Mutant p53 Gain of Oncogenic Function: In Vivo Evidence, Mechanism of Action and Its Clinical Implications

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Abstract  
p53 is an indispensable tumor suppressor and exerts this function by transactivating numerous downstream target genes that play vital roles in controlling cell proliferation, apoptosis, senescence, and DNA repair. Mutations in the p53 gene, which are frequently seen in human tumors, impair its tumor suppressor function. Several of these tumor-derived p53 mutants can confer further aggressive oncogenic properties such as exacerbated malignant transformation and metastatic phenotype when overexpressed in p53-null cells. This oncogene-like behavior of mutant p53 is referred to as gain of function. The exact mechanism underlying gain-of-function phenotypes, however, remains enigmatic. Recently, we have generated mice with a point mutation (p53R172H) in their endogenous p53 loci as a model for the human Li-Fraumeni syndrome. The mutant p53R172H knock-in mice spontaneously develop tumors with high frequency of metastasis, contrary to that observed in mice with p53 deletion, indicating gain of function by the mutant p53R172H. In addition, our results show that other p53 family members, p63 and p73, are involved in the gain-of-function phenotypes. We further demonstrate that mutant p53R172H is inherently unstable and its stabilization is required for its gain-of-function phenotypes. This review focuses on recent reports regarding the potential molecular pathways for mutant p53 gain of oncogenic function and discusses its clinical implications.

1. p53, an essential tumor suppressor

Various environmental stresses induce mutations or abnormal expression of genes that play crucial roles in cellular homeostasis. Cancer, a life threatening disease with uncontrollable cell growth, can result from such alterations of genes that regulate cell proliferation, apoptosis, DNA repair, and senescence. The tumor suppressor p53 is one of the key guardians for these events. p53 functions as a transcription factor via tetramer formation, regulating numerous downstream target genes such as p21\textsuperscript{Waf1/Cip1} for cell cycle arrest, Bax and Puma for apoptosis, and p53R2 and Gadd45 for DNA repair.

Under conditions of homeostasis, wild-type p53 is very unstable with a half-life of less than 20 minutes, mainly due to degradation by its E3 ubiquitin ligase, MDM2. However, genotoxic stresses and subsequent post-translational modifications of p53 such as phosphorylation and acetylation prevent it from the MDM2-mediated degradation. Mice lacking Mdm2 are early embryonic lethal. This lethality is completely rescued by concomitant deletion of p53, indicating that Mdm2-null lethality is p53-dependent.
and Mdm2 is crucial for regulating p53 activity in vivo\(^5\).

The importance of the p53 pathway in tumorigenesis is emphasized by the clinical observations that p53 is the most frequently mutated tumor suppressor with more than 50% of human tumors having p53 mutations\(^7\)~\(^9\). Mutations in the p53 gene are also observed in over 70% of patients with Li-Fraumeni syndrome (LFS), a human familial cancer-prone disease that is characterized by early onset of various types of sarcomas and carcinomas\(^10\)~\(^11\). Studies involving p53\(^{-/-}\) and heterozygous (p53\(^{+/}\)) mice demonstrate spontaneous tumor development at a very high frequency\(^12\)~\(^13\). Additionally, overexpression of MDM2 is found in 30~40% of human sarcomas through gene amplification and other as yet unknown mechanisms\(^5\)~\(^14\). Transgenic mice with Mdm2 overexpression also spontaneously develop tumors\(^15\)~\(^16\). Collectively, these studies indicate that the regulation of p53 activity is critical for cellular homeostasis and tumor suppression; high level of p53 leads to cell death or cell cycle arrest, while its insufficiency results in tumor development.

### 2. Oncogenic function of mutant p53

p53 was first identified in 1979 as a cellular protein that complexes with the SV40 large T protein\(^17\)~\(^18\). Several initial studies suggested that p53 was an oncogene, since overexpression of p53 enhanced cellular transformation or tumorigenicity\(^19\)~\(^23\). Even in studies using L12 cells that lack endogenous p53, reconstitution of p53 conferred these cells with a fully transformed phenotype\(^24\). Subsequent studies, however, revealed that the p53 gene used for these studies contained missense mutations. Moreover, overexpression of p53 cDNA derived from normal cells rather suppressed cellular transformation induced by oncogenic ras\(^25\)~\(^26\). Since then, years of research and accumulated studies have defined wild-type p53 as a bona fide tumor suppressor rather than an oncogene\(^27\).

