Genes & Cancer
2(4) 466–474

DOI: 10.1177/1947601911408889
http://ganc.sagepub.com
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Mutations in the p53 Tumor Suppressor Gene: Important Milestones at the Various Steps of Tumorigenesis

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Abstract

Inactivation of the p53 tumor suppressor is a frequent event in tumorigenesis. In most cases, the p53 gene is mutated, giving rise to a stable mutant protein whose accumulation is regarded as a hallmark of cancer cells. Mutant p53 proteins not only lose their tumor suppressive activities but often gain additional oncogenic functions that endow cells with growth and survival advantages. Interestingly, mutations in the p53 gene were shown to occur at different phases of the multistep process of malignant transformation, thus contributing differentially to tumor initiation, promotion, aggressiveness, and metastasis. Here, the authors review the different studies on the involvement of p53 inactivation at various stages of tumorigenesis and highlight the specific contribution of p53 mutations at each phase of cancer progression.

Keywords: p53, mutant, cancer, tumorigenesis

Introduction: Mutations in p53 Are a Frequent Event in Cancer

The evolution of a normal cell toward a cancerous one is a complex process, accompanied by multiple steps of genetic and epigenetic alterations that confer selective advantages upon the altered cells. The alterations underlying tumorigenesis are considered to endow the evolving tumor with self-sufficiency of growth signals, insensitivity to antigrowth signals, evasion from programmed cell death, unlimited replicative potential, sustained angiogenesis, and finally, the ability to invade and metastasize.[1]

Despite massive research efforts and the very impressive progress made over the past several decades, full molecular understanding of cancer still remains a major challenge to the biomedical community. Back in 1947, Isaac Berenblum and Philippe Shubik discovered that chemical carcinogenesis consists of two stages: initiation and promotion.[2] More than 2 decades later, Knudson proposed a theory for tumor development known as the "Knudson two hit hypothesis."[3] This theory suggested a genetic model for retinoblastoma development, according to which the inherited *RB* gene mutation is described as the first hit and the tumor-restricted mutation as the second hit. This model was later expanded to include additional genetic aberrations, such as inactivation of a tumor suppressor and activation of an oncogene, as hits.

Despite the huge diversity in the genes implicated in tumorigenesis, the p53 transcription factor (encoded by the human gene *TP53*) stands out as a key tumor suppressor and a master regulator of various signaling pathways involved in this process.[4,5] The many roles of p53 as a tumor suppressor include the ability to induce cell cycle arrest, DNA repair, senescence, and apoptosis, to name only a few.[6] Indeed, *TP53* mutations were reported to occur in almost every type of cancer at rates varying between 10% (e.g., in hematopoietic malignancies[7]) and close to 100% (e.g., in high-grade serous carcinoma of the ovary[8]). For further information, see the IARC TP53 mutation database version R15, November 2010.[9] The importance of p53 as a cardinal player in protecting against cancer development is further emphasized by Li-Fraumeni syndrome (LFS), a rare type of cancer predisposition syndrome associated with germline *TP53* mutations.[10] Unlike the majority of tumor suppressor genes, such as *RB*, *APC*, or *BRCA1*, which are usually inactivated during cancer progression by deletions or truncating mutations, the *TP53* gene in human tumors is often found to undergo missense mutations, in which a single nucleotide is substituted by another.[11] Consequently, a full-length protein containing only a single amino acid substitution is produced. The cancer-associated *TP53* mutations are very diverse in their locations within the p53 coding sequence and their effects on the thermodynamic stability of the p53 protein. However, the vast majority of the mutations result in loss of p53's ability to bind DNA in a sequence-specific manner and activate transcription of canonical p53 target genes.[12]

TP53 mutations are distributed in all coding exons of the *TP53* gene, with a strong predominance in exons 4-9, which encode the DNA-binding domain of the protein. Of the mutations in this domain, about 30% fall within 6 "hotspot" residues (residues R175, G245, R248, R249,

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R273, and R282) and are frequent in almost all types of cancer.[13] The existence of these hotspot residues could be explained both by the susceptibility of particular codons to carcinogen-induced alterations and by positive selection of mutations that render the cell with growth and survival advantages.

