Published in final edited form as:

Biochim Biophys Acta. 2016 April ; 1865(2): 220–227. doi:10.1016/j.bbcan.2016.03.001.

Long story short: p53 mediates innate immunity

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Abstract

The story of p53 and how we came to understand it is punctuated by fundamental insights into the essence of cancer. In the decades since its discovery, p53 has been shown to be centrally involved in most, if not all, of the cellular processes that maintain tissue homeostasis. Extensive functional analyses of p53 and its tumor-associated mutants have illuminated many of the common defects shared by most cancer cells. As the central character in a tale that continues to unfold, p53 has become increasingly familiar and yet remains surprisingly inscrutable. New relationships periodically come to light, and surprising, novel activities continue to emerge, thereby revealing new dimensions and aspects of its function. What lies at the very core of this complex protagonist? What is its prime motivation? As every avid reader knows, the elements of character are profoundly shaped by adversity – originating from within and without. And so it is with p53. This review will briefly recap the coordinated responses of p53 to viral infection, and outline a hypothetical model that would explain how an abundance of seemingly unrelated phenotypic attributes may in the end reflect a singular function. All stories eventually draw to a conclusion. This epic tale may eventually leave us with the realization that p53, most simply described, is a protein that evolved to mediate immune surveillance.

Keywords

P53; adenovirus; DNA damage; interferon

1. Introduction

“I ask you to judge me by the enemies I have made.”

— Franklin D. Roosevelt

“We do not merely destroy our enemies; we change them.”

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Conflict of interest

The authors declare no conflicts of interest related to this manuscript.

— George Orwell, 1984

The preface to the story of p53 is set in the early twentieth century. Pioneering virologists had recognized that the study of relatively simple viruses could illuminate the dark recesses of the tumor cell. In the decades that followed, the molecular dissection of retroviruses would reveal the fundamental genetic nature of cancer and the role of dominant genes (1).

Because of their highly specialized life cycles, the RNA viruses exhibit the phenotypes of the genes they carry but provide limited information about the normal processes of the cells that they infect. In contrast, viruses that use DNA as their genetic material uniquely rely on the enzymatic capabilities of the host cell, and their own ability to evade the cellular responses to exogenous DNA. The small DNA tumor viruses thus provided the first experimentally tractable model systems for the study of human RNA transcription, DNA replication and the DNA damage response (DDR¹). These compact viruses, like all viruses, evolved to harbor a minimal set of genes and a spare repertoire of functional proteins. The interactions between the polyomaviridae, adenoviridae and papillomaviridae, and their host cells have revealed much about the fundamental mechanisms of cell proliferation and their dysfunction in cancer (2, 3). After more than 35 years of study, studies of the DNA tumor viruses continue to provide new insights to the common characteristics of cancer cells and the functions of p53.

Since its discovery as a cell-encoded factor bound by viral antigens, p53 has the distinction of being among the most intensively investigated of all human proteins. Early studies revealed that p53 is highly responsive to cellular stressors that threaten the integrity of the genome (4). DNA strand breaks, crosslinks, oxidative DNA damage, DNA adducts and aberrant DNA structures such as unsheltered telomeres and DNA replication intermediates all cause p53 to become post-translationally modified and stabilized (5–8). Upon its activation in the nucleus, p53 binds to numerous promoters and thereby mediates a functionally expansive transcriptional program (9, 10). Ionizing radiation - a frequently-employed stimulus of p53 activity in both the laboratory and the clinic - creates DNA strand breaks and triggers the p53-dependent induction of about 500 genes and the repression of a smaller number of genes within 4–8 hrs of treatment (9, 11). The transcriptional profile of cells with activated p53 varies significantly by cell type and tissue context. Several of the most common and robustly-induced targets of p53 are directly involved in the shift away from a proliferative state towards one favoring growth arrest and cell death. The functions of many other reported target genes - particularly those that appear to be regulated by p53 only in specific cells or unique circumstances - are poorly understood, and their relevance to tumorigenesis is therefore uncertain (12).