Unlike other tumor suppressors, missense mutations of p53 are more frequent events compared to gene deletion in human cancers\(^28\). A majority of these mutations are found in the DNA binding domain, and at least partially result in loss of the wild-type p53 function as a transcription factor. However, in agreement with previous observations of p53 as an oncogene, some mutants induce more aggressive oncogenic phenotypes including malignant transformation, upregulation of drug resistance-related genes, genomic instability, and metastasis in p53\(^{-/-}\) cells\(^29\)~\(^31\). These oncogenic properties of several p53 missense mutants can not be explained simply by the loss of wild-type p53 and are referred to as “gain of function”. Clinically, expression of missense mutant p53 is usually high in tumors and is associated with poor disease prognosis\(^32\)~\(^39\). Further, patients with certain p53 missense mutations have worse prognosis than patients with deletion of p53\(^39\)~\(^39\).

Taken together, mutations in the p53 gene not only result in loss of the wild-type tumor suppressor function, but also frequently confer oncogenic gain-of-function properties. This can explain why mutations are selected for over deletions in tumors and why p53 was initially believed to function as an oncogene.

### 3. Mutant p53 gain of function in mouse models of Li-Fraumeni syndrome

A p53 missense mutation from arginine (R) to histidine (H) at codon 175 (p53\(^{R175H}\)) is a hotspot mutation in sporadic cancer and in the germline of LFS patients. The p53\(^{R175H}\) mutant not only completely loses function as a transcription factor but also has been shown to exert gain-of-function phenotypes\(^39\)~\(^45\). Recently, we and others\(^41\)~\(^42\) have generated p53 knock-in mice as a model of LFS by introducing an arginine (R) to histidine (H) missense mutation at codon 172 of the p53 gene (p53\(^{R172H}\), equivalent to the human p53\(^{R175H}\) mutation). Our group generated p53\(^{R172H}\) knock-in mice in C57BL/6 background, while
the other group backcrossed to 129S4/SvJae background. By comparing phenotypes of mice with p53 mutant or null alleles, p53+/R172H mice exhibit more aggressive and metastatic tumor phenotypes in both C57BL/6 and 129S4/SvJae backgrounds than p53+/− mice (Fig. 1A)41,42. Such metastatic phenotype in mice carrying p53R172H closely mimics human LFS. Further, on the 129S4/SvJae background, p53+/R172H mice exhibit increased numbers of carcinomas and B-cell lymphomas compared to p53+/− mice, which are not observed in the C57BL/6 background42. These results indicate that the in vivo gain-of-function phenotypes by mutant p53R172H are affected by the genetic background of the mice.

To address the mechanism of gain of function, both groups independently demonstrated that endogenous mutant p53 interacts with other p53 family members, p63 and p73, that play a central role in regulating cell proliferation and apoptosis41,42. Further, down-modulation of mutant p53R172H results in increase of the p21Waf1/Cip1 promoter activity by p63 and/or p73 as well as upregulation of the p21Waf1/Cip1 RNA expression. Importantly, down-modulation of p63 and p73 in p53−/− mouse embryonic fibroblasts (MEFs) increases oncogenic ras-induced cell transformation to the level seen in p53R172H/R172H MEFs (Fig. 1B)41. These data support the notion that inactivation of p63 and p73 by mutant p53 is involved in the gain-of-function phenotypes.

To demonstrate the functional interactions of p53 with p63 or p73 in vivo, Flores et al.43 generated

![Figure 1](image-url)
compound knockout mice of p53 and p63 or p73. Interestingly, p53+/p63−/− and p53+/p73−/− mice develop metastatic tumors at high frequencies, whereas very few tumors from p53−/− mice show metastasis. These in vivo results suggest that inactivating p63 or p73 in p53-null background promotes tumor metastasis, similar to that seen in p53−/−R172H mice. Taken together, these observations appear to support our hypothesis that p63 and p73 are involved in the gain-of-function phenotypes by mutant p53R172H. Nevertheless, the exact mechanism involved in the mutant p53 gain of function needs further investigation.