In addition to the loss of function that a mutation in *TP53* may cause, many p53 mutants are able to actively promote tumor development by several other means. In a heterozygous situation, where both wild-type (WT) and mutant alleles exist, mutant p53 can antagonize WT p53 tumor suppressor functions in a dominant negative (DN) manner. The inactivation of the WT p53 by the mutant p53 in a DN mechanism stems from the fact that the transcriptional activity of WT p53 relies on the formation of tetramers, whose DNA binding function may be interfered by mutant p53.[14-16] However, such a heterozygous state is often transient, as *TP53* mutations are frequently followed by loss of heterozygosity (LOH) during cancer progression. LOH is often seen in the case of tumor suppressors where, at a particular locus heterozygous for a mutant and WT allele, the WT allele is either deleted or mutated. The LOH of the short arm of chromosome 17, where *TP53* is located,[17] implies a selective force driving the inactivation of the remaining WT allele, suggesting that the DN activity of mutant p53 is not sufficient to completely inactivate WT p53.

Furthermore, accumulating evidence supports the concept that many mutant p53 isoforms can exert additional oncogenic activity by a gain-of-function (GOF) mechanism. This term refers to the acquisition of oncogenic properties by the mutant protein, compared with the mere inactivation of the protein.[18,19] Both the DN and GOF effects may play a significant role in the positive selection of missense mutations in *TP53* during tumorigenesis.

When Is p53 Inactivated in Malignant Transformation?

The notion that mutations in *TP53* may occur at different stages along the process of malignant transformation raises the possibility that mutated p53 may contribute differently to various steps of this process. It is still an open question whether *TP53* mutations are involved in the initiation of malignant transformation or perhaps only at more advanced stages of cancer, leading to additional growth and aggressiveness advantages. It appears, however, that the timing of the mutation during tumorigenesis is extremely variable from one cancer to another. In this review, we revisit the questions of when p53 mutations occur during malignant transformation and how these mutations affect the cancerous phenotype at different stages of tumorigenesis.

Mutations in p53 in Late Stages of Cancer

Different studies have set out to model the tumorigenesis process and describe the order of events that take place throughout this process. In the early 1990s, the Vogelstein lab used colorectal cancer (CRC) as a model system to study the sequence of genetic alterations that take place during cancer development.[20] They analyzed the different stages of CRC, starting with healthy epithelium, progressing to early, intermediate, and late adenoma and eventually carcinoma and metastasis. This analysis led them to suggest a multistep progression model. This model argues that colorectal tumorigenesis has a clonal nature and that p53 is usually inactivated at the transition from late adenoma to carcinoma, rather than at an earlier stage. Nevertheless, the model highlights the fact that the order of the tumorigenic events may vary, whereas the combined accumulation of these changes is central. Already in that early study, several exceptions to the concept of the late timing of loss of the short arm of chromosome 17 (17p), which contains the *TP53* gene, were noted: These include loss of 17p as early as in small adenomas and 17p deletions followed by other chromosomal deletions.[20] Further evidence for the variations in the order of mutations came from additional studies. For example, while in the Vogelstein model, APC gene mutation and beta-catenin accumulation preceded the loss of chromosome 17p, another study suggested that in fact the aberrant accumulation of beta-catenin in tumors results from p53 inactivation.[21]

Another cancer progression model was suggested for pancreatic cancer. This model follows the progression from normal ductal epithelium to duct lesions and eventually to invasive ductal adenocarcinoma. This succession is once again associated with multiple genetic aberrations, including mutation in *k-ras*, over expression of *HER-2/neu*, and inactivation of *CDKN2A* (*p16*), *DPC4*, *BRCA2*, and *TP53*. In this model, the *TP53* gene was suggested to be lost late in the development of pancreatic neoplasia.[22]

In addition, examination of breast cancers reveals that *TP53* mutations are rare at T1 stage tumors, which are less than 2 cm in diameter and significantly more frequent in T3 stage tumors, which are greater than 5 cm.[23]

Further evidence for aberrations in p53 occurring late in tumorigenesis can be found in other cancer types such as hepatocellular carcinoma,[24-26] prostate cancer,[27,28] and bladder cancer.[29]

Despite these data, it seems that for the majority of cancer types, the determination of the *TP53* mutation timing is quite ambiguous and varies greatly between the different studies, cohorts examined, and methods of analysis.