The experimental stabilization or overexpression of p53 leads to profound anti-proliferative phenotypes in cells from tumors and normal tissues alike. The discovery of these basic anti-proliferative processes and their links to the downstream transcriptional targets of p53 fed a developing narrative that was fairly easy to grasp. In essence, cancer results from the

¹Abbreviations: DDR, DNA damage response; IFN, interferon; ISG, interferon-stimulated gene; ISRE, interferonstimulated response element; SASP, senescence-associated secretory phenotype; PML, promyelocytic leukemia protein; SUMO, small ubiquitin-like modifier; CMV, cytomegalovirus; ER, endoplasmic reticulum; SERCA, sarco/ER Ca²⁺-ATPase

expansion of clones that harbor genetic alterations. Intuitively then, a sensor of DNA damage that impairs cell growth would be an ideal mechanism to suppress tumorigenesis. The subsequent discovery of multiple roles for p53 in replicative senescence and DNA repair provided further evidence that p53 is a central node in a broad signaling network that integrates cell birth, cell death and genomic stability (13).

Upon a brief recounting, the story of p53 can seem deceptively straightforward. But many observations do not seem to readily fit into a simple narrative. Recently identified transcriptional targets and non-transcriptional signaling pathways have linked p53 to basic processes such as metabolism, secretion and cell-to-cell communication (14–16). It now appears that the full range of p53 functions transcends its well-described role as a stress-activated regulator of transcription that operates in a solely cell-autonomous manner. As one attempts to integrate all of these attributes into a coherent model, it becomes difficult to imagine how one gene and one protein (multiple isoforms and family members notwithstanding) can have so many effects on cells and tissues. Recent developments may at long last foreshadow a unifying conclusion, in which all the loose ends and subplots in the larger narrative can be tied together.

2. Lessons from adenovirus

The first chapter of the p53 story begins in 1979, when a 53 kDa cellular protein was co-precipitated by antisera that recognized the large T antigen protein expressed by the polyomavirus SV40 (17). Unrelated to the RNA-based retroviridae, SV40 and the other DNA tumor viruses were known to be associated with tumorigenesis (18). To varying extents, these viruses can experimentally transform normal cells with a finite proliferative capacity into immortal cells that phenotypically resemble those explanted from naturally-occurring tumors. It was evident that the genes encoded by the DNA tumor viruses can cause cellular changes that are highly relevant to tumorigenesis, and therefore the potential importance of this novel cellular protein-viral protein interaction was immediately appreciated. Within a short period of time, p53 was found to associate with proteins expressed by other DNA tumor viruses, including the 55 kDa protein expressed by the adenovirus gene E1B (E1B-55K), and the E6 protein expressed by papillomaviruses.

The discovery of p53 in virus-infected cells and its elevated expression in many cancers shaped an initial hypothesis that *TP53* was a proto-oncogene. It was only with the seminal finding that wild type *TP53* alleles are selectively lost during colorectal tumorigenesis that the true role of p53 as a tumor suppressor was definitively revealed (19). Viewed in this new light, the interaction between the host and viral proteins was reinterpreted, and a new chapter of the p53 story began. The DNA tumor viruses were not stimulating p53; they were inhibiting p53, and thereby creating a local environment that facilitated their unchecked replication.

The functional characterization of the proteins expressed by the DNA tumor viruses has provided a unique window into the inner workings of the proliferating cell and its responses to foreign DNA. Within this broad class of viruses, the high-risk serotypes of human papillomaviruses are the most strongly associated with tumorigenesis. At the other end of

the pathogenic spectrum, adenovirus infections cause mild inflammatory illnesses that are generally self-limiting. The adenoviral genome has been genetically tractable for many years, and more recent technical developments have further simplified the generation of specific adenoviral mutants (20, 21). Adenoviruses can be readily modified in ways that further limit their pathogenicity but which preserve their replication-competence. For all of these reasons, the adenoviruses are a widely-employed model system.

The responses of p53 to adenoviruses and their mutants have been - and continue to be - particularly informative. The species C adenoviruses are large relative to the other DNA tumor viruses. The double-stranded DNA genomes encode as many as 40 distinct proteins from open reading frames that in several cases overlap. More than 20 of these proteins are expressed after infection but prior to the onset of viral DNA synthesis (22).

Two of the viral proteins expressed during the immediate-early phase of infection are directly involved in p53 degradation (Figure 1). In the extensively-studied serotypes 2 and 5, the E1B-55K protein binds to p53. Along with host proteins recruited by viral E4-ORF6, E1B-55K assembles an active E3 ubiquitin-ligase complex. This virus/host hybrid enzyme marks p53 for degradation by the proteasome (23, 24).

