Pierre Hainaut · Magali Olivier
Klas G. Wiman *Editors*

p53 in the Clinics

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Foreword

Initially ignored by many researchers as an outsider molecule of minor importance in carcinogenesis, p53 took over 10 years to rise to the status of "guardian of the genome (1992)" and "molecule of the year" (1993). Yet, despite all the hype of the 1990s, the path from molecular discoveries to clinical application has been frustratingly long and winding for p53. It is only in recent years that breakthroughs have started to accumulate, opening doors to applications of the huge amount of knowledge accumulated on p53 into clinical practice. If p53 is not yet part of the everyday cancer management, with the rapid diversification of translational research on p53 detection and treatment, the road towards major clinical applications is now rapidly clearing up.

Discovered in 1979, the p53 protein has now reached the full maturity age of 33—which does not necessarily mean that it is ready to deliver healing miracles in public health care. In 2005, the first p53 book entitled "25 Years of p53 Research" highlighted the development of the p53 field and its profound impact on concepts in cancer research. The book emphasized the role of p53 as a regulator of cell cycle checkpoints and cell death in response to multiple cellular stresses. It also reviewed experimental models for studying p53, as well as knowledge on the prognostic and predictive value of tumor-associated p53 mutations and emerging therapeutic strategies for restoring p53 function in tumors. Since the publication of the first p53 book, the p53 field has developed at an ever-increasing pace and p53 researchers have uncovered novel and entirely unexpected functions and aspects of p53. There has been substantial progress on the significance of the genetic diversity of p53 and on mutations as biomarkers in molecular pathology. Research on p53-based therapy has intensified dramatically with the development of several drugs that target the p53 pathway. Thus, there have been considerable advances on the potential applications of p53 in the clinic.

The goal of this new p53 book is to capture these developments as the field moves into a next phase with strong emphasis on translational research. The present

book covers the most striking advances in p53 research and clinical applications that emerged over the last few years. These advances include:

1. Establishing a role for p53 in biological processes such as energy metabolism and fertility: Although p53 was previously thought to act mainly as a cellular trigger for cell cycle arrest or cell death, accumulating evidence has shown that p53 also regulates cell oxidative metabolism and the cellular response to nutrient deprivation. In addition, p53 has a role in fertility. These and other findings have led to the notion that p53's main task is to ensure the fidelity of a wide range of biological and physiological processes.
2. Uncovering the fundamental role of p53 in stem cell biology and in generation of induced pluripotent stem cells (iPS): Stem cells and regenerative medicine is a dynamic research area with great promises for clinical application. Studies have demonstrated that p53 controls the division and renewal of stem cells and that p53 influences the efficiency of induction of pluripotent stem cells from differentiated somatic cells. Therefore, p53 is a key protein to be considered in new strategies for implementing stem cell-based therapy in the clinic.
3. Understanding the interconnections between p53 family members, p63 and p73, and particularly their roles in cancer: Although not frequently mutated in cancer, both p63 and p73 are involved in cancer development through altered regulation and expression of isoforms that lack the N-terminal transactivation domain. Novel insights into p63 and p73 may lead to improved diagnosis and better prediction of prognosis and therapy response in cancer.
4. Pioneering translational research on the impact of p53 status on prognosis and clinical outcome in human cancer: It is now well established that p53 mutation is associated with poor prognosis in most cancers, particularly in breast cancer. Current challenges are (1) to better assess the function of p53 in tumors through new powerful techniques such as genome-wide analysis of the p53 pathway; (2) to determine in which context p53 status is clinically predictive of response to therapy and disease outcome; and (3) to develop diagnostic tests adapted to routine clinical practice.
5. Identifying germline *TP53* mutations not just as the basis of a rare form of familial cancer, but as one of the main forms of inherited predisposition to cancer: Germline *TP53* mutations are more common than previously considered (occurring in about 1 to 2,500 to 5,000 births) and are also extremely diverse in their impact on cancer risk. Furthermore, the discovery of a common founder mutation predisposing to cancer in Brazil provides a paradigm for searching for other population clusters in which germline *TP53* mutations might occur at a high frequency.
6. Developing p53-based methods for cancer therapy and bringing them to clinical trials: These approaches include long-awaited developments in gene therapy using viral vectors and their evaluation into several clinical contexts. Moreover, p53 researchers have identified small molecules that either activate wild-type p53 in wild-type p53-carrying tumors or restore wild-type function to mutant p53 in mutant p53-carrying tumors. In most cases these molecules are still at a preclinical

stage. However, at least two compounds, one that targets the p53-Mdm2 complex and induces wild-type p53 expression, and one that targets mutant p53 and restores its conformation and pro-apoptotic activity, have recently been tested in phase I clinical trials. Further studies should clarify whether these molecules have potent anticancer efficacy in larger cohorts of patients. Also, a number of novel molecules that reactivate wild-type or mutant p53 are under way.

This short survey makes it clear that p53 is ripe for clinical application. In fact, p53 is already in the clinic, although not yet included in routine analysis and still far from being used for targeted therapy. Exactly how could p53 be exploited for improved cancer diagnosis and treatment in oncology clinics around the world? This book shall address this and its related questions and hopefully provide information that will be useful and inspiring to both basic cancer researchers and clinicians. We strongly believe that the coming 10 years will be the decade of "p53 in the clinic," with major benefits for early detection, prognosis, allocation of personalized treatments, and, ultimately, cancer patient survival.

8 Nov 2011

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Chapter 1
TP53: Coordinator of the Processes That Underlie the Hallmarks of Cancer

Pierre Hainaut

1 Introduction

Over 10 years ago, Hanahan and Weinberg developed the concept of "Hallmarks of Cancer" as an organizing principle for rationalizing the molecular and mechanistic complexity of human cancer (Hanahan and Weinberg 2000). Initially, these Hallmarks included six major biological characteristics acquired during the process of multistep cancer development: sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis. During the last decade, technical advances in the comprehensive analysis of the patterns of genetic and epigenetic changes in cancers have led to a deeper understanding of these Hallmarks as well as to an extension of the concept to include new, emerging Hallmark processes such as reprogramming of energy metabolism and evading destruction by the immune system. Moreover, the biological changes that contribute to each Hallmark are promoted and accelerated by genome instability and inflammation, two conditions that can be seen as the cornerstones of cancer development (Hanahan and Weinberg 2011). Further, the development of Hallmark capabilities takes place within a complex network of interactions between neoplastic and normal cells, defining the concept of "tumor microenvironment." Not the least merit of the Hallmark concept is to provide a rationale for developing novel, molecular approaches to cancer therapy: in principle, each Hallmark has as molecular "signature" a number of molecular targets that can be specifically addressed using selective pharmacological agents, thus allowing for a radical departure from the classical approaches of cancer eradication by conventional cytotoxic therapies.