Occurrence of p53 Mutations at Early Phases of the Tumorigenesis Process

Despite the ample evidence for the occurrence of *TP53* mutations and loss of WT alleles late in tumorigenesis, many other studies suggest otherwise. For example, mutant p53 has been found in ductal carcinoma in situ (DCIS), a human premalignant breast lesion.[30,31] In liver cancer, *TP53* is thought to be eliminated along with the RB and C/EBPα tumor suppressors following elevation of *gankyrin* at early stages of

tumorigenesis.[32] *TP53* was also reported to be lost or mutated early in astrocytoma tumorigenesis.[33,34]

In a noteworthy study, Barrett esophagus (BE) patients were biopsied prospectively over time. These patients have a premalignant condition predisposing them to esophageal adenocarcinoma, and it is recommended that they have endoscopic surveillance for early detection of cancer. The study demonstrated the evolution of the neoplastic cell lineages in BE and showed that inactivation of *TP53* by mutation and 17p LOH seems to be a relatively early event in neoplastic progression in BE. This is because it develops in diploid cells before aneuploidy, thus priming the cells for the formation of a dysplastic lesion.[35] Such prospective studies have a great advantage over the majority of studies, which are performed retrospectively and might only show the results of clonal selection of *TP53* mutations in higher grade cancers and overlook mutations that occur at an earlier stage.

Carcinogens and *TP53* Mutations

A mutation in *TP53* at an early stage of cancer progression can occur due to exposure to a carcinogen. This has been described extensively in the case of exposure to dietary aflatoxin B1. Contamination of food by this carcinogenic mycotoxin has been implicated as a risk factor for hepatocellular carcinoma (HCC) in regions of eastern Asia and sub-Saharan Africa, where HCC is a major cause of cancer death.[36] Several studies presented evidence that aflatoxin B1 induces a G:C to T:A transversion in codon 249 of the *TP53*.[37-39] Aflatoxin B1 was also found to be enzymatically activated in human hepatocytes and to bind to the third base of codon 249.[40,41] The expression of the 249 serine mutation was further shown to inhibit p53-dependent apoptosis and transcription and enhance liver cell growth *in vitro*.[42] This mutation was also found in nontumorous liver in correlation with aflatoxin B1 intake,[43] highlighting the notion that this mutation can occur early in the process of malignant transformation.

Another important example of carcinogen-induced mutations in the *TP53* gene was observed in lung cancer, where *TP53* was reported to be mutated in approximately 50% of non-small-cell lung cancer cases and more than 70% of small-cell lung cancers.[44] Tobacco smoke is the best-known and studied mutagen involved in lung carcinogenesis, and *TP53* mutational patterns differ between smokers and nonsmokers, with an excess of G to T transversions in smoking-associated cancer.[45,46] This transversion, which is uncommon in most cancers with the exception of HCC, is found to be associated with specific carcinogenic agents. The most prominent carcinogens in tobacco smoke, polycyclic aromatic hydrocarbons (PAHs) and especially benzo(a) pyrene, were found to be able to form DNA adducts in the coding region of the *TP53* gene. In addition, there is a correlation between the mutational hotspots of *TP53* in lung cancer (at codons 154, 157, 158, 245, 248, and 273) and the hotspots of adducts formation by PAHs in tobacco smoke. An additional examination of the lung cancer p53 hotspot mutants revealed that they are all defective for transactivation ability with less than 20% of WT activity on all p53-responsive elements.[47] It seems, therefore, that both a specific transversion associated with PAH adducts and loss of transactivation are the major driving forces in shaping the p53 mutation pattern in this type of cancer.