The E1B-55K-dependent degradation of p53 was long thought to be a mechanism employed by adenoviruses to circumvent the anti-proliferative effects of the genes that are transcriptionally induced by p53. While the transient overexpression of E1B-55K could indeed result in the decreased expression of p53 target genes (25), the initial hypothesis was ultimately proven incorrect. Adenoviruses with deletions that eliminate E1B-55K retain their ability to replicate, even in host cells that express wild type p53 (26, 27). As would be expected, wild type p53 protein is stabilized after infection with E1B-55K-deficient adenoviruses. However, the resulting accumulation of p53 fails to induce the transcription of its target genes (27). This surprising phenotype was later attributed to the small protein E4-ORF3, which forms a polymeric nuclear scaffold that selectively silences the promoters of p53 targets (28). These groundbreaking studies raise an obvious question. Why would adenoviruses employ one mechanism to degrade p53 and another to prevent its engagement with its target promoters? One interpretation is that a single mechanism would simply not be sufficient to fully inactivate a robust p53-mediated antiviral response in the nucleus. If this is correct, then it is difficult to understand the selective evolutionary pressures that would have shaped two entirely different but complementary modes of p53 pathway inactivation, instead of a single, optimized p53 degradation system. An alternative explanation for the two very different mechanisms of control employed by adenoviruses is that they serve two very different requirements for productive infection.

p53 proteins are found in at least two distinct locales (Figure 2). In addition to the major pool of nuclear p53 that controls transcription, a second pool of functional p53 is located in the perinuclear regions of the cytoplasm. Distinguished by unique posttranslational modifications, cytoplasmic p53 enhances the permeability of the mitochondrial outer membrane and thereby promotes apoptosis and autophagy (29–32).

Adenoviral proteins usurp control of p53 localization and specifically interfere with cytoplasmic p53. In addition to its role in the ubiquitin-mediated degradation of nuclear p53, E1B-55K also conjugates the small ubiquitin-like modifier 1 (SUMO1) to p53 in a manner that is E4-ORF6 independent (33, 34). By SUMOylating p53, E1B-55K promotes its association with nuclear bodies that contain the promyelocytic leukemia (PML) protein, an important co-factor for p53-mediated transcription (35, 36). However, PML nuclear bodies (also known as nuclear domain 10) are disrupted following viral infection by the formation of the E4-ORF3 nuclear scaffold, and their components dispersed (37). This redistribution of PML protein is required for efficient viral replication, and results in the export of p53 from the nucleus to the cytoplasm (33), a process that is dependent on the exportin CRM1 (33, 34). E1B-55K itself is SUMOylated and then similarly exported in a CRM1-dependent manner (38).

Cytoplasmic p53 is found at the mitochondria but, interestingly, the pro-apoptotic function of p53 is retained in mutants that lack the domain required for mitochondrial localization (39). Very recent studies have identified a new role for p53 at the mitochondria-associated membranes that functionally link mitochondria to the endoplasmic reticulum (ER) (40). The balance of pro- and anti-apoptotic signals is at least partially mediated by the flux of Ca^{2+} into and out of the ER lumen by the sarco/ER Ca^{2+} -ATPase (SERCA) pumps (41). The pool of p53 localized to these membranes has been reported to directly bind SERCA and change its oxidation state (40). Upon its interaction with cytoplasmic p53, SERCA increases the intraluminal accumulation of Ca^{2+} and causes the increased flow of Ca^{2+} to the mitochondria. This Ca^{2+} signal primes mitochondria for subsequent permeabilization and thus lowers the threshold for the onset of apoptosis. The pool of E1B-55K exported from the nucleus suppresses mitochondrial destabilization by p53 (42). It is possible that E1B-55K would antagonize the interaction between p53 and SERCA and thereby preserve reticular Ca^{2+} homeostasis, but such an activity remains to be experimentally demonstrated.

In adenovirus-infected cells, E1B-55K causes the degradation of nuclear p53. But a substantial fraction of p53 exits the nucleus and, together with E1B-55K, accumulates in a characteristic perinuclear structure known as the cytoplasmic body (43). The cytoplasmic body is a distinct compartment that is actually created by E1B-55K; mutant forms of E1B-55K that fail to form this structure can no longer inhibit p53-mediated apoptosis (44). The formation of a cytoplasmic body that contains p53 is a common finding in cells that have been infected by diverse types of DNA viruses, including cytomegaloviruses (CMV) (45). The redistribution of PML protein from nuclear bodies, temporally related to nuclear export, is a similarly common result of infection with these diverse viruses (46, 47).

