The Evolution of TP53 Mutations: From Loss-of-Function to Separation-of-Function Mutants

Madison Miller1, Nitin Shirole1,2, Ruxiao Tian1, Debjani Pal1,3, and Raffaella Sordella1,2,∗
1Cancer Center, Cold Spring Harbor Laboratory, USA
2Graduate Program in Genetics, Stony Brook University, USA
3Graduate Program in Molecular and Cellular Biology, Stony Brook University, USA

Abstract

As the most mutated gene in cancer, it is no surprise that TP53 has been the center of cancer biology discourse since its discovery in the late 1970s. Although early demonstrations of p53's role in the modulation of cell proliferation and survival solidified its classification as a tumor suppressor and transcription factor, our conceptualization of p53 is ever-evolving. Here, we present novel evidence of the role of alternative splicing isoforms, truncating/separation-of-function mutations, and hot-spot silent mutations in the regulation of p53's activities.

ABBREVIATIONS

CypD: Cyclophilin D; GOF: Gain-of-Function; LOF: Loss-of-Function; SOF: Separation-of-Function; MPTP: Mitochondria Permeability Transition Pore; LFS: Li-Fraumeni Syndrome

INTRODUCTION

p53 has long been revered by cancer biologists as the “Guardian of the Genome” [1]. It first emerged as a character in cancer discourse in the late 1970s and has since become one of the most studied genes with more than 80,000 related publications to date [2]. The functional complexity of p53 is well reflected by the scientific community’s evolving understandings of its role in cancer biology. Initially, p53 was conceptualized as a proto-oncogene; however, further investigations revealed thatp53primarily functions as a tumor suppressor [3-6]. While p53’s activities as a tumor suppressor have since been validated, recent findings indicate that certain p53 mutations could actually have pro-tumorigenic activities. These contemporary discoveries once again challenge the scientific community’s classification of this gene [1,3,6-9].

As a tumor suppressor, p53 regulates a number of cellular functions that are necessary for cell growth and survival. Specifically, activated p53 can induce transient growth arrest, DNA repair, autophagy, senescence, apoptosis and necrosis [10-17]. p53 is also shown to regulate physiological processes such as development, reproduction, self-renewal, and metabolism [18-23].

While p53 regulates the majority of the aforementioned processes by modulating the expression of downstream target genes as a transcription factor, p53 regulates several other processes in a transcription-independent manner (Figure 1) [12-15]. Structure-function analysis of p53 demonstrated the presence of five distinct domains that enable its transcriptional activities: i) a transcriptional activation domain (TAD); ii) a proline-rich domain (TAD-II/PRD); iii) a sequence-specific DNA-binding domain (DBD); iv) a tetramerization domain (TET) and; v) nuclear localization sequences (NLS) (Figure 1) [24]. In addition, p53 has also been shown to interact with and modify the function of other proteins, including but not limited to: BCL-2, BCL-XL, Bax, mTOR, AMPK, PTEN, SMAD3, p73 and p63 [10,19,24-31].

The breadth of p53’s biological functions and interactions is centered on its crosstalk with multiple signaling networks that ensure the precise regulation of its expression, subcellular localization and stability via multiple covalent modifications (e.g. phosphorylation, acetylation, methylation, ubiquitination) and protein-protein interactions (Figure 1) [24]. In particular, one of the best-characterized primary negative regulators of p53 is the MDM2 gene. MDM2 encodes for an E3 ubiquitin ligase that induces proteasome-based degradation of p53. MDM2 also inhibits the transcriptional activity of p53 by directly binding to its transactivation domain [10,11,32].

In addition to the previously mentioned regulators of p53’s activity, it is now well known thatp53’s function may be amended...
by means of alternative splicing. In fact, TP53 produces multiple protein isoforms with unique biochemical properties (Figure 2) [28,33]. For example, alternative splicing of intron-9 produces the p53-beta and p53-gamma isoforms, while the alternative splicing of intron-2 generates the Δ40p53-alpha, Δ40p53-beta, Δ40p53-gamma isoforms [34]. The expression of some of these isoforms is documented in multiple types of cancer such as breast, AML, melanoma, ovarian and head and neck cancers [33]. Although their specific functional roles are still under investigation, recent data suggest these p53 isoforms participate in tumorigenesis and metastatic dissemination [35].