4. Molecular pathways for mutant p53 gain of function

Studies from several groups propose two potential mechanisms for the gain-of-function effects of mutant p53. One mechanism proposes that mutant p53 could interact with and modulate the activities of proteins including transcription factors regulating cell proliferation and survival as well as proteins related to DNA repair. A recent report indicates that two common p53 gain-of-function mutants (p53R248W and p53R273H) interact with Mre11 following DNA double-stranded break damage, which prevents recruitment of the Mre11–Rad50–Nbs1 (MRN) complex to DNA breaks, resulting in impaired ATM activation and induction of genomic instability. Further, these mutants along with p53G245S are shown to cause genomic instability by interacting with topoisomerase I (Topo I)149,150. Our findings showing the inhibition of transcription factors p63 and p73 by mutant p53R172H also supports this mechanism. Other transcription factors including Sp1, Ets-1 and NF-Y also interact with mutant p53. The interaction of Sp1 with mutant p53 (p53V143A and p53R273H) is shown to function cooperatively in transactivation of the HIV LTR. Additionally, several p53 mutants (p53V143A, p53R175H, p53R248W, p53R273H, and p53G248W) cooperate with Ets-1 for upregulation of MDR-1 expression. A recent report demonstrates that p53R175H and p53R273H mutants interact with NF-Y on the promoter regions of NF-Y target genes including cyclin A, cyclin B2, cdk1, and cdc25c that regulate cell cycle progression. Interestingly, following exposure of cells with genotoxic reagent adriamycin, mutant p53 recruits the transcription coactivator p300 to the NF-Y mutant p53 complex in place of histone deacetylase 1 (HDAC1), which results in aberrant cell cycle regulation.

The alternative mechanism proposes that mutant p53 directly binds to unidentified DNA sequences and alters RNA expression of genes that modulate tumor phenotypes and progression. Genes in this group include MSP/MST−1, CD95, inhibitor of differentiation 2 (ID2), and EGR1. Interactions of mutant p53 with promoter regions of genes such as MSP/MST−1 (p53R175H and p53R273H), CD95 (p53R175H, p53R248W, and p53R273H), and ID2 (p53R248W and p53R273H) cause repression of these gene expressions, whereas interaction of p53R175H with the EGR1 promoter region upregulates EGR1 expression that contributes to enhanced transformed properties and resistance to apoptosis.

Thus, interactions of mutant p53 with both protein and DNA partners alter protein functions and RNA expression profiles in cells. It is also possible that partners of mutant p53 and the consequent phenotypes might change depending on the cellular context including different cell- or tissue-types and the presence of different stresses.

5. Accumulation of mutant p53 induces its gain-of-function phenotypes

Although compound knockout mice of p53 family members show a similar metastatic phenotype to mutant p53R172H knock-in mice, they are not identical in the kinetics of tumorigenesis: p53+/+ p63−/− and p53+/+ p73−/− mice succumb to tumors earlier than p53−/− mice, whereas tumor onset of p53−/−R172H mice is similar to p53+/+ mice in spite of the presence of metastasis. We did not have an exact answer for this difference, but a characterization of tumors from p53 mutant mice provided a possible explanation.
Immunohistochemistry for p53 in tumors from p53+/R172H or p53R172H/R172H mice revealed 70–80% of the primary tumors to be positively stained for p53, whereas almost all normal tissues showed negative p53 staining (Fig. 1C)62). These results indicate that mutant p53 is inherently unstable and its accumulation requires additional signals14. Importantly, more tumors at metastatic lesions (7 out of 8, 87.5%) showed positive p53 staining62, suggesting a possible link between accumulation of mutant p53 and metastasis. These results led us to hypothesize that accumulation of mutant p53 is required for its gain-of-function phenotypes. This hypothesis might explain why p53+/R172H mice show a similar tumor onset to p53+/- mice, since accumulation of mutant p53 in tumors may occur at later stages of tumor development. To test this hypothesis, we asked if γ-irradiation or genetic alterations could stabilize mutant p53 similarly to wild-type p53 and if gain-of-function phenotypes could be uncovered when mutant p53 was accumulated. Upon irradiation, mutant p53R172H was accumulated in both thymocytes and splenocytes (Fig. 2A, B), suggesting that mutant p53R172H is stabilized similarly to wild-type p5362. To examine whether loss of Mdm2 facilitates the accumulation of mutant p53R172H, we generated mutant mice in an Mdm2-null background (p53R172H/R172H Mdm2-/-). As expected, the level of mutant p53R172H was clearly elevated in all normal organs except for the liver of p53R172H/R172H Mdm2-/- mice in early age, suggesting a tissue specificity of mutant p53 accumulation. Interestingly, irradiation did not further enhance the accumulation of mutant p53R172H in thymocytes and splenocytes (Fig. 2A, B), suggesting that Mdm2 is the major molecule for the degradation of mutant p53R172H in these cell types. To address the critical question whether mice with accumulated mutant p53R172H show gain-of-function phenotypes, we compared tumor development between p53R172H/R172H and p53R172H/R172H Mdm2-/- mice. p53R172H/R172H Mdm2-/- mice developed tumors significantly earlier than p53R172H/R172H mice (Fig. 2C). Given that tumor onset and spectra are almost identical among p53+/+, p53+/- Mdm2-/- and p53R172H/R172H mice in our previous studies41,63, earlier onset of tumor development in p53R172H/R172H Mdm2-/- mice is due to stabilized mutant p53R172H. Importantly, 17% of tumors from p53R172H/R172H Mdm2-/- mice showed metastasis.

