription of genes belonging to the mevalonate pathway [105]. This is achieved by a mechanism that involves direct interaction of mutant p53 with SREBPs and recruitment on target gene promoters. Noteworthy, pharmacological inhibition of different steps of the mevalonate pathway leaded to significant attenuation of the aggressive phenotype ascribed to mutant p53 in breast cancer cell lines. Particularly interesting was the effect of Simvastatin and Mevastatin, two lipophilic statins that inhibit HMG-CoA reductase, the rate-limiting step of cholesterol biosynthesis [106], which not only reduced proliferation but also induced cell death *in vitro*. Due to their ability to reduce cholesterol levels, statins are used in the clinic to treat hypercholesterolemia but they are not yet used to treat cancer despite evidences showing tumor suppressive activities [107]. The observation that patients with high levels of the 17 genes from the mevalonate pathway identified as transcriptional targets of mutant p53 were associated with poor clinical outcome provides further support to this strategy [105].

Mutant p53 may also engage in inhibitory interactions, among which complex formation with p63 or p73 have been extensively studied. The ability to form these complexes was largely proposed as a GOF mechanism. This hypothesis stemmed from the observation that mutant p53 inhibited p63 and p73 activity as transcription factors and that wt p53 was unable to form similar complexes with any of the family members [108–110]. Nevertheless, understanding their precise biological effects have been complicated by the presence of multiple isoforms for both p53 family members. Studies that specifically addressed the role of TA isoforms have showed that loss of TAp63 or TAp73 enhanced tumorigenesis *in vivo* [111,112] arguing for their role as tumor suppressors. Accumulating evidences confirmed that individual downregulation of both proteins may phenocopy oncogenic at least some mutant p53 effects [11,60,113]. Consequently, interfering with sequestration by mutant p53 may unleash the tumor suppressive capabilities of p63 and p73.

In the case of p73, complex formation with mutant p53 was shown to inhibit TAp73-induced apoptosis in response to DNA-damaging drugs [114,115]. Supporting a potential clinical use of therapies targeting the mutant p53/p73 complex a small molecule, RETRA, was identified in a cell based screening of a chemical library looking for compounds able to induce transcription of a wt p53 specific reporter [116]. This molecule induces cell death in mutant p53-bearing cells *in vitro* and in mouse xenografts by counteracting TAp73 sequestration. Similarly, it was reported that short synthetic peptides derived from the sequence of p73 DBD inhibit the interaction with p53R175H, activating p73 transcriptional function. The ability of those peptides to induce cell death specifically in cells expressing mutant p53 further supports the therapeutic potential of this strategy [117].

Inhibition of the mutant p53/p63 complex may be particularly relevant for breast cancer, where mutant p53 was proposed to repress the expression of several TAp63 target genes important for suppression of metastasis like *SHARP1*, *CCGN2* [60] and *DICER* [59]. Targeting mutant p53 partners may also counteract complex formation. For example, Pin1 was also reported to enhance the mutant p53/p63 interaction and to reduce the expression of *SHARP1*, *CCGN2* and *DICER* in cells harboring endogenous mutant p53 [59]. In addition, TopBP1 promotes the interaction between mutant p53 and p63 or p73 and contributes to the inhibition of the transcriptional activity of both p53 family members [91].

3.4. Targeting the crosstalk with other oncogenic pathways

Besides altering intracellular processes, mutant p53 is able to capture external signals to drive tumor aggressiveness (Fig. 1). Revealing evidences from several groups have shown that p53 mutants may enhance the tumor promoting activities of TGF-β, Integrin, RTK and NF-κB signaling pathways. Paradoxically, this promiscuous ability may also provide the basis for novel therapies. In particular, combined strategies using simultaneously drugs targeting mutant p53 and inhibitors of the altered pathways may enhance specificity and reduce effective drug concentrations (Fig. 2).

