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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*