Not all of these viral functions make obvious sense. Why do E1B-55K and functionally orthologous proteins go to great lengths to deliver p53 to a repository in the cytoplasm? In the case of adenovirus, it would seem much more economical for E1B-55K to simply promote the rapid and complete ubiquitin-mediated degradation of p53 molecules, regardless of their location. On the contrary, the half-life of cytoplasmic p53 has been reported to be significantly extended at the later stages of CMV infection (45, 48). That unrelated viruses have convergently evolved complex mechanisms to coordinate the nuclear export and stable retention of p53 in a defined space in the cytoplasm suggests that this pool

of p53 must somehow support the viral life cycle. The nature of this stabilized, extranuclear p53 function - and whether it relates to the recurrent gain-of-function phenotypes of the p53 mutant proteins often found in tumors (49, 50) – is unknown.

Also unknown is whether the DNA tumor viruses idiosyncratically target p53, or if p53 might play a more general role in anti-viral innate immunity. The p53 protein is reportedly stabilized in response to diverse viruses, including the human immunodeficiency virus (51), influenza A virus (52, 53) and Sendai virus (54), and has been proposed to play a role in host restriction. Interestingly, *Tp53*-null mice exhibit increased susceptibility to viral infection, while “super-p53 mice” mice with an extra copy of p53 exhibit enhanced resistance (55).

3. The interferon response and p53

Among the primary barriers to viral infection is the local release of interferons (IFNs), a broad class of cytokines that collectively serve as an intercellular signal of pathogen detection. The IFNs induced by viruses are predominantly produced by cells in the monocyte lineage, and are detected by epithelial cells that express a cell surface complex known as the IFN- α/β receptor. The engagement of IFN with its receptor activates an intracellular signaling cascade involving the JAK-STAT pathway that ultimately results in the increased expression of numerous genes that contain an IFN-stimulated response element (ISRE). These genes are accordingly referred to as IFN-stimulated genes (ISGs). The ISGs mount a range of antiviral responses that block infection, inhibit replication, and degrade viral genomes. Thus, the ISGs collectively mediate innate immunity (56, 57).

The *TP53* promoter contains a functional ISRE, and *TP53* can therefore be considered an ISG (58). This observation fits in well with the ongoing p53 narrative. It was well understood that mutant viruses can trigger p53-dependent apoptosis, and therefore logical to conclude that the regulation of *TP53* by IFN was simply a mechanism to enhance this apoptotic response. However, the ability of p53 to suppress the replication of RNA viruses is notably retained in cells that fail to undergo apoptosis following infection (54). Even the basal level of p53 present in the absence of DNA damage is apparently sufficient to elevate the level of ISG expression.

This plot twist introduced a new chapter, one as mysterious and interesting as any that had come before. Previously viewed as a downstream effector that initiates anti-proliferative responses to viral infection, p53 was now revealed as a central mediator of the global innate immune response. The seminal observation of p53 as an activator of ISGs (54, 59) provides a new perspective on its antiviral role, and thereby shapes a new hypothesis: as an ISG gene product that upregulates other ISGs, p53 serves to amplify the intracellular IFN response.

ISG promoters do not generally contain p53 consensus binding sites, and so ISG induction by p53 must occur by an indirect mechanism. Munoz-Fontela et al. (54) identified IFN regulatory factor 9 (IRF9), a component of the ISG factor 3 (ISGF3) complex, as a p53 target gene. ISGF3 directly induces the expression of ISRE-containing genes, and so could represent a mechanistic link between p53 and ISG induction. Several additional IFN-

stimulated mediators of ISG expression, including IFN regulatory factor 5 (IRF5), immune-stimulated gene 15 (ISG15) and the Toll-like receptor 3 (TLR3), have been identified as direct p53 target genes (60–62).

Adenoviruses have evolved an array of distinct mechanisms to evade the host immune system. The most prominent immunosuppressive phenotypes map to the E3 region (63, 64). The small E3 proteins, including gp19K, 10.4/14.5K and 14.7K, function to inhibit NF- κ B signal transduction and the secretion of arachidonic acid, and to hamper the expression of MHC-I, Fas and TRAIL at the cell surface. The net effect of these disparate activities is the downregulation of the inflammatory and apoptotic responses that would otherwise be activated by viral infection. E3-deleted mutant viruses replicate efficiently in culture, which reflects the primary role for E3 in shaping the adaptive host response to adenoviral antigens.