Recently, Senturk et al., identified a thirteenth p53 splicing isoform. This novel isoform is generated by the use of a cryptic acceptor site in intron-6, which leads to a frame shift in the coding region that introduces a stop codon in exon-7 (Figure 2) [36]. As a result, this alternative splicing generates a truncated gene product, named p53-psi, that is primarily expressed in tissues exposed to chronic stress conditions and in tumors with high metastatic potential [36]. Further molecular analysis of p53-psi showed that this isoform lacks the majority of the DNA binding domain, nuclear localization sequences, and tetramerization domain that are present in the wild-type protein. Thus, p53-psi is devoid of transcriptional activities and canonical tumor suppressor functions [36]. However, p53-psi is still able to localize to the mitochondria where it can interact with Cyclophilin D (CypD), a major regulator of the mitochondria permeability transition pore (MPTP) [36] (Figure 3). Consequently, p53-psi increases the mitochondria permeability and the levels of mitochondria reactive oxygen species [36]. Interestingly, Senturk et al., also demonstrated that the p53-psi/CypD interaction is necessary for the reprogramming of cells to acquire mesenchymal features (Figure 3) [36].

Considering the critically important roles p53 plays in the modulation of cell growth and survival in response to a variety of cellular cues, it is not surprising that p53 is the most mutated gene in cancer [37]. In fact, a mutated version of TP53 is detected on average in 50% of tumors [38]. The types of mutations commonly affecting TP53 are loss of function (LOF), gain of function (GOF) and, as will be introduced in the present review, separation of function (SOF) mutations [39,40]. Most LOF mutations in TP53 adhere to the two-hit hypothesis proposed by Alfred G. Knudson in 1971 [41]. In fact, the most common cause of TP53 loss of function is an inactivating missense mutation in one allele and simultaneous deletions in regions of the 17p chromosome encompassing the TP53 locus [42,43].

It has become apparent that certain TP53 GOF mutations confer p53 with capacities that are different than those resulting only from the loss of tumor-suppressing functions. Interestingly, these GOF mutations occur at higher than expected frequencies and, consequently, are usually referred to as “hot-spot” mutations [44,45]. Knock-in mouse models of some of these p53 GOF hot-spot mutations have been shown to produce carcinomas, adenomas and osteosarcomas [44-47]. These models’ outcomes differ from those of TP53 null models, which primarily develop lymphoma. The mechanism behind these processes is highly complex and primarily centered on changes that occur in the transcriptional activities of p53 [44-47].

Functional differences between GOF and LOF mutations in the TP53 gene are perhaps best exemplified by Li-Fraumeni Syndrome (LFS). LFS is a relatively rare and hereditary disorder that renders those affected susceptible to the development of various cancers [46-48]. LFS patients harbor different germline mutations in the TP53 gene (Figure 4). While LFS patients harboring LOF TP53 mutations tend to develop tumors later in life, carriers of the GOF TP53 mutation have an increased probability of developing cancers as children and adolescents [49].

Recent studies from our laboratory have demonstrated that several TP53 truncating mutations occur at the exon-6/exon-

Figure 3 Comparison of p53-WT p53-psi activities. A) Diagram depicting p53-WT’s role in regulating apoptosis, DNA damage repair and cell-cycle checkpoints. P53-WT’s activities are attributed to its possession of a functional DNA-binding domain, nuclear localization sequences, and a tetramerization domain. B) Diagram depicting p53-psi’s ability to modulate cell survival, epithelial-to-mesenchymal transition (EMT), invasion and metastasis. Unlike p53-WT, p53-psi is generated by the use of an alternative 3’ splice site in intron 6 and lacks the functional domains necessary to promote canonical tumor suppressor functions.