Mutant p53 fosters TGF-β1 induced invasiveness by opposing p63 anti-metastatic activity, but in this case a ternary complex including SMAD2 is formed [60]. As a consequence, transcriptional induction of p63 target genes *SHARP1* and *CCGN2* is impaired which in turn promotes cell migration. Outstandingly, low expression of these two genes was found to correlate with higher risk of recurrence in breast cancer patients and was proposed as a prognostic tool. Accordingly, downregulation of endogenous mutant p53 severely impaired TGF-β1 induced migration and invasion *in vitro* and reduced the metastatic potential of human tumor-derived cell lines in mice [60]. Noteworthy, formation of the inhibitory complex between SMAD2, p63 and mutant p53 requires active TGF-β signaling and phosphorylation of mutant p53 on S6 and S9, which is enhanced by Ras signaling. Therefore, in tumors where the crosstalk between TGF-β signaling and mutant p53 is active, therapies using TGF-β and Ras signaling inhibitors combined with drugs inducing mutant p53 inactivation may prove successful. In support to this idea, treatment of cell lines with the MEK1 inhibitor PD98059 inhibited TGF-β induced migration [60].

Other external signals, like EGF, HGF and fibronectin may be exploited by mutant p53. A central role in this process is played by integrin heterodimers that regulate the coordinated transduction of external signals through their ability to crosstalk with receptor tyrosine kinase (RTK) – activated signaling. Trafficking of integrin heterodimers through the endosomal pathway governs their signaling activity [118,119]. Upon internalization of receptors, endosomal membranes act as platforms where different signaling complexes that regulate downstream signaling are conformed. For example, activated α5β1 integrin heterodimers may form complexes with EGF receptor (EGFR) which are internalized and then recycled to the plasma membrane in a process that depends on the interaction of these complexes with Rab-coupling Protein (RCP), thereby activating AKT signaling [120]. Mutant p53 increases the rate of integrin and EGFR recycling to the plasma membrane, without affecting internalization, and, as a consequence promotes random migration, loss of polarity and invasion [113]. Pharmacological inhibition of EGF receptor with PD153035 inhibited invasion *in vitro* specifically in cells expressing mutant p53 [113], suggesting that inhibition of the EGF pathway may be more effective in treatment of tumors expressing p53 point mutants. Further contributing to an invasive phenotype, mutant p53 makes use of a similar mechanism to foster HGF-induced cell scattering. In this case, recycling of the HGF receptor MET is enhanced through a α5β1 and RCP dependent process that results in activation of MAPK instead of AKT signaling [121]. Other RTKs such as IGF-1 and PDGF-β receptors are unable to bind RCP and they do not affected invasiveness in mutant-p53 expressing cells, despite being able to activate AKT, suggesting that integrin/RCP dependent recycling is specific for some RTKs. Binding of receptors to RCP is required in both cases, suggesting that targeting this interaction may block the invasive behavior. Of note, an indirect mechanism was suggested for the effect of mutant p53, since no interaction was observed between mutant p53 and RCP. Interestingly, for both receptors p63 downregulation phenocopies the effect of mutant on EGF or HGF-driven migration and invasion while p63 overexpression opposes it [113,121].

Another remarkable connection is the crosstalk between mutant p53 and the NF-κB pathway. The family of NF-κB transcription factors is the central hub of a complex network able to promote apoptosis inhibition, proliferation as well as migratory and invasive behavior in response to a broad range of signals including growth factors an cytokines [122]. Persistent activation of NF-κB pathway is the hallmark of chronic inflammation and is also frequently found in human cancers [123]. A connection between mutant p53 and NF-κB was initially proposed basing on evidences showing that mutant p53 enhanced transcription of *NF-κB2* and that p52/p100 (NF-κB2) protein levels were found elevated in cells expressing mutant p53 [124,125]. More important, downregulation of p52/p100 severely impaired mutant p53 activities such as enhanced proliferation, migration and chemoresistance [125]. Other evidences showed that mutant p53 enhances NF-κB signaling by promoting p50/p65 nuclear translocation and that it contributes to inhibit TNFα induced apoptosis [126]. In addition mutant p53 enhances transcription of chemokines regulated by the NF-κB pathway like CXCL5, CXCL8 and CXCL12 [127,128]. Therefore, by stimulating the ability to sense pro-oncogenic signals while at the same time taking advantage of NF-κB pro-inflammatory responses mutant p53 may endow tumor cells with an effective strategy to better exploit their microenvironment. Recent evidences confirmed that the alteration of NF-κB signaling by p53 mutants fosters tumor progression by exacerbating the inflammatory response [129]. In cultured cell lines, mutant p53 extends NF-κB pathway activation elicited by TNF-α, transforming a self-limiting pro-inflammatory response into a chronic state. This prolonged response was also observed in animal models of dextran sodium sulfate (DSS) induced Inflammatory Bowel Disease (IBD), which resemble human colitis-associated cancer (CAC). In those mice mutant p53 promoted chronic inflammation and tissue damage *in vivo*, and sustained elevated expression levels of pro-inflammatory cytokines. Moreover, progression to colon carcinoma in mice harboring mutant p53 was exceptionally more frequent. In contrast, mouse models for sporadic colorectal cancer (CRC) combining DSS with azoxymethane (AOM) treatment, showed no difference between mice harboring wt or mutant p53. P53 mutations in CAC often precede the development of neoplastic lesions [130] but in sporadic CRC seem to be rather late events [131]. Consequently, these evidences are suggestive of a role in tumor initiation for mutant p53 in IBD-associated cancer.