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The *TP53* tumor suppressor gene, encoding the p53 phosphoprotein, occupies a special place in the gallery of molecular factors that contribute to the Hallmarks of Cancer. This multifunction gene encodes a jewel of molecular integration, with much functional complexity packed up in a small amino acid sequence (393 residues) and simple molecular architecture (Hollstein and Hainaut 2010). One can only marvel at the simple beauty of this extremely efficient, almost minimalistic, use of molecular structures to provide multiple and adaptative functional diversity. These characteristics endow p53 with the capability to play critical roles in each of the ten Hallmarks of Cancer. As a result, the p53 signaling pathway can be seen as a molecular device integrating the Hallmark processes into a coherent biological program. A direct consequence of this role is that p53 function is at least partially disabled in most, if not all, cancers. To date, mutation in *TP53* remains the most frequent genetic change identified in human cancers, irrespective of organ site, histology, or natural history (Petitjean et al. 2007). In cancers that retain normal *TP53* alleles, the expression, stability, or activity of the p53 protein is often altered by multiple mechanisms. Such is the central role of p53 in carcinogenesis that it may be considered that alteration of p53 function is required, although not sufficient, for any form of cancer to develop. In the global world of molecular carcinogenesis, p53 occupies the main market place, marking the closest spot we currently know to the point where all cancer trade routes begin or end. This unique place among cancer genes makes it an attractive focal point for directing interventions aimed at controlling cancer—in particular in the clinics.

This introduction chapter briefly describes how p53 may operate as growth suppressor within the general framework of the ten Hallmarks of Cancer as recently redefined by Hanahan and Weinberg (2011). The aim of this outline is not to scoop through the daunting amount of literature accumulated on p53 since its discovery in 1979. Rather, my approach will be to use the beautiful conceptual framework of the Hallmarks as a magnifying lens to scrutinize aspects of p53 biology that might be amenable to clinical intervention.

2 The Long Road to p53 Function(s)

The p53 protein came under the limelight in a modest way, almost as a supporting role in the cast of the big show of molecular cancer research. It first emerged in 1979 as a protein of cellular origin which coprecipitated with the large T antigen of the Simian Virus SV40. At about the same time, it was identified as an antigen recognized by antisera raised against chemically transformed mouse cells (which have accumulated the p53 protein as the result of a chemically induced mutation in the *TP53* gene). In the late 1980s, the emergence of the concept of tumor-suppressor gene, largely based on studies of the retinoblastoma gene, paved the way for a paradigm shift that totally changed our understanding of p53 and of the molecular mechanisms of carcinogenesis (Hainaut and Wiman 2009; Lane and Levine 2010). First, it was shown that wild-type p53 cDNA clones could suppress transformation of

rodent cells in culture, whereas point mutant versions of p53 were transforming (Levine et al. 2004; Eliyahu et al. 1989). Second, from 1989, there has been a rapid accumulation of reports showing that missense mutations in *TP53* and/or loss of *TP53* alleles were common in colorectal, lung, breast, liver, and many other cancers. Third, inherited *TP53* mutations were found to be the underlying genetic defect of Li–Fraumeni syndrome (LFS), a familial syndrome of predisposition to multiple early cancers (Malkin et al. 1990).

Today, the vision of p53 biochemical roles and functions has considerably diversified. A selection of landmark p53 publications that trace the main advances in the field is given in Hainaut and Wiman (2009). During the early 1990s, convergent studies established that p53 was activated in response to DNA damage, justifying its 1992 nickname of "guardian of the genome," an expression that captures its capacity to arrest the proliferation of cells with genomes damaged by carcinogens by forcing them to undergo cell-cycle arrest or apoptosis (Lane 1992). At the turn of 2012, there are over 60,000 articles containing "p53" as keyword indexed in PubMed, demonstrating the extraordinary success and diversification of p53 research. In addition to its role in response to many forms of DNA damage, p53 has now gained recognition as a critical oncogene-induced barrier against progression of cancer beyond its early stages and as an important inducer of replicative senescence. In 2007, a study by Scott Lowe and collaborators provided one of the most compelling examples to date of p53 function as a critical tumor suppressor (Xue et al. 2007). To determine the consequences of reactivating the p53 pathway in tumors, these authors introduced into liver progenitor cells an inducible RNA interference system that conditionally regulates endogenous p53 expression. Their results show that switching off p53 function could activate the growth of large liver tumors and that even brief (24 h) reactivation of endogenous p53 could produce complete tumor regression. Furthermore, they found that the primary response to p53 reactivation was not apoptosis, but the induction of a cellular senescence program associated with differentiation, upregulation of inflammatory cytokines, and activation of innate immune response contributing to the clearance of tumor cells (Xue et al. 2007). Although there is no doubt that cancer involves complex combinations of genetic and epigenetic alterations in multiple genes, the extent to which loss of p53 function is required to sustain cancer growth suggests it has a huge potential as target for clinical cancer control (Martins et al. 2006).

3 An Evolutionary Perspective on p53

For over about 20 years, *TP53* has appeared to stand alone among oncogenes and tumor suppressor genes by not being a member of a multigene family. Until the late 1990s, it was even unclear whether a true equivalent to *TP53* was present in organisms other than vertebrates. This view has considerably evolved over the past 15 years with the discovery of two homologues of *TP53*, *TP63*, and *TP73* (Aylon and Oren 2011; Allocati et al. 2012). Although p53, p63, and p73 share structural

and sequence similarities, p63 and p73 are rarely mutated in tumors, in contrast with p53. Instead, the *TP63* locus is amplified in 20–30 % of squamous cell carcinomas of the head and neck, esophagus, or lung, and p73 is overexpressed in diverse types of cancers. While mice deficient for p53 function develop early multiple cancers, p63- or p73-deficient mice die tumor free from complex and diverse developmental and physiological defects (Melino et al. 2003). Although experimental, ectopic expression of p63 or p73 in cultured cells can activate roughly equivalent biological effects as p53, these two proteins play different roles than p53 in physiological conditions. Due to the presence of internal promoters and/or alternative splicing mechanisms, *TP63* and *TP73* encode two different classes of protein isoforms: those containing an N-terminal transactivation (TA) domain, similar to the one of p53, and those that lack this transactivation domain, commonly termed Delta-N isoforms. High expression of Delta-Np63, for example, is essential for the morphogenesis and maintenance of proliferative and renewal capabilities of many tissues, including the epidermis, squamous, respiratory or urothelial epithelia, as well as basal/myoepithelial cell compartments of salivary, lachrymal, mammary, and prostate mucosa. Moreover, Delta-Np63 is critical for the asymmetric division and proliferative potential of epidermal stem cells (Nekulova et al. 2011).