Exposure to sunlight and UV radiation has also been implicated in genetic transitions in *TP53* in the skin, leading to cancer development. About 50% of skin cancers exhibit *TP53* mutations that are characterized by specific C to T and CC to TT transitions, a signature of UVB-induced mutagenesis.[48,49] Among the 3 types of skin cancer—melanoma, basal cell carcinoma, and squamous cell carcinoma—only the arginine 248 mutation was found in common. However, each of these 3 types of skin cancer had specific characteristic hotspot mutations.[48] *TP53* mutations generally seem to be an early genetic change in the development of UV-induced skin cancers. This notion is supported by the finding of frequent *TP53* mutations in both normal-appearing sun-exposed skin and premalignant actinic keratosis lesions, which are considered precursors of squamous cell carcinoma. These findings suggest that *TP53* mutations drive the formation of precancerous lesions, which may convert into malignant full-blown squamous cell carcinomas. This might also be used as biomarkers for skin cancer susceptibility.[50] UV radiation was further shown to induce the growth and proliferation of the cells by stimulating the production of growth factors and cytokines, thus allowing clonal expansion of mutant p53-bearing cells that are resistant to apoptosis.[50]

Additional carcinogens are suspected to induce mutations in *TP53* in various tissues such as bladder, liver, and colon.[51] Overall, early mutations in the *TP53* gene caused by various carcinogens are a typical example of the possible involvement of mutant p53 in tumor initiation and in early stages of tumor development.

p53-Specific Antibodies and Free Circulating DNA as Biomarkers for Cancer and Early Detection

A humoral response against the p53 protein in animals was discovered more than 30 years ago.[52-55] Human anti-p53 antibodies were first described in the case of breast cancer patients.[56] Over the years, these antibodies were shown to be found frequently in human cancer patients and to be associated mainly with *TP53* missense mutations and accumulation of mutant protein in the tumor.[57,58] Such antibodies were found in the serum of patients with various types of cancer, including lung,[59,60] esophageal,[61,62] oral,[63] colorectal,[64] liver,[65] and more. p53-specific antibodies were also found in the

saliva of oral cancer patients.[66] There seems to be a correlation between p53 antibodies and poorly differentiated tumors, a trend already observed for *TP53* mutations.

Overall, these reports suggest that the humoral response is an early event and that p53 antibodies may be used as a marker for the early detection of cancer.[59,63] Nevertheless, the use of p53 antibodies for clinical purposes remains controversial.[67]

Several studies have examined the relationship between the status of p53 antibodies and tumor eradication during therapy. Zalcman *et al*.[68] showed that the titers of p53 antibodies in the serum decrease as lung cancer therapy progresses. Such correlation was also found in additional cancers such as esophageal carcinoma.[69] In another study, surgical resection of colorectal cancer in patients who had p53 antibodies prior to surgery eliminated the detection of such antibodies in the serum.[70] p53 antibodies were also detected prior to the manifestation of tumor relapse; specifically, Lubin *et al*.[57] found that p53 antibodies were detected 3 months prior to the detection of breast cancer relapse. This suggests that the continuous detection of anti-p53 antibodies in the serum is dependent on the accumulation of the p53 protein in the tumor cells. A notable disadvantage of assaying serum antibodies is its lack of sensitivity since only 30% of patients with p53 mutations develop p53 antibodies. Thus, this assay is not sufficient as a method to screen for cancers in healthy individuals.[58] On the other hand, analyzing p53 antibodies may provide a biomarker both for the efficiency of the cancer treatment and for possible relapse.

Another biomarker suggested for the early detection of tumors with mutated *TP53* is p53 DNA found in the sera and other body fluids. For example, DNA containing mutations in *TP53* can be found in the serum of colorectal and liver cancer patients,[71,72] stool of colorectal and pancreatic cancer patients,[73,74] urine of bladder cancer patients,[75,76] saliva of head and neck squamous cell carcinoma patients,[77] sputum of lung cancer patients,[78] and more. In many of the cases studied, the mutations found in the body fluid DNA were identical to the ones found in the primary tumor tissue of the patient, thus confirming their tumoral origin.

Overall, similar to the detection of p53 antibodies, the detection of tumor DNA in body fluid specimens was found to correlate with the tumor status and thus may be useful for early detection and therapy follow-up. The fact that both of these biomarker types can be found at early time points indicates that *TP53* mutations may occur very early in cancer progression, thus acting as one of the initial driving forces in this multistep process.