3.5. Reactivating wt function in mutant p53

Restoration of lost tumor suppressive activities would seem the most logical approach to fight cancer. In the case of p53, for example, persuasive experimental evidences from mouse models that allow controlled re-expression of wt p53 in p53-null tumors showed a remarkable tumor regression upon protein restoration [132–134]. From a clinical perspective, however, to re-establish wt p53 function appears technically challenging. One approach that has reached clinical trials in several types of cancer is gene therapy [135]. Although at least partial clinical responses were observed, this approach shows several technical limitations regarding efficiency of gene delivery and accessibility.

Unexpectedly, tumor cells harboring *TP53* mutations may carry their own destructor. Regarding *nonsense* mutations it was reported that aminoglycoside antibiotics, including G418 and gentamicin, enable ribosomes to read through premature stop codons and to recover to some extent full length protein expression [136]. For some mutations it was further shown that aminoglycoside treatment specifically induced apoptosis in cell culture. However, *nonsense* mutations account for only 8% of *TP53* mutations. What about missense mutations? Even if tumors may benefit from the neomorphic activities p53 pint mutants, they still have a full length protein. Any condition able to restore wt structure to those mutants may unleash a cytotoxic response (Fig. 2). The attractive idea that loss of wt activity may be reversible was suggested more than a decade ago by the observation that the activity of point mutants may be rescued at low temperature, and was largely supported by work from several groups that identified small molecules or peptides able to rescue several aspects of wt p53 function [137,138]. Pharmacological reactivation of wt-like functions in p53 point mutants is expected to achieve specific tumor clearance with low toxicity. Moreover high mutant p53 expression levels in tumor cells should favor a strong response and oncogenic alterations should contribute to activate and stabilize the regenerated p53 protein. The key to this functional plasticity seems to reside in the structural characteristics of the DBD of p53. This domain is intrinsically unstable, being prone to partial unfolding at temperatures slightly higher than 37 °C [139,140]. Such a flexible architecture was proposed to allow wt p53 to engage in multiple interactions with DNA and proteins. Cancer-related mutations are concentrated preferentially in the DBD and can be classified in two categories: contact mutations, which affect aminoacids directly involved in contacts with DNA and that may have little or no effect on protein conformation, and structural mutations, that severely affect protein structure. Mutants from both groups usually lose the ability to bind DNA or to transactivate wt p53 target genes. Strikingly, small molecules with different structures were identified by rational design or by library screenings that are able to recover wt-like functions in mutant p53, suggesting that different mechanisms may be involved in each case. We do not intend to discuss exhaustively this issue, but rather to consider some aspects that we believe may be relevant for therapy design.