![Fig. 2](image-url)
while no metastatic tumors were found in p53R172H/R172H mice. We also examined how the loss of p16INK4a, which frequently occurs in human cancer, affected mutant p53 accumulation by generating p53R172H/R172H/p16INK4a–/– mice, since recent studies indicate that loss of p16INK4a indirectly activates wild-type p53 via the RB/E2F pathway (Fig. 3)64,65. Several normal tissues from p53R172H/R172H/p16INK4a–/– mice, but not as many as p53R172H/R172H Mdm2–/–, showed accumulation of p53R172H, suggesting tissue specific context contributes to the accumulation of mutant p53R172H. Importantly, p53R172H/R172H/p16INK4a–/– mice developed tumors much earlier than p53R172H/R172H mice (Fig. 2D), and 33% of sarcomas from p53R172H/R172H/p16INK4a–/– mice developed metastatic tumors62. Thus, mutant p53R172H is inherently unstable, and its stabilization is a prerequisite for its gain-of-function phenotypes. These studies reveal that mutant p53R172H is accumulated similarly to wild-type p53 but with dramatically different consequences.

6. Mechanism of action

Based on the findings described above, we propose a model for the mechanism of action for mutant p53 gain of function (Fig. 3). In the absence of any genotoxic stresses, oncogenic stresses, or genetic alterations, mutant p53 is unstable, mainly due to the presence of MDM2. Upon varieties of stresses, mutant p53 gets accumulated and interacts with various proteins or associates with promoter regions of genes that play roles in tumor development and progression. Therefore, cells expressing high levels of mutant p53 display gain-of-function phenotypes, which are not induced simply by loss of wild-type p53.

In many studies, phenotypes of gain of function by mutant p53 have been demonstrated together with

![Fig. 3](Mechanism of action of oncogenic mutant p53 (Mutp53). In the absence of genotoxic stresses, mutant p53 is unstable mainly due to MDM2 and therefore no obvious gain-of-function phenotypes are observed. In the presence of genotoxic stresses, oncogenic stresses, or genetic alterations such as loss of 16INK4a and MDM2, mutant p53 is accumulated similarly to wild-type p53. Accumulated mutant p53 displays gain-of-function phenotypes via interactions with proteins (e.g. p63, p73, Mre11, DNA topoisomerase I, NF-Y, Ets-1, and Sp1) or promoter regions of genes (e.g. MSP/MST-1, EGR1, CD95, and ID2) that affect tumor phenotypes.)
easily detectable levels of mutant p53 using cells in tissue culture. Also in our study, mutant p53 is easily detected in MEFs, whereas it is undetectable in most tissues of p53R172H mice. These results suggest that tissue culture manipulations induce some stresses to the cells, which increases levels of mutant p53, allowing investigators to identify gain-of-function phenotypes.

Further studies using various mutant p53 knock-in mice in combination with other genetically engineered mice will provide clearer answers for questions, such as “are all mutant p53 inherently unstable?” “are other p53 mutants accumulated by genetic changes or genotoxic stresses similarly to p53R172H?” “is MDM2 the only critical negative regulator?” “do all p53 mutants show similar gain-of-function phenotypes in vivo?” and “does every mutant share the mechanism of gain of function?”

7. Clinical implications

Our studies indicate that stabilizing inherently unstable mutant p53 induces its gain-of-function phenotypes including tumor metastasis. Approximately 50% of all human tumors have wild-type p53, some of which express high levels of MDM2. It is of great clinical interest to reactivate wild-type p53 in these tumors by releasing wild-type p53 from MDM2. Use of Nutlin and other reagents that inhibit the p53–MDM2 interaction is a promising therapy for these tumors. However, in the event that tumors contain mutant p53, preventing the mutant p53–MDM2 interaction could induce gain-of-function phenotypes, possibly promoting tumor malignancy and metastasis. Other clinical treatments including chemotherapy or radiation therapy can also stabilize mutant p53 via its post-translational modifications such as phosphorylation. Although some p53 mutants may not be accumulated by these approaches, it would be important to determine the mutation status of p53 in each tumor prior to treatment.