The properties of adenoviruses that allow them to suppress the innate immune response have come into focus more recently. Here, the E1B-55K protein once again emerges as a foil to p53. Upon infection, the E1B-55K-null virus known as H6 triggers the robust expression of antiviral ISGs (65), thus demonstrating that wild type E1B-55K must normally suppress the type 1 IFN response. Accordingly, the replication of H6 and other viral mutants that destabilize or otherwise functionally alter the expressed E1B-55K protein is markedly suppressed by the addition of exogenous IFN, despite the presence of normal quantities of replication proteins (66). The evasion of the IFN response orchestrated by E1B-55K must be independent of p53-mediated transcription, because this process is suppressed by the E3-ORF3 nuclear scaffold (28). Expressed from a plasmid in a non-infected cell, E1B-55K can suppress p53-mediated transcription (25, 67) but has no effect on ISG expression (67). These detailed studies of E1B-55K elegantly reveal the host responses to infection and the evolutionary adaptations that allow the adenovirus to circumvent these responses.

Several broad conclusions can be drawn. As the inhibition of ISG expression by E1B-55K must be independent of p53-mediated transcription, non-transcriptional mechanisms must be predominant in the host response to viruses that transfer DNA. The direct induction of IRF9 by p53 may be a component of the host responses to RNA viruses (54), which have apparently not developed similarly elaborate mechanisms to inhibit p53-mediated transcription. Could the antiviral signal for ISG induction originate in the cytoplasm, from the transcriptionally inactive pool of p53? An intriguing possibility is that the cytoplasmic pool of p53 – laboriously exported, stabilized and localized by the combined activities of E1B-55K and E3-ORF3 – is somehow required for DNA tumor virus-mediated ISG suppression. A mechanism that would account for such suppression remains obscure.

4. The DDR distinguishes between chromosomal DNA damage and viral infection

Many of the most entertaining and enduring stories conclude with a battle between good and evil. This climactic struggle breaks down all illusion and pretense, and reveals the protagonist's true character. In the cellular battle between good (genomic stability, tissue homeostasis, viability) and evil (genomic instability, cancer, degeneration and aging), p53 is obviously a leading combatant. The p53 protein does not function in isolation, of course. It

is a highly interconnected nodal component of the DDR, an extensive network of sensors, signaling proteins/posttranslational modifiers and effectors that together maintain the integrity of the human genome and suppress tumorigenesis (68). Upon its activation, the DDR coordinately interrupts cell growth, promotes the initiation of DNA repair and lowers the apoptotic threshold for the elimination of critically damaged cells. New insights into the role of the DDR in innate immunity provide new clues as to the essential character of p53.

The DDR and the IFN responses have traditionally been studied separately, by two communities of investigators with different perspectives and questions. Thus, two narratives have been written in parallel. However, recent findings have revealed that these two basic pathways are intimately connected. ATM, an apical serine/threonine kinase in the DDR and the primary responder to double-strand DNA breaks, actively suppresses type 1 IFN signaling, and thereby maintains ISG expression at a basal level (69). These constitutively low levels of IFN signaling are believed to maintain the innate immune system in a “ready to go” state that can be quickly boosted in response to an invading pathogen (70). In the absence of functional ATM, double-strand break repair is defective, and damaged DNA accumulates in the nucleus. Some of these fragments are released into the cytoplasm, where they activate a recently described cytosolic DNA sensor known as Stimulator of interferon genes (STING) (71). The activation of STING by chromosomal DNA fragments creates a state of innate immune hyperactivity that could represent a “danger” signal, or result in chronic inflammation (69). These new findings provide an explanation for the long-recognized inflammatory component of the autosomal recessive disorder Ataxia-telangiectasia, a cancer predisposition syndrome caused by germline mutations in the *ATM* gene (72).