Most mutant p53 reactivating compounds were identified through screenings of chemical libraries that used as read out mutant p53-dependent cytotoxicity, DNA binding to p53RE or wt protein conformation [137,138]. All of them regained the ability to transactivate wt p53 targets as well as DNA binding to different extents. Particularly curious is the observation that most of these compounds are able to reactivate both, structural and contact mutants, suggesting that different mutants may share similar characteristics. A paradigmatic example of reactivating compounds is PRIMA-1, which was identified through a cell-based screening [141]. PRIMA-1 and a more active analog, PRIMA-1MET (Commercial name APR-246), reactivate transactivation of pro-apoptotic targets like *PUMA*, *NOXA* and *BAX* in p53 mutants and induces apoptosis preferentially in cells harboring mutant p53 [141,142]. They inhibit growth of human tumor xenografts in mice [141,143] as well as growth of mouse tumors in syngeneic hosts [144]. In addition, they showed citotoxic effects in cells from AML and CLL cells *ex vivo* [145,146]. The encouraging results in pre-clinical models along with its low toxicity and suitable pharmacokinetic properties allowed PRIMA-1^{MET} to reach clinical trials. Nevertheless, a disadvantage of cell-based screenings is that they do not provide detailed information on the mechanism of action, which is critical for optimization of potency and target selectivity. In the case of PRIMA-1 and PRIMA-1^{MET} it was reported that they are rapidly converted to other compounds. One of them, MQ, binds covalently to the p53DBD, but this modification is not sufficient to activate pro-apoptotic responses. Instead it was proposed that MQ would stabilize a wt-like conformation or promote binding to DNA through cysteine modification.

As an alternative, a rational approach was used to find compounds able to target p53 carrying Y220C, which is the ninth most frequent p53 mutation found in human cancer with increased frequency specifically in breast cancer [147]. This mutation creates a unique surface crevice that is particularly suited for pharmacological manipulation. *In silico* screening based on the crystal structure of Y220C DBD allowed to identify PhiKan083, a molecule able to specifically bind and stabilize this mutant [148]. A similar strategy combining structural information with NMR analysis lead to the isolation of PK7088, a molecule that induces expression of p21 and NOXA in cell lines harboring Y220C mutation, and elicited proliferation arrest and cell death [149]. PK7088 shows a notable specificity for Y220C mutant and it is not active with other p53 mutants.

A group of compounds from the thio-semicarbazone family that shows allele-specific reactivation of mutant p53 was identified by a novel approach based on the NCI60 anti-cancer drug screen from the national Cancer Institute [150]. The screening determined growth inhibition half maximal inhibitory concentrations (IC_{50}) of 48,129 compounds on 60 human tumor cell lines with known p53 status. Basing on these data, a score function was developed to identify compounds with low IC_{50} in cell lines harboring mutations in hot spot codons 175, 248 or 273 comparing with wt or null p53 cell lines. Since scoring relies on data from several cell lines expressing p53 mutants, this approach reduces cell type-specific effects providing a model that takes into consideration the molecular heterogeneity found in human cancer. Surprisingly, the compounds identified showed a marked specificity for mutations in codon 175, particularly R175H. One of these compounds, NSC319726, favored a wt conformation, induced transcription of wt p53 target genes and apoptosis in cell lines harboring R175H. Also, the drug inhibited the growth of xenograft tumors in mice derived from human cell lines. Even if the mechanisms of action is not yet understood, it is interesting to note that the apoptotic effect of NSC319726 was inhibited by the reducing agent NAC but enhanced by diamide, and oxidizing agent, suggesting a role for ROS in the process.

The small molecule RITA was identified in a cell-based screening for molecules able to suppress cell proliferation in a wt p53-dependent manner and only in transformed cells [151,152]. Upon binding RITA induces accumulation of wt p53 through disruption of p53-MDM2 interaction in tumor cells. Surprisingly, it was shown that RITA is able to induce an apoptotic response also in tumor cells bearing p53 mutations, expanding its applicability in the clinics [153]. The apoptotic response elicited by RITA is dependent on the presence of mutant p53 and it is sustained by the reactivation of p53's apoptotic machinery in cell lines expressing different mutants. Wt p53 target genes are also induced upon RITA treatment in mutant p53 expressing cells and wt p53 inhibitors abrogate apoptosis induction. Moreover, considering that Pin1 is required for RITA-induced cytotoxicity [152] and that it is able to bind both wt and mutant p53 proteins [59], it would be interesting to determine if tumors harboring mutant p53 and high Pin1 levels are more sensitive to RITA treatment.

In addition to small molecules, several peptides were described that are able to induce wt-like activities in p53 mutants. In pioneering studies a synthetic peptide derived from the p53 C-terminus, peptide 46, was shown to restore transactivation of wt p53 targets and to induce apoptosis in mutant p53 expressing cells [154]. Another peptide, CDB3, that was rationally designed to bind the DBD also rescued the ability to transactivate wt p53 target genes and elicited a weak apoptotic response [155]. Also, an unbiased screening leaded to the identification of several peptide aptamers able to bind selectively p53R175H and to induce apoptosis only in mutant p53 expressing cells [156]. Comparing with small molecules, peptides have obvious disadvantages as therapeutic agents since their production is more complicated and expensive and they pose several obstacles for administration and delivery. However, it is worth noting that CDB3 can enter cells [157] and that a cell-penetrating peptide derived from the p53 C-terminus reactivated mutant p53 and opposed tumor development in mouse models [158]. Moreover, peptides may provide key information on the molecular mechanisms involved.