Sequences homologous to p53/p63/p73 have now been identified in organisms other than vertebrates, including, for example, *Drosophila*. Strikingly, the pattern of expression of all family members into two classes of isoforms, TA and Delta-N, appears to be highly conserved. The existence of Delta-N isoforms of p53, some of them extremely similar to Delta-Np63 or Delta-Np73 in their mechanisms of production, their architecture, and their biochemical activities, suggests that the ancestral function of the family might be related to stem cell maintenance, renewal, and proliferation (Marcel et al. 2011). According to this view, p53 might have evolved from a blueprint of genes essential for stem cell maintenance to specialize in functions that are not essential for development per se, such as sensing environmental changes in the cell ecosystem. Taking into account the role of p53 in response to oxidative stress and its capacity to control many aspects of oxidative metabolism (Hafsi and Hainaut 2011), it is interesting to speculate that *TP53* has evolved to adapt cells to hosting mitochondria, a supreme source of damaging radicals, as their main energy production system (Wang et al. 2012).

4 Implications of p53 in the "Hallmarks of Cancer"

Studies on genome-wide mutation patterns in cancer cells have confirmed that *TP53* is one of the most, if not the most, consistently altered gene in human cancer (Pfeifer and Hainaut 2011). Taking into account the multiplicity of mechanisms other than mutation that may inactivate p53 functions (such as enhanced protein degradation through increased expression of cellular or viral proteins that target p53 for destruction by the proteasome), it may be considered that p53 function is inactivated, or somehow made deficient, in the vast majority of cancers. The fact that this deficiency

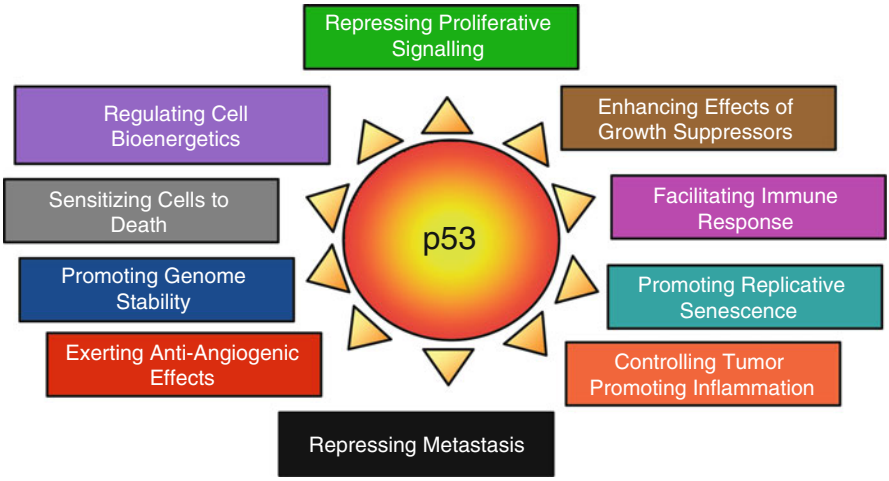

Fig. 1.1 A Copernican view of p53 in the "Hallmarks of Cancer." This model depicts p53 as the central point of a solar system in which each Hallmark process represents a zodiac sign. The Hallmark boxes are colored according to the original scheme given in "Hallmarks of Cancer: The next Generation" (Hanahan and Weinberg 2011)

may occur—or may show its effects—at any step in the carcinogenic process from precancer condition to highly aggressive, metastatic lesions, makes it very complex to address the prognostic or predictive significance of measurable p53 alterations. Furthermore, such a sweeping role for a single molecule in such a mechanistically complex, multifactorial disease raises the suspicion that p53 does not act as a front-line driver but as a kind of facilitator of carcinogenesis. In the following section, I briefly discuss some of the mechanisms by which p53 interferes in each of the ten Hallmarks of Cancer processes, supporting the view that loss of p53 function not only make cells permissive to the acquisition of Hallmark capabilities but also removes a critical brake that prevents the expression of Hallmark capabilities. In this model, p53 is not a specific component of any particular Hallmark process but rather a molecular device that organizes the Hallmark processes into a coherent biological program (Fig. 1.1).

4.1 Repressing Proliferative Signaling

Cancer cells have systematically acquired the capability to sustain proliferative signaling, and they can do so in dazzling number of ways. Although p53 is not on its own a component of proliferation signal transduction cascades, it influences and controls them by entertaining dialogues with regulators of growth and survival. Loss of p53 function thus enhances proliferation by at least three main mechanisms. First, p53 regulates the transcription of *PTEN* (phosphatase and tensin homolog),

a well-established tumor suppressor gene that is mutated, deleted, or epigenetically silenced in a variety of cancers (Yin and Shen 2008). Induction of *PTEN* induces apoptosis and controls cell growth by inhibiting the PI3K/AKT pathway. Second, downstream of the same pathway, p53 is inhibiting mTOR (mammalian target of rapamycin) (Huang et al. 2001). In metazoans, TOR is a central integrator that receives signals arising from growth factors, nutrients, and cellular energy metabolism. The mechanisms by which p53 regulates mTOR involves AMP kinase activation and requires TSC1/2 (tuberous sclerosis 1 and 2 complex), two factors that respond to energy deprivation (Matthew et al. 2009). Thus, cross-talks between mTOR and p53 interconnect growth factor signaling and sensing nutrient bioavailability with growth suppression. Third, when faced with excessive or untimely proliferative signals such as the activation of an oncogene, cells are capable of activating antiproliferative signals acting as a safeguard against excessive proliferation (Lomazzi et al. 2002). Excess proliferative signaling, as for example in response to oncogene activation, increases the expression of $p14^{ARF}$, the alternative reading frame product of the *INK4a/CDKN2a* locus, encoding p16, another canonical tumor suppressor gene and located on chromosome 9p22. In turn, $p14^{ARF}$ sequester Mdm2 and prevents it from binding and degrading p53, thus causing an increase in nuclear levels and suppressive activity of p53. $p14^{ARF}$ also exerts p53-independent function as tumor suppressor. In this context, loss of p53 helps cells to evade senescence and to maintain a high proliferative capacity. Deletion of *INK4a/CDKN2a* may represent a double blow, with ablation of both p16, a negative regulator of G1 phase of the cell cycle, and of $p14^{ARF}$, a tumor suppressor that links cell-cycle progression with p53-mediated growth suppression (Dominguez-Brauer et al. 2010).

4.2 Enhancing the Effects of Growth Suppressors

The main mechanisms by which loss of p53 functions helps cells to evade growth suppressor are by impairing the expression of *CDKN1/p21WAF1*, encoding the $p21^{WAF1}$ cyclin kinase inhibitor with multiple roles in controlling G1/S, G2, and M phases. This gene was the first one to be identified as a transcriptional target of p53 and is a central effector of the growth suppressive effect of p53 in response to intracellular stress signals such as DNA damage, levels of nucleotide pools, or availability of oxygen (el-Deiry et al. 1993; Sherr and Roberts 1999). However, *p21WAF1* has complex activities, both pro- and antioncogenic, and its genetic or epigenetic inactivation is not common in human cancer.