Mechanistic Views of How Mutant p53 Exerts Its Function

It is well established that p53 inactivation and mutant p53 expression can grant cells with additive growth and survival advantages, such as increased proliferation, evasion of apoptosis, and chemoresistance.[16,18] In an effort to further study the mechanisms that underlie the role of mutant p53 at the various steps of tumor progression, it was important to establish animal models that express mutant p53 in a controlled manner. Indeed, recent data obtained through the use of such *in vivo* models support the notion of GOF properties acquired by mutant p53, which drive cells toward migration, invasion, and metastasis. Earlier work revealed that although p53 knockout mice develop tumors at a high frequency,[79] they exhibit a rather low occurrence of metastasis or invasive growth.[80] In contrast to this, mice knocked in with p53 R270H or R172H, corresponding to the human hotspot mutants p53R273H and p53R175H, respectively, developed highly metastatic tumors.[81,82]

In addition, recent work demonstrates that mutant p53 can augment cell migration and invasion in *in vitro* assays.[83,84] Importantly, the data imply that although selection for oncogenic Ras and mutant p53 occurs in early neoplasms to promote growth and survival, they play an equally important role at late stages of tumor progression in empowering TGFβ-induced metastasis.[84]

Initiation of metastasis has many phenotypic similarities with epithelial-to-mesenchymal transition (EMT), including loss of cell-cell adhesion and an increase in cell motility. Although WT p53 was shown to inhibit EMT,[85,86] mutant p53 was found to promote EMT by facilitating the function of the key transcriptional regulators of this process, TWIST1 and SLUG.[85,87,88] An additional mechanism through which mutant p53 was shown to augment cell invasion is via the inhibition of TAp63, thus promoting TGFβ-induced metastasis and boosting integrin recycling pathways that promote invasiveness.[83,84] Another possible GOF effect of mutant p53 on tumor progression may be achieved through the positive regulation of angiogenesis, as tumors generated following mutant p53 knockdown tend to be less vascularized.[89]

Taken together, it appears that in certain cancers, p53 is mutated late in the tumorigenesis process or plays a significant role in those advanced stages, leading to a more aggressive and invasive tumor.

During the past years, we have established an *in vitro* model in which various steps in tumor progression can be dissected and associated with defined molecular events.[90] Using a genomic approach, we were able to identify distinct transcriptional signatures that can be associated with p53 inactivation or mutant p53 expression at either early or late stages of tumorigenesis. Specifically, p53 inactivation as a single event results in the induction of expression signatures associated with increased proliferation rate.[90-92] In contrast, inactivation of p53 in conjunction with oncogenic H-Ras expression activates the expression of a large set of chemokines and interleukins reported to promote angiogenesis, invasion, and metastasis.[93,94] These data

support the hypothesis that *TP53* mutations at early stages of tumorigenesis contribute mainly to uncontrolled proliferation, a feature of both benign and malignant tumors, whereas mutations at later stages synergize with additional oncogenic events to drive invasion and metastasis, the hallmark of malignant tumors.

Cancer-Predisposing p53 Mutations

Li-Fraumeni syndrome is a cancer predisposition syndrome first described in 1969.[95,96] Although most cancer predisposition syndromes are associated with specific tumor sites, LFS is characterized by a wide spectrum of tumor types occurring over a wide age range, starting at a young age. *TP53* germline mutations were found to be the underlying genetic defect in almost all LFS families.[10,97] Mutations in codons 175, 245, 248, 273, and 282 are the most common in both sporadic tumors and familial ones, although their ranking is different among the two types.[98] The distribution of cancers in carriers of a germline *TP53* mutation is very different from the expected cancer distribution in the general population.[98] This again highlights the diversity in the manifestation of p53 mutations, implying that the specific cancer type and time of the mutation occurrence may be interdependent.