7 boundary. These splicing mutations, deletions, and nonsense mutations produce mutants that induce cell proliferation and the acquisition of metastatic features in cancer cells [50]. Notably, these p53-truncated proteins have molecular features that are nearly indistinguishable from those of the p53-psi isoform (Figure 5) [50]. Like p53-psi, these exon-6 truncating mutants retain the capacity to partially localize to the mitochondria, to interact with CypD and to regulate the MPTP [50].

Given the similarity of the molecular and phenotypic features of exon-6 truncating mutants and p53-psi, these truncating mutations are perhaps best characterized as separation-of-function (SOF) [50]. In fact, SOF mutations produce stable proteins that are marked by the loss of certain biochemical properties, while leaving other activities of the wild-type allele undisrupted. Notable examples of SOF mutations are certain RAG2 mutants. RAG2 is a nuclease that cleaves and rejoins different segments of the V(D)J locus during V(D)J recombination. Indeed, it has been observed that several RAG2 mutants resulting from SOF mutations retain the capacity to perform cleavage despite being severely defective in the joining step in V(D)J recombination [51].

Analysis of multiple cancer genome datasets indicated that, much like TP53 GOF mutations, SOF mutations occur at higher than expected frequencies at particular hot-spot locations. Although these p53 SOF mutations are not druggable, CypD
Figure 4 Distribution of TP53 missense and nonsense mutations in patients with Li-Fraumeni Syndrome (LFS). A) Histogram featuring p53 missense mutations present in the germline of LFS patients. B) Histogram featuring truncating mutations present in the germline of LFS patients. Together, these plots show that the majority of TP53 mutations occur in the core domain. Labeled residues are mutation hot-spots. Exon-6/exon-7 truncating mutations are indicated in purple.

Figure 5 Tumor Distribution of TP53 truncating mutations. A) Histogram depicting the frequency of somatic TP53 truncating mutations in tumors. This plot demonstrates that exon-6/exon-7 truncating mutations are particularly abundant and occur at higher than expected frequencies. Exon-6/exon-7 truncating mutation hot-spots are indicated in orange.

Figure 6 Distribution of TP53 silent mutations. A) Histogram depicting the frequency of somatic TP53 silent mutations in tumors. This plot demonstrates that silent mutations occur at higher than expected frequencies at certain locations on the TP53 gene. Labeled residues are mutation hot-spots.
inhibitors are readily accessible and have been developed by pharmaceutical companies for other indications. Hence, these observations consummately imply that these SOF mutations could constitute a good precision medicine target. Notably, the frequency of SOF mutations in tumors for which the current therapeutic opportunities are very limited, such as pancreatic and ovarian cancers, occur at frequencies comparable to other actionable precision medicine targets like the EGFR oncogenic-mutation in non-small cell lung carcinomas [52].

Interestingly, as has also been suggested by the reports of Cartegni et al. [53], our analysis of the IARC TP53 database indicated that certain TP53 silent mutations also occur at higher than expected frequencies at specific hot-spots (Figure 6) [54]. In principle, this observation suggests that the presence of these mutations could confer an advantage of growth to tumors. Though these silent mutations do not impact the amino acid composition of p53, the resulting nucleotide changes could instead lead to alterations in splicing patterns and/or impinge upon p53 expression and activity. The effects of these TP53 silent mutations may mirror certain mutations in the MAPT gene that have been documented in cases of frontotemporal dementia with Parkinsonism [55].

More than 40 years have elapsed since the initial discovery of p53; nonetheless, progress continues to be made in the characterization of the most studied gene in cancer biology. As was presented, a growing body of evidence indicates the importance of novel alternative splicing isoforms, truncating/separation-of-function mutations, and possibly hot-spot silent mutations in the modulation of p53’s activity. These findings challenge pre-existing conceptions of p53 as a mere tumor suppressor protein and transcription factor. Importantly, these results also provide a basis for the development of novel therapeutic options for multiple tumor types that could ultimately bring us closer to a cure for cancer.

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Email: sordella@csht.edu

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