In cultured cells, one of the mechanisms that suppresses proliferation is cell-to-cell contacts (contact inhibition). This mechanism involves the reorganization of cell surface molecules and of their coupling with cell surface receptors, the extracellular matrix and the cytoskeleton. One of the main factors involved in these processes is Merlin, the product of the *NF2* (neurofibromatosis type 2) tumor suppressor gene. Merlin orchestrates contact inhibition by coupling cell surface adhesion molecules to receptor tyrosine kinases and by strengthening the adhesivity of cadherin-mediated

cell-to-cell attachments (Curto and McClatchey 2008). Merlin increases p53 stability and activity by inducing the degradation of Mdm2, thus leading to p53-dependent apoptosis in response to serum starvation or to DNA damage (Kim et al. 2004).

Yet another important role of p53 in regulating responses to growth suppressors is underlined by its connection with the tumor suppressor *LKB1*, which is genetically inactivated in patients with Peutz–Jeghers syndrome and somatically mutated in a variety of cancers. *LKB1* is responsible for the maintenance of epithelial cell polarity and can, for example, overrule the mitogenic effects of the *MYC* oncogene. In contrast, ablation of *LKB1* leads to disruption of epithelial structures and enhanced cell proliferation mediated at least in part by a decline of p53 and p16 growth suppressor pathways (Liang et al. 2010).

Perhaps one of the most elaborate antiproliferative pathways is the one controlled by TGF-beta. Far more than just an inhibitory pathway, TGF beta can induce profound re-programming of cell fate, as for example during the Epithelial to Mesenchymal Transition (EMT) (Wendt et al. 2009; Xu et al. 2009). The TGF-beta signaling pathway has long been considered as largely independent upon p53 but recent results have highlighted roles for p53, not only in the modulation of distal biological responses such as EMT but also in more proximal aspects of TGF-beta-mediated growth inhibition. For example, the *SnoN* proto-oncogene product negatively regulates TGF-beta signaling through its interactions with Smad complexes. The same factor also interacts with the promyelocytic leukemia (PML) protein and the accumulation of SnoN in PML nuclear bodies induces the stabilization and activation of p53, leading to premature senescence (Lamouille and Derynck 2009).

4.3 Suppressing Replicative Immortality Through Senescence

When maintained in culture, normal cells undergo a phase of expansion through division until they enter senescence, reaching a crisis during which cells die unless they acquire an unlimited replicative potential that confer them immortality in culture. The rheostat that determines how many division cycles a cell can achieve before reaching replicative senescence is a structure located at the end of chromosomes and composed of tandem repeats of six nucleotides, the telomeres. Each normal division cycle is accompanied by telomere erosion. In human cancer, telomere erosion is compensated by activation of telomerase, a specialized DNA polymerase that adds telomere repeats. This enzymatic activity is almost absent in normal, postmitotic cells but is expressed at high levels in 90 % of immortalized and transformed human cells (Shay and Wright 2001). The p53 protein exerts at least three complementary roles in the regulation of telomeres.

First, over-expression of wild-type p53 down-regulates the enzymatic activity of telomerase through transcriptional repression of the gene encoding its catalytic subunit, human telomerase reverse transcriptase (*hTERT*) (Kanaya et al. 2000). This repression appears to require p53 DNA binding activity and to involve $p21^{WAF1}$. Furthermore, it is attenuated by inactivation of the *RB* family or by dominant-negative

mutation of *E2F1*. Mutation of *TP53* eliminates this repression and may therefore contribute to high telomerase activity in cancer cells.

Second, telomere erosion activates a complex growth suppression program which is controlled by p53. Mice deficient for telomerase acquire dysfunctional telomeres and develop a dwarf phenotype associated with p53 activation of senescence or apoptosis in several tissues including skin. This dwarf phenotype is rescued by ablation of p53, which also restitutes skin renewal and wound healing responses (Flores and Blasco 2009). There is evidence that telomere erosion induces a form of DNA damage detected by p53, thus inducing a senescence response in stem/progenitor cells that controls their capacity to contribute to tissue growth and regeneration, thus affecting tissue and organismal fitness (Sahin et al. 2011). Furthermore, p53 deficiency enhances the efficiency of somatic cell reprogramming to a pluripotent state. Cells with mutant *TP53* have to undergo reprogramming much more efficiently than cells lacking *TP53*. Moreover, reprogrammed cells expressing mutant p53 tend to give rise to malignant tumors much more than cells lacking *TP53*, suggesting that mutant p53 exerts a gain-of-function effect on reprogramming (Sarig et al. 2010).

Third, p53 regulates cell-cycle progression in response to telomere uncapping. In normal cells, telomeres are protected by protein complexes that specifically bind to telomeric DNA. Uncapping occurs transiently in each G2 phase of the cell cycle, following DNA replication. Telomere caps are reassembled at the G2/M transition under the control of the p53/p21^{WAF1} DNA damage response pathway. Human or mouse cells lacking p53 or p21^{WAF1} progress into mitosis prematurely with uncapped telomeres which make chromosomes prone to religate into deleterious end-to-end fusions (Thanasoula et al. 2010).

4.4 Sensitizing Cells to Death

Induction of apoptosis is one of the best characterized biological effects of p53 (Oren 2003). It has long been recognized that wild-type p53 directly controls the transcription of several classes of genes involved at different levels in apoptosis signaling. The apoptotic circuitry is commonly described as composed of two complementary programs: the extrinsic and the intrinsic apoptotic programs (Moffitt et al. 2010). The extrinsic program involves extracellular signaling through death-mediating receptors. The p53 protein directly activates the transcription of genes encoding death receptors such as *APO1/FAS/CD95* and *KILLER/DR5* (Wu et al. 2000). It also controls the expression of genes regulating the bioavailability of survival factors, such as *IGF-BP3*, which encodes a protein that binds and neutralizes IGF1 and IGF2 (Buckbinder et al. 1995; Butt et al. 1999). The intrinsic program integrates multiples signals of intracellular origin, with the mitochondria as centerpiece. The role of p53 in this program involves both transcription-dependent and independent aspects. Among the products of genes transcriptionally regulated by p53, several interact with the antiapoptotic factor Bcl2 and/or its family members. These p53 target genes include *PUMA*, *NOXA*, and *BAX*, encoding proteins with BH3 motifs that interact

with Bcl2 and disrupt the integrity of the outer mitochondrial membrane, leading to the release of apoptosis signaling factors among which the most important is cytochrome C (Shamas-Din et al. 2011). Nontranscriptional functions may involve indirect effects such as those responsible for down-regulating aspects of base-excision repair (BER), therefore sensitizing cells to death in response to DNA damage (Gatz and Wiesmuller 2006).

Separately from its functions as a transcription factor, p53 is also capable to be translocated to the mitochondria (Vaseva et al. 2009), presumably by interacting with Tid1, a mitochondrial DnaJ-like chaperone. In breast cancer cells, depletion of Tid1 by short hairpin RNA (shRNA) leads to absence of p53 accumulation at mitochondria and resistance to apoptosis under hypoxic or genotoxic stress (Ahn et al. 2010; Trinh et al. 2010).