Genotype-phenotype analysis of LFS families revealed that families carrying a germline missense mutation within the core DNA binding domain of the *TP53* gene show a more penetrant cancer phenotype than families with other *TP53* mutations or no mutation. Families with the former mutation type also exhibited a higher cancer incidence and an earlier age of diagnosis, compared with families carrying protein truncations or other inactivating mutations.[99] The enhanced oncogenic potential of missense *TP53* mutations is in common in both sporadic cancer occurring in somatic cells and LFS and again highlights the GOF and DN properties of these mutant isoforms.

Additional lessons can be learned when looking at mouse models. Although p53–/– mice develop tumors at a higher incidence than p53+/+ mice,[79] p53R172H/R172H did not exhibit GOF and showed similar survival curves as p53–/–.[81]

However, p53+/R172H mice, used as a model for LFS, developed tumors that were found to be more metastatic than tumors derived from p53+/– mice.[81] Moreover, p53+/R172H and p53+/R270H mice developed allele-specific tumor spectra, distinct from that of p53+/– mice.[81,100,101] On one hand, when examining mice homozygous to R172H, it cannot be concluded that the mutant form has an additional GOF over p53 knockout since p53R172H/R172H and p53–/– mice both exhibit the same tumor incidence. On the other hand, comparing the p53+/– with p53+/R172H or p53+/R270H mice suggests that the effect of the mutant protein is mostly in the later stages of cancer development affecting the aggressiveness of the tumors. This could be explained by a DN effect of the mutant form over the WT form, counteracting the WT p53 tumor-suppressive activity, or by a GOF mechanism of the mutant form, which promotes tumor metastasis. Nevertheless, the difference in tumor spectra indicates that the mutant form also has an influence on the initiation stages of the cancer in specific tissues.

Importantly, mutant p53 does not accumulate in normal tissue of neither mice knocked in for the mutant form nor LFS patients, yet it does accumulate in most tumors.[67,81,100-102] However, Mdm2–/– mice, which lack the E3 ubiquitin ligase that regulates WT p53,[103,104] knocked in with mutant p53, do accumulate mutant p53 in some normal tissue.[102] Together, these findings suggest that TP53 inactivating mutations alone are insufficient for the accumulation of mutant p53. This expression of mutant in Mdm2–/– mice significantly reduces survival, thus demonstrating that mutant p53 accumulation is important for its GOF potential. These results could also explain why LFS patients, who are heterozygous for mutant p53, do exhibit an earlier age of cancer onset. Unlike the mice grown in the lab, patients are more subjected to environment mutagens and carcinogens and therefore might accumulate mutant p53, leading to GOF of the mutant form, resulting in malignant transformation.

Overall, studies based on the analysis of cancer predisposition models, such as LFS patients and mutant p53 knock-in mice, provide a strong case for p53 mutations acting as the initial driving force on the road to tumorigenesis, leading to general genomic instability and additional genetic aberrations, as well as contributing to more aggressive tumor features at late stages of tumor progression.

Cancer and Stem Cells

Among the many theories trying to explain the process of tumor initiation and progression, the cancer stem cells theory has been receiving growing attention in the past several years. Cancer cells and stem cells are comparable in several aspects.[105] While stem cells are defined as having the capacity to self-renew and differentiate, cancer cells are alike as they obtain properties of proliferation and high plasticity. Also, aggressive poorly differentiated human tumors were shown to have an embryonic stem cell–like gene expression profile.[106] Moreover, advanced tumors often tend to be less differentiated. The similarity between cancer cells and stem cells has led to the speculation that tumors are derived and maintained by cancer stem cells.

The origin of cancer theory is divided into two main schools. The first argues that normal differentiated cells that are becoming malignant acquire certain stemness traits during the transformation process, thus granting them additional aggressive properties. The second school proposes that progenitor or stem cells, residing within the tissue, accumulate oncogenic properties and transform into cells, which initiate the tumor.[107,108]

Recent studies point to a new role for WT p53 in balancing both differentiation and de-differentiation in a cell type– and

cell fate–dependent manner.[109,110] The regulation of differentiation may be crucial in guarding the cell from aberrant maturation or reprogramming that might lead to cancer stem cell formation.