Immunohistochemistry is obviously not a good screening method for mutant p53, since p53 staining is negative in about 30% of tumors carrying mutant p53. Sequencing the entire coding region of the p53 gene would be a better approach. Further, it may be critical to target mutant p53 function during treatment of tumors carrying mutant p53. Several studies suggest down-modulating mutant p53 slows down tumor growth or malignancy. Therefore, reagents that can down-modulate mutant p53 as well as PRIMA and P53R3, compounds that can alter conformation of mutant p53 and restore wild-type p53 function, will greatly improve therapy for cancers with mutant p53.

8. Conclusion

Increasing evidence indicates that several hotspot p53 mutants have oncogenic phenotypes. At least one missense mutant p53R172H is proven inherently unstable in most of normal mouse tissues using LFS mouse models. However, p53R172H can be accumulated by various genotoxic stresses similarly to wild-type p53, leading to induction of gain-of-function phenotypes. Although it is not yet determined whether all p53 mutants are inherently unstable and which mutants are accumulated following genotoxic stresses similarly to wild-type p53, our study emphasizes the importance of characterizing mutation status of the p53 gene in each tumor prior to treatment, especially the therapeutic strategies involved in release of mutant p53 from MDM2.

Molecular targeted therapy is getting an ever-increasing attention in both basic and clinical research. Pertinent basic studies based on clinical trial of Iressa (Gefitinib), a drug that targets EGFR for lung cancer, found that mutations in the EGFR gene affect clinical response to this drug. Therefore, taking advantage of rapidly evolving technologies that enable to sequence the entire genome using small-sized samples from tumors, information about mutations in genes involved in tumor development such as EGFR and p53 can be incorporated into future cancer treatment. In order to move towards the concept of
personalized medicine, a common dream of cancer researchers and medical doctors, we will need to clarify several issues raised in this review and utilize our advanced knowledge about mutant p53 gain of oncogenic function.

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References


(References with numbers in bold are listed as important ones for readers.)
腫瘍抑制遺伝子産物 p53 の機能獲得型変異：
その表現型, メカニズム, 臨床的意義

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環境ストレスは細胞の恒常性に重要な役割を果たす遺伝子の突然変異を誘発する。中でも癌遺伝子や腫瘍抑制遺伝子に生じる変異は発癌の発端になりうる。腫瘍抑制遺伝子の一つである Tp53 遺伝子産物 p53 は、細胞増殖、細胞死、細胞老化、DNA 修復などに関与する遺伝子群を転写制御することによりその機能を果たしている。しかし、p53 の遺伝子上に生じた突然変異により、その転写活性化能は失われる。Tp53 遺伝子の突然変異がヒトの腫瘍の約 50％で認められることは、いかに p53 がヒトの腫瘍抑制に重要な働きをしているかを示唆している。腫瘍由来の変異 p53 の中に、p53 の欠損した培養細胞株に導入された際に、腫瘍の悪性度を高めるものが存在することが知られている。このような腫瘍遺伝子的な機能を有する p53 の変異を機能獲得型変異と呼ぶ。しかしながら、変異 p53 の機能獲得のメカニズムは未だ不明である。最近我々は、遺伝子組換え技術を用いて、ヒトの遺伝性腫瘍症候群の 1 つであり、70％以上の症例で p53 に異常の認められるリー・フラウメディ症候群のマウスモデルを作出した。これは、マウスの p53 の遺伝子内にアルギニンからヒスチジンへの点突然変異を導入したもので、Tp53 遺伝子欠損マウスとは異なるものである。Tp53 遺伝子欠損マウスは転移性腫瘍を発生しないことが知られているが、点突然変異を導入したマウスは高頻度に転移性腫瘍を発生する。このマウスに認められた腫瘍の転移は、p53 の機能獲得変異によって誘発されたものである。我々はまた、p53 のホモログである p63 と p73 が p53 の機能獲得変異に関与することを示した。さらに我々は、変異 p53 が元来不安定な蛋白質であるが、野生型の p53 と同様に様々なストレスにより安定化し、それが転移性腫瘍誘発することを示した。化学療法や放射線療法は現行の腫瘍の治療に頻繁に用いられているが、これらの治療法は、変異 p53 を安定化し、より悪性度の高い腫瘍を誘発する可能性がある。それゆえ、治療前に個々の腫瘍内での Tp53 の突然変異の有無を調べることは重要な意味があると思われる。近年、変異 p53 の高次構造を変化させ、野生型 p53 の機能を回復させる薬剤が報告されてきている。これらの薬剤は、現行の腫瘍の治療法に大きく貢献することが期待される。