An interesting lesson from mutant p53 reactivating compounds is that differences exist regarding the particular activities recovered by each molecule or the biological response elicited, arguing that different compounds may evoke different aspects of wt p53 function rather that completely recover the whole range of p53 activities. Taking into consideration the amazing variety of cellular processes in which p53 was proposed to be involved [4], including the promotion of cell survival, the question arises as to which p53 activities are desirable to restore for an anti-cancer therapy. At first glance, induction of senescence or apoptosis would seem the best choice to ensure tumor clearance. However, considering that each biological response may affect in radically different ways the microenvironment, it may be desirable to induce specifically one or the other in particular tumor types. Moreover, the growing knowledge on the biology of tumors, showing the presence subpopulations with different characteristics among transformed cells may suggest the need to activate other p53 activities, as for example expression of cell surface or secreted proteins that may impinge on cell communication.

4. Concluding remarks

The enormous effort on cancer research in the last decades have showed that some recurrent alterations, like the presence of p53 point mutants, may be at the base of the oncogenic mechanisms in a large number of tumor types. Compelling evidences showing that mutant p53 is able to promote aggressive and metastatic phenotypes has undoubtedly placed it as a promising target for anti-cancer therapies. One the most interesting aspects of p53 mutants is that they show abnormally elevated expression levels almost exclusively in tumor cells, potentially endowing any drug

acting on mutant p53 with an exquisite specificity and enhanced sensibility. The challenge now is to understand in what tumors mutant p53 exerts an oncogenic effect and how does it do it.

Several clinical studies tried to address whether if p53 mutation behaves as an independent prognostic and/or predictive factor. Recent studies that identified p53 mutations by direct sequencing showed a general trend that confirmed the association between p53 mutation and poor clinical outcome [8,147]. As mounting evidences suggest, the cooperation with other cancer-related alterations is necessary to fully unleash mutant p53 pro-oncogenic potency. Hence, studies that account for such cooperation are necessary to identify in which cases the disease depends on mutant p53-based mechanisms. An outstanding example of the multiple clinical interactions that mutant p53 may display is represented by the distribution of p53 mutations in breast cancer where p53 mutation has a prognostic value despite of the rather low frequency of p53 mutation in the overall population [147]. Breast tumors may be classified basing on their expression profile [159] and this stratification is able to distinguish groups with different clinical outcomes [160]. Strikingly, p53 mutation frequency dramatically increases in some of those subclasses, approaching values between 70% and 80% of cases in ERBB2+ and Basal-like groups, which are correlated with reduced survival [160,161]. Basal-like subclass is particularly relevant since it is the most frequent molecular subtype among TNBC, which have a higher risk of recurrence and whose management still represents a clinical challenge. Hence, it would be interesting to analyze the potential of mutant p53-based therapies in TPNBC.

On the other hand, understanding the exact molecular mechanisms activated by mutant p53 in each tumor type would provide more chances to specifically deactivate the pathological process. At first glance the variety of molecular mechanisms in which mutant p53 was involved seems confusing. Surprisingly, despite the multiplicity of mechanisms described, inactivation of different p53 point mutants often results in similar biological outcomes in cell lines and in animal models. Considering the complexity or tumor progression it does not seem illogical that tumor cells could make use of strategies involving multifunctional proteins able to wire redundant circuits in tight cooperation with other mechanisms of malignancy. Such a strategy would maximize the capacity of tumors to progress under a wide range of external conditions. In addition, the possibility that mutant p53 may play different roles in different steps of tumor progression should also be considered.

The ability to transform an efficient tumor suppressor pathway into a network that promotes tumor aggressiveness by introducing only a missense mutation is one of the finest examples of the efficient use of resources by tumor cells. Hopefully, the double-sided personality of the p53 pathway may provide the key to improve the clinical management of cancer.

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