Mutant p53 was also shown to be involved in regulation of differentiation. More specifically, it was demonstrated that mutant p53 exerts a differentiation-blocking activity and affects proper cellular maturation. One such example was provided by studying the process of B cell maturation, where an early pre–B cell line that lacks p53 expression was reconstituted with either WT or mutant p53. Although the introduction of WT p53 resulted in the maturation of these cells and a lower incidence of tumors upon injection into mice, the mutant p53-producing cell lines were blocked for differentiation and gave rise to highly proliferative lethal tumors.[111] Similarly, although WT p53 enhances macrophage differentiation, various types of mutant p53 exert different effects on this differentiation pathway, either blocking or facilitating it.[112,113]

Wang and coworkers[114] have used mice engineered to have an internal deletion mutation in exons 5-6 of *TP53* specifically in neural stem and progenitor cells. They found that a majority of mice developed malignant brain tumors and that mutant p53 was detected in the tumor cells but not in normal cells. Mutant p53 protein was accumulated in a minority of proliferative neural stem cells 2 months after birth. They suggested that these cells start to proliferate, giving rise to transit-amplifying progenitor-like cells expressing an aberrant pattern of neural progenitor markers, which initiate glioma formation. Thus, the accumulation of a mutant form of p53 leads to improper maturation of neural stem cells and gliomagenesis. This and other studies indicate that aberrant differentiation of progenitor and stem cells, facilitated by mutant p53, may lead to malignant transformation.

We and others have also set out to examine the role of p53 in the reprogramming process, whereby somatic cells are de-differentiated into induced pluripotent stem (iPS) cells. p53 deficiency was found to facilitate the reprogramming process.[115-121] Our study further indicated a novel GOF activity for mutant p53, enhancing the reprogramming efficiency compared to p53 deficiency.[119] Importantly, when using only two reprogramming factors, the reprogrammed clones expressing mutant p53 lost their *in vivo* pluripotent capacity and generated malignant tumors, unlike the p53 knockout clones that retained pluripotency. Furthermore, the malignant tumors derived from mutant p53-expressing cells exhibited invasive growth and accumulated p53 in the undifferentiated regions of the tumor.[119] Overall, it appears that mutant p53 exhibits an additional GOF activity in the reprogramming process, whereby it allows genomically unstable cells to be reprogrammed into cells that are pluripotent *in vitro* but possess malignant tumor-forming properties. Altogether, the role of mutant p53 in the regulation of differentiation and de-differentiation highlights its potential role in the initiation of cancer. Reprogramming of cells, facilitated by mutant p53, gave rise to malignant tumor-initiating cells, thus potentially supporting the first theory for the origin of cancer, according to which normal cells undergo de-differentiation and form cancer-initiating cells.

Concluding Remarks

Inactivation of WT p53 is very diverse with regard to the type and location of the mutations, the types of cancers in which it is involved, the chronology of the mutation along the tumorigenesis process, and its contribution to the distinct steps of malignant progression. This diversity represents an infinite number of ways in which a p53 mutation might be selected during cancer progression, affected by many factors such as oncogenic stress, specific carcinogens, LOH, DN and GOF advantages, and much more. The findings summarized in this review also support the notion that the accumulation of genetic aberrations, rather than the chronology of their manifestation, determines tumor progression and aggressiveness. It therefore appears that knowing the status of p53 in the tumor cannot inform about the stage of the tumor. However, assessing p53 status may very well be beneficial in early detection and monitoring of tumor relapse, by detecting p53 antibodies and mutant p53 DNA. Furthermore, analysis of p53 status can serve as a tool in the prediction of effective therapeutic regimens, whereas p53 itself, particularly mutant p53, may represent targets for cancer therapy.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

Research in our laboratories is supported in part by a grant from the Flight Attendant Medical Research Institute (FAMRI) Center of Excellence (to VR and MO) and a grant from the Israel Science Foundation (ISF) (to VR). Prof. Varda Rotter is the incumbent of the Norman and Helen Asher Professorial Chair Cancer Research at the Weizmann Institute. Prof. Moshe Oren is the incumbent of the Andre Lwoff Professional Chair in Molecular Biology at the Weizmann Institute.

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