Distinct from its role in sensitizing cells to apoptosis, p53 is also active in the control of autophagy, an intracellular catabolic process during which cells degrade their own components through the lysosomal machinery. Autophagy is a double-edged sword. It is a stress-induced cell death mechanism, in particular in response to nutrient deprivation. It is also a metabolic switch through which cells reallocate energetic resources to different biological processes (Eng and Abraham 2011). Deletion, depletion, or inhibition of p53 induces autophagy in human, mouse, and nematode cells. In the latter organism, deletion of the p53 homologue cep-1 increases life span through an increase in baseline autophagy. The effects of p53 on induction of autophagy may be, at least in part, a consequence of the cross-talks between p53 and the PTEN/PI3K/AKT/mTOR pathway, which plays a critical role in nutrient sensing (Galluzzi et al. 2010; Feng 2010). Other mechanisms by which p53 induces autophagy have emerged with the discovery that p53 directly transactivates *SESN2*, encoding Sestrin2, a controverted cysteine sulfinic acid reductase thought to be involved in responses to stress, to accumulate with age, and to inhibit mTOR activation (Maiuri et al. 2009). On the other hand, p53 also transactivates *DRAM* (damage-regulated autophagy modulator), which encodes a protein with six putative transmembrane domains that co-localizes with cathepsin D in the lysosome. Silencing *DRAM* inhibits both p53-mediated autophagy and apoptosis, suggesting these two outcomes are mechanistically linked (Crighton et al. 2006, 2007).

4.5 Regulating Cell Bioenergetics

One of the most spectacular developments in our understanding of p53 functions is the discovery that it plays a fundamental role in cell bioenergetics. Loss of p53 activity may be pivotal for the Warburg effect, or aerobic glycolysis, the long-known capacity of many cancer cells to generate energy mainly through glycolysis even in the presence of nonlimiting levels of oxygen (Gottlieb and Vousden 2010; Hafsi and Hainaut 2011). Overall, p53 appears to down-regulate glucose usage and to favor oxidative metabolism. These effects include down-regulation of glucose transporters at the plasma membrane through direct inhibition of GLUT-1 and GLUT-4

(Schwartzenberg-Bar-Yoseph et al. 2004) and indirect inhibition of GLUT-3 by a mechanism involving NF-KappaB (Kawauchi et al. 2008). Further downstream, p53 regulates the synthesis of two rate limiting enzymes in the glycolytic pathway. It induces *TIGAR* (*TP53*-induced glycolysis and apoptosis regulator), an enzyme with fructose bi-phosphatase activities that counteracts the activity of 6-phosphofructo-1 kinase (Bensaad et al. 2006). In contrast, it down-regulates *PGM* (phosphoglycerate mutase), which converts 3-phosphoglycerate into 2-phosphoglycerate during the late ATP-generating steps of glycolysis (Kondoh et al. 2005). In parallel with its capacity to limit glycolysis, p53 inhibits the pentose phosphate pathway (PPP). The p53 protein binds to glucose-6-phosphate dehydrogenase (G6PD), the first and rate-limiting enzyme of the PPP, and prevents the formation of the active dimer. Tumor-associated mutant p53 mutants lack this G6PD-inhibitory activity (Jiang et al. 2011). p53 also promotes oxidative phosphorylation through at least two mechanisms. First, it activates *AIF*, encoding apoptosis inducing factor, a bifunctional protein with oxido-reductase function contributing to the assembly and function of complex I of the respiratory chain (Vahsen et al. 2004). Second, p53 transactivates *SCO2* (encoding synthesis of cytochrome c oxidase 2), a copper-dependent chaperone protein that is required for the assembly of complex IV of the respiratory chain (Wanka et al. 2011).

Another critical effect of p53 on energy metabolism and antioxidant defenses is mediated by its transactivation of *GLS2*, encoding a mitochondrial glutaminase catalyzing the hydrolysis of glutamine to glutamate (Hu et al. 2010). GLS2 regulates energy metabolism by increasing the production of glutamate and alpha-ketoglutarate, which in turn results in citric acid cycle, mitochondrial respiration, and ATP generation. GLS2 also contributes to antioxidant defenses by increasing reduced glutathione (GSH) which in turn protects cells from oxidative stress. Overall, loss of p53 through mutation in cancer cells may thus promote a profound switch in metabolism, facilitating the capacity of cancer cells to generate energy and to maintain high pools of reduced nucleotides for DNA synthesis under oxygen-poor conditions.

4.6 Promoting Genetic and Genomic Stability

Following the recognition that it regulates cell-cycle progression and apoptosis, p53 has been rapidly identified as a main contender in maintaining genetic stability, a property that earned it its nickname of "guardian of the genome." Indeed, by arresting cell cycle and controlling DNA replication in cells with genetic damage, p53 protects cells from acquiring further DNA defects that may initiate cancer or drive its progression.

Nucleotide excision repair (NER), the mechanism which removes the vast majority of UV-induced DNA damage, is regulated by the ATR/p53 checkpoint via modulation of XPA (*Xeroderma pigmentosum* group A protein) nuclear import in a cell cycle-dependent manner in G1 and S phases. XPA, one of eight factors implicated

in XP disorders, is an indispensable factor for both transcription-coupled NER (TC-NER) and global genome NER (GG-NER) which play roles in verifying DNA damage, stabilizing repair intermediates, and recruiting other NER factors to the damage site. The DNA damage-induced response of XPA nuclear import is significantly slower in p53-deficient cells than in p53-proficient cells, consistent with the notion that loss of p53 function may decrease the elimination of cyclopentane pyrimidine dimers, a typical UV photoproduct, thus enhancing UV-induced mutagenesis (Li et al. 2011a, b).

In contrast to NER which recognizes bulky distortions of the DNA helix, BER is a mechanism that removes smaller base lesions that could otherwise cause mispairing or strand breaks during DNA replication. BER is initiated by DNA glycosylases that remove specific damaged bases. The resulting apurinic/apyriminidic (AP) sites are cleaved by the AP endonuclease APE1/Ref1 and the resulting single strands are processed by either short-patch (where a single nucleotide is replaced) or long-patch BER. The p53 protein contributes to BER at several levels, both in a positive and in a negative fashion (Gatz and Wiesmuller 2006; Offer et al. 2001). First, p53 regulates the transcription of *hOGG1*, encoding the glycosylase responsible for the excision of 7,8-dihydro-8-oxoguanine (8-oxoG; the main product of guanine oxidation by reactive oxygen species) (Chatterjee et al. 2006). Second, it regulates the transcription of *MGMT* (O^6-methyl-guanine-DNA-methyl-transferase), the primary enzyme that repairs alkyl adducts at the O^6 position of guanine. The effect of p53 on *MGMT* expression is somehow biphasic: while wild-type p53 is required for enhanced *MGMT* expression, transfection and over-expression of p53 represses *MGMT* (Grombacher et al. 1998). Third, p53 induces *PPM1D* encoding protein phosphatase 1D, a serine/threonine phosphatase that interacts with Ung2 (uracil DNA glycosylase 2) and may suppress BER probably via dephosphorylation of Ung2 (Lu et al. 2004a, b). Fourth, p53 activates the expression of genes such as *GADD45* (growth arrest and DNA damage inducible gene) which product interferes with BER by controlling nuclear localization and activity of APE1/Ref1. Finally, p53 interacts with APE1/Ref1 and may modulate its stability and activity (Seemann and Hainaut 2005). The general message emerging from these observations is that p53 may enhance BER as part of its genome-stabilizing, tumor-suppressive activities. However, after activation of p53 in response to stress, high levels of p53 may repress BER to return DNA repair to the deactivated state and therefore participate to maintenance of basal DNA damage capacity. In conditions where p53 becomes activated to very high levels, such as for example in response to severe DNA damage, p53 may stop BER to accelerate apoptosis of cells that have accumulated DNA damage beyond repair capacity.

The involvement of p53 in mismatch repair (MMR) is demonstrated by the identification of *MSH2* (MutS homologue 2), MLH1 (MutL homologue 1), and *PMS2* (postmeiotic segregation 2) as transcriptional p53 target genes [reviewed in Gatz and Wiesmuller (2006)]. MMR is the main mechanism that corrects DNA following DNA polymerase errors, removing mismatches in heteroduplex DNA during recombination and preventing homologous recombination (HR). *TP53* mutation increases HR by several orders of magnitude and experiments with *TP53* hotspot mutants in cultured cells have revealed severe HR inhibitory defects.

Despite its wide implication in DNA repair, there is only limited evidence that impaired p53 function leads to an increased mutation load (mutator phenotype). Spontaneous tumors in a mouse model of LFS show only modest increase in mutation loads (Hill et al. 2006). In humans, there is a highly significant increase in copy number variations among carriers of germline *TP53* mutations with a familial cancer history (Shlien et al. 2008). However, *TP53* mutation carriers do not appear to be hyper-sensitive to mutagens although radiosensitivity has been demonstrated in cultured cells and observed in some LFS patients who received radiation-based treatments [discussed in Palmero et al. (2010)].

4.7 Controlling Tumor Promoting Inflammation

Inflammation is a complex biological response involving vascular structures, immune system and tissue microenvironment aimed at removing injurious stimuli and at initiating healing processes. This is achieved by mobilizing a wide set of bioactive molecules, including reactive oxygen and nitrogen species acting both as chemical cleansers and as signaling molecules, cytokines, growth factors, and survival factors that promote cell regeneration, and angiogenic and extracellular matrix remodeling factors that restructure the microenvironment towards wound healing. Excess or persistent inflammation, however, turns these protective responses into a cancer-promoting process. Many aspects of chronic inflammation involve p53 in a direct or indirect way (Kamp et al. 2011). First, p53 is activated in response to DNA damage inflicted by reactive oxygen and nitrogen species generated during inflammation, thus mediating growth suppressive responses that characterize several chronic inflammatory lesions [reviewed in Hafsi and Hainaut (2011)]. Second, p53 regulates the expression of genes encoding enzymes involved in the production or detoxification of reactive species. These genes include up-regulation of *COX2* (cyclooxygenase 2) (de Moraes et al. 2007), down-regulation of *SOD2* (mitochondrial superoxide dismutase) (Forrester et al. 1996), and activation of *GPX1* (glutathione peroxidase) (Tan et al. 1999) and of *ALDH4A1* (aldehyde dehydrogenase 4 A1) (Yoon et al. 2004). Third, p53 represses the expression of *NOS2* (inducible nitric oxide synthase) (Ambs et al. 1998; Forrester et al. 1996), and *TP53* mutation is correlated with increased expression of *NOS2* and enhanced damage by nitrogen species in cancers developing in a context of chronic inflammation (Ambs et al. 1999; Vaninetti et al. 2008). Fourth, p53 entertains a complex network of functional cross-talks with NF-KappaB, the main transcription factor involved in the regulation of inflammatory responses. For example, p53 and NF-kappaB cooperate in the regulation of *COX2*, which provides a survival mechanism in chronically inflamed tissues (de Moraes et al. 2007; Ryan et al. 2000). Recently, p53 has been found to attenuate lipopolysaccharide (LPS)-induced NF-kappaB activation and acute lung injury in mice (Liu et al. 2009). Moreover, loss of p53 function impairs the repression of NF-kappaB target gene transcription by glucocorticoids and severely impairs glucocorticoid rescue of death in a mouse model of LPS shock (Liu et al. 2009).

The functional cross-talk between p53 and NF-kappaB may play an important role in regulating organismal senescence and life span: it seems that the efficiency of p53 signaling declines during aging whereas that of NF-kappaB is clearly enhanced.

Overall, the involvement of p53 in inflammatory responses may be such that p53 operates as a natural brake against excess DNA damage and abnormal cell proliferation in the context of chronic inflammation. Loss of p53 function may therefore upset the balance of inflammatory signals towards tumor-promoting effects, with excessive cell proliferation, escape from senescence and loss of control over genetic and genomic stability in the face of high levels of damaging reactive oxygen species.

4.8 Repressing Metastasis

The invasion–metastasis cascade is taking place according to a sequence of discrete steps encompassing local invasion, intravasation by cancer cells and transit into blood and lymphatic vessels, extravasation into the parenchyma of distant tissues, formation of micrometastases and their growth into macroscopic tumors, this last step being termed "colonization." Many aspects of this multistep process are directly or indirectly influenced by p53 and mutation of *TP53* is commonly observed in metastatic cancers. In recent years, several studies have uncovered an important role of p53 in regulating epithelial to mesenchymal transition (EMT), a developmental morphogenesis and wound healing program that underlies the capacity of epithelial cancer cells to acquire metastatic properties. EMT is activated by multiple pathways (beta-Catenin, Notch, EGFR, Ras/MAPK) and is controlled by transcription factors such as Snail/Slug and ZEB family members (Brabletz 2012; Jing et al. 2011; Xu et al. 2009). EMT confers a combined stemness and motility phenotype to cancer cells, defining a subpopulation of migrating cancer stem cells as potential source of metastasis. The miR-200 family of microRNAs consists of two gene clusters (miR-200a, b and 429, Chr1; miR-141 and 200c, Chr12) acting as orchestrators of EMT through their capacity to inhibit EMT activators such as ZEB factors, thereby inducing a reverse process to EMT, the mesenchymal to epithelial transition (MET). MiR-200 family members not only counteract EMT but also suppress stem cells factors, such as Bmi1. The p53 protein regulates both EMT and EMT-associated stem cell properties through transcriptional activation of the miR-200c promoter (Chang et al. 2011). Loss of p53 correlates with a decrease in the level of miR-200c, associated with an increase in EMT and stemness markers, and development of a high tumor grade in a cohort of breast tumors.

Another important connection between p53, EMT, and metastasis involves the Twist1 protein, a regulator of embryogenesis. Twist1 has been shown to induce EMT and is over-expressed in a large fraction of human cancers (Ansieau et al. 2008). A common cancer-derived mutant p53 protein, p.R175H, up-regulates Twist1 expression in several cancer cell lines, suggesting that increased Twist1 might occur in cancer cells as the consequence of gain-of-function mutations in *TP53* (Kogan-Sakin et al. 2011).

4.9 Exerting Antiangiogenic Effects

Tumor progression almost systematically involves the activation of an "angiogenic switch" causing the normally quiescent vasculature to resume sprouting new vessels that supply cancer cells in nutrients, growth factors, and oxygen. Angiogenesis is induced by hypoxic conditions and regulated by the hypoxia-inducible factor 1 (HIF-1), an hetero-dimer transcription factor which regulates the expression of vascular endothelial cell growth factor (VEGF) degradation and activity. p53 interacts with the alpha subunit of HIF1 (HIF-1alpha) and regulates its degradation. Mutant p53, in contrast, promotes the stabilization of HIF-1aplha and the expression of VEGF, thus enhancing angiogenesis (Khromova et al. 2009; Choi et al. 2003).

Antiangiogenic drugs targeting the VEGF pathway have shown clinical effects in delaying or slowing down metastatic disease in some patients. Antiangiogenic therapy appears to be sensitive to p53 status in tumors, implicating a role for p53 in the regulation of angiogenesis. One of the mechanisms of this regulation may involve the transcriptional activation by p53 of *alpha(II)PH*, the gene encoding alpha(II) collagen prolyl-4-hydroxylase. This activation leads to the extracellular release of antiangiogenic fragments of collagen type 4 and 18. As a result, conditioned medium of cells expressing high p53 levels inhibits the growth of human endothelial cells (Teodoro et al. 2006). Another collagen-derived antiangiogenic factor, Arresten, is processed from alpha 1 collagen 4 (encoded by *COL4A1*). Recent studies show that p53 induces the expression of *COL4A1* and the release of fragments of extracellular matrix containing Arresten. The p53 protein directly activates the transcription of *COL4A1* and also increases the metalloproteinase-mediated release of Arresten, thus controlling the production of an important antiangiogenic factor (Assadian et al. 2012).

4.10 Facilitating Innate and Adaptative Immune Response

Recently, the panel of genes regulated by p53 has extended beyond the already large set of cell cycle, DNA repair, and apoptosis regulators to include the control of human Toll-like receptor (*TLR*) gene expression (Menendez et al. 2011). The *TLR* gene family mediates innate immunity to a wide variety of pathogenic agents through recognition of conserved pathogen-associated molecular motifs. The role of p53 in regulating *TLR* expression appears to be extremely diverse and complex. The promoters of most *TLR* family members contain both canonical and noncanonical p53 response elements, thus defining a potentially rich repertoire of dose- and context-dependent stress responses to p53 activation (Menendez et al. 2011). Furthermore, several lines of evidence suggest that p53 regulates antiviral immunity. Mice lacking p53 have impaired and delayed antiviral response to influenza A virus (IAV), caused by disruption of both innate and adaptive immunity (Munoz-Fontela et al. 2011). In cells infected by hepatitis C virus (HCV), the HCV core protein induces the

p53-dependent expression of *TAP1* (encoding transporter associated with antigen processing 1) and consecutive major histocompatibility complex (MHC) class I up-regulation, leading to a significant down-regulation of the cytotoxic activity of natural killer (NK) cells against HCV-infected cells and facilitating the establishment of a chronic infection (Herzer et al. 2003).

Some other p53 activities might also directly contribute to help cancer cells to escape immune destruction. Many early transformed cells express ligands for the natural killer cell immunoreceptor NKG2D, which sensitizes them to recognition and elimination by cytotoxic lymphocytes (Textor et al. 2011). The expression of the NKG2D ligand ULBP2 is controlled by the tumor-suppressive microRNAs (miRNA) miR-34a and miR-34c, which are themselves regulated by p53. Therefore, p53 represses ULBP2 through miR-34a and miR-34c and loss of this function may contribute to eliminate an innate barrier against tumor development (Heinemann et al. 2012).

5 p53 and the Coordination of Hallmark Processes

This rapid and selective survey of the multiple roles of p53 in processes underlying the Hallmarks of Cancer show that these processes are deeply interconnected, representing nodes in a web-like network rather than independent functional units. Its implication in each of these nodes supports that p53 represents a unifying factor between Hallmark processes. While acquisition of one or several Hallmark capabilities drives cells towards cancer, loss of p53 disintegrates the coherence between the Hallmark processes and removes molecular obstacles to malignancy. Thus, in cancer cells having lost p53 function, DNA damage can accumulate without inducing cell growth arrest, accompanied by a metabolic switch that confers energetic autonomy, bypassing replicative senescence, activating embryonic developmental programs such as EMT, promoting tumor inflammation and avoiding immune destruction. In cells with normal p53 competence, transient activation of any of these capabilities will be compensated by p53-dependent down-regulation of other capabilities, thus maintaining homeostasis.

5.1 A Pivotal Connection: From Telomeres to Stem Cell Maintenance and Aging

This pivotal role of p53 in coordinating Hallmark processes is illustrated in the sequence of molecular events that links telomere dysfunction, DNA damage, p53 activation, metabolic adaptation, progressive tissue functional decline and atrophy, and aging (Fig. 1.2) (Sahin et al. 2011; Sahin and Depinho 2010). Mice with constitutively dysfunctional telomeres accumulate relatively low levels of persistent DNA damage in a variety of organs including hematopoietic stem cells, heart, and liver.

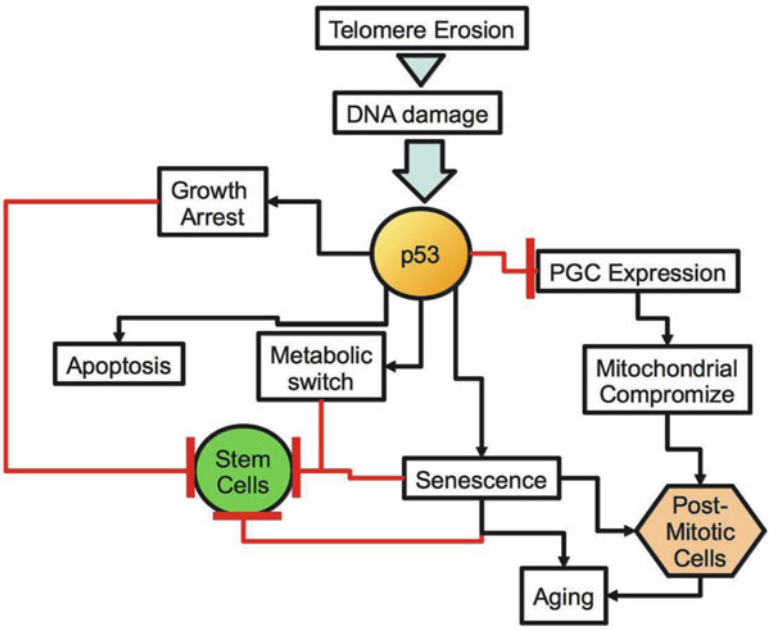

Fig. 1.2 Roles of p53 in the molecular and cellular circuitry linking telomere erosion, stem cell maintenance, senescence, energy metabolism, and aging. This model shows how DNA damage resulting from telomere attrition or dysfunction induces p53, which itself activates coordinated growth arrest and metabolic responses that reduce stem and progenitor cell capabilities and compromises the metabolic fitness of postmitotic cells, leading to organismal aging. Based on Sahin et al. (2011), adapted and augmented

This DNA damage results in p53 activation. In such conditions, p53 induces the repression of *PGC-1alpha* and *PGC-1beta*, encoding peroxisome proliferator-activated receptor gamma coactivators 1 alpha and 1 beta (Sahin et al. 2011). These two factors are master regulators of mitochondrial physiology and metabolism. As a result, p53 interconnects telomere dysfunction with impaired mitochondrial biogenesis and function, decreased gluconeogenesis, cardiomyopathy, and increased production of reactive oxygen species. These conditions favor replicative senescence and the depletion of pools of stem/progenitor cells responsible for tissue renewal and, ultimately, organismal aging. Ablation of *TP53* substantially restores *PGC* expression and the functionality of the subsequent redox and energy metabolic network, restituting mitochondrial respiration, cardiac function, and gluconeogenesis in liver cells. These observations demonstrate that p53 couples telomere attrition and subsequent DNA damage with metabolic effects, senescence, and aging. However, even in the face of normal telomere function, lack of p53 function has, on its own, major effects on mitochondrial determinants of fitness and exercise capacity. p53 interacts with TFAM (mitochondrial transcription factor A), a nuclear-encoded gene important for mitochondrial DNA (mtDNA) transcription and maintenance

(Park et al. 2009). p53-deficient mice show decreased mtDNA content compared to their p53-competent counterparts, with reduction in aerobic versus glycolytic skeletal muscle capacity. Such molecular mechanisms may provide a basis for the inverse correlation between cancer incidence and cardiorespiratory fitness observed in population studies (Wang et al. 2012).

5.2 p53 and Orchestration of the Microenvironment

Hallmark processes form a highly coherent biological program that operates within cells and also between cells, providing an orchestration score for choral interactions between multiple cell types and the extracellular matrix. Acquisition of Hallmark capabilities distorts these interactions and modifies the microenvironment in a way conducive for cancer development. Maintaining the integrity of the microenvironment is therefore a potentially critical tumor suppressive mechanism. Indeed, it is still debated whether cancer cells become metastatic because of their acquired endogenous Hallmark capabilities or because of permissive conditions occurring in the microenvironment. Understanding the tumor suppressive role of the microenvironment may therefore hold the key to tumor reversion and control of metastasis, the holy grail of cancer therapeutic research.

How p53 participates in the control and orchestration of microenvironment signaling is starting to emerge. An interesting lead in this respect is the identification of *TSAP-6* (tumor suppression associated pathway 6) as a target gene of p53 (Amzallag et al. 2004). The product of *TSAP-6* is a 5–6 transmembrane domain protein which interacts with and enhances the secretion of TCTP (translationally controlled tumor protein, also called histamine-releasing factor). This secreted protein participates in inflammatory responses by promoting the release of histamine. TSAP-6 promotes the incorporation of secreted factors into exosomes (Lespagnol et al. 2008). Exosomes are a family of secreted microvesicles which have recently been shown to play a role in remodeling the tumor microenvironment and in priming the metastatic niche, perhaps carrying addressing molecules that target specific signals to particular cells or structures in the microenvironment (Peinado et al. 2011). These observations identify *TSAP-6* as a "multipass" membrane protein with a general role in the regulation of vesicular trafficking, secretion and, beyond, signaling within the microenvironment.

A recent study by Carol Prives and collaborators has provided a very impressive demonstration of the role of p53 as orchestrator of cell–cell and cell–matrix interactions (Freed-Pastor et al. 2012). These authors have used three-dimensional culture models of epithelial breast cells in which nonmalignant cells form spheroids reminiscent of normal breast acinar structures, whereas cancer cells with mutant *TP53* form disrupted structures. In this model, depletion of mutant p53 protein phenotypically reverts breast cancer cells to a more acinar-like morphology. Furthermore, through genome-wide expression analysis, they identified the mevalonate pathway

as significantly up-regulated by mutant p53. This pathway (also known as the isoprenoid pathway) serves as the basis for the biosynthesis of molecules used in isoprenoid synthesis, protein anchoring into membranes, and also initiates the steroid biosynthesis pathways. Mutant p53 associates with sterol gene promoters at least partly via SREBP transcription factors and mutation in *TP53* correlates with highly expressed sterol biosynthesis genes in human breast tumors. These observations provide a mechanism linking p53, essential aspects of breast cell metabolism, cell membrane dynamics, and cell-to-cell as well as cell–matrix communications in forming organized, microenvironmental structures.

6 Conclusions: Controlling Hallmark Capabilities Through p53-Based Therapy

One of the main attractions of the Hallmarks of Cancer is that this paradigm provides a rationale and a framework for developing and combining molecular targeted therapeutic interventions. Each Hallmark capability may be approached as a potential drug target. Furthermore, combining drugs that target different Hallmark capabilities is expected to result into increased therapeutic efficacy. So far, most of the promising small-drug based therapies have been designed to target particular factor within defined signaling pathways. Typical examples are tyrosine kinase inhibitors specific for mutant, activated EGFR or monoclonal antibodies to V-EGFR that inhibit angiogenesis signaling. Redundancy between pathways and the existence of multiple bypass mechanisms across the web of Hallmarks are major obstacles for the long-term efficacy of such forms of treatment.

The identification of p53 as a coordinator of Hallmark processes points to a new paradigm for developing combined therapeutic approaches. When associated with drugs addressed at neutralizing or correcting specific Hallmark capabilities, drugs targeting the p53 pathway may restitute critical interconnections between biological processes which, together, cooperate towards effective tumor suppression. The molecular methods to target the p53 pathways are many and diverse: they range from gene-based therapies to small drugs that bind to mutant p53 and restore its activities, or to specific drugs that enhance wild-type p53 function through neutralization of its degradation. The most promising options, however, may stem from recent studies in which p53 has lead to re-discover, under a new light, long-known aspects of biochemistry and bioenergetics. Given the wide possibilities for identifying simple molecules that interferes with metabolic intermediates in these p53-dependent biochemical pathways, the potential for new and effective drugs has never been brighter. Developing and clinically testing such new drugs will require mastering the measurement of a wide range of p53 targets as biomarkers of effects.

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