A recent study from Clodagh O’Shea and her colleagues (73) further demonstrates a fundamental requirement for the DDR in innate immunity and also reveals a fascinating mechanism of viral recognition. At the site of a chromosomal double-strand DNA break, the MRN complex, comprised of MRE11, RAD50, and NBS1, acts as a sensor for the rapid activation of ATM. ATM rapidly phosphorylates many downstream substrates, including p53 and the histone variant H2AX. The H2AX phosphoprotein (known as γ H2AX) marks an extensive region of chromatin flanking the double strand break site, and thus triggers a global DDR (74).

This DDR is actively suppressed by wild type adenovirus 5. Analogous to its effects on p53, the ubiquitin ligase formed by the early viral proteins E1B-55K and E4-ORF6 promotes the proteasomal degradation of MRE11 (24, 73), while the nuclear scaffold created by E4-ORF3 sequesters MRN, thus excluding it from compartmentalized viral replication centers (28, 75). Interestingly, adenoviral mutants deficient for E1B-55K and E4-ORF3 reveal an alternative DDR that is activated by MRE11 and which recognizes viral DNA. This alternative DDR is localized to viral genomes and characterized by a distinct lack of γ H2AX (73). Thus, in the context of viral infection, the MRN complex is able to specifically restrict viral DNA replication. These exciting new findings explain why the disruption of MRN is a prerequisite for viral DNA synthesis (76–78) and more broadly reveal the DDR as an intrinsic mediator of antiviral immunity. Thus the IFN and DDR networks join forces to battle a common enemy.

The evolutionary game of cat and mouse played by adenoviruses and their cellular hosts reveals a heretofore obscure aspect of the DDR, and one that may be central to its evolved purpose. A bifunctional DDR not only senses chromosomal DNA damage and foreign DNAs, but also allows the cell to distinguish between them. Thus defined, the need for such a system becomes immediately obvious. The cellular genome is continuously damaged by stochastically-arising DNA strand breaks, and is also constantly under siege by invading viruses. The unrelenting activation of a global DDR by viral DNAs would presumably prevent the proliferation of cells that are required for physiological tissue regeneration (73). By the same token, the cross-activation of an antiviral DDR by any random chromosomal break or stalled DNA replication fork would lead to a state of immune hyperactivity that would be similarly detrimental. The DDR – and by extension, p53 – appears to have developed multiple functional modes to appropriately confront these diverse challenges.

5. The loose plot lines: glucose metabolism and cell-to-cell communication

Among the many interesting attributes of p53 that have been recognized in the past decade is its ability to promote the metabolism of glucose by the process of mitochondrial oxidative phosphorylation (79, 80). Recent studies have indicated that this highly oxidative metabolic state, in addition to being highly efficient, is intrinsically antiviral in nature.

The loss of p53 function during tumorigenesis contributes to the tendency of cancer cells to employ a glycolytic pathway to ferment glucose to lactic acid, even when the local level of oxygen is not limiting. The upregulation of the oncoprotein MYC during tumorigenesis also contributes to this common glycolytic shift, known as the Warburg effect. The phenotypic tendency of tumor cells to utilize aerobic glycolysis increases their adaptability at the expense of efficiency, and is considered a fundamental hallmark of cancer (81, 82). Notably, the mutant *TP53* alleles that most frequently cause Li-Fraumeni syndrome exhibit a gain-of-function phenotype that causes mouse and human carriers to exhibit an increased capacity for oxidative respiration at the expense of their glycolytic capacity (83). This important clinical observation demonstrates the extent to which p53 controls metabolism at the level of the whole organism.

A cell that primarily employs oxidative respiration would be ill-equipped to survive in the changing microenvironment of a developing tumor. Interestingly, that cell would also be relatively resistant to viral infection. Adenoviruses rapidly induce glycolysis upon infection of host cells, and thus cause a metabolic shift that closely resembles the Warburg effect in cancer cells (84). In addition to the mechanisms they have evolved for the inactivation of p53, adenoviruses also cause an increase in the expression of MYC and its engagement of glycolytic targets. This activity has recently been mapped to the E4-encoded protein E4-ORF1, which appears to promote MYC stabilization via a direct interaction (84). Thus, virus-infected cells are shifted to a glycolytic mode that is also commonly found in cancer cells. The advantages to viruses of a glycolytic environment are not completely clear, but may include the increased availability of nucleotides and other glucose metabolites required for viral replication. Once again, adenoviruses provide a new perspective on a common cancer phenotype.

Like metabolism, innate immunity is not a cell-autonomous attribute, but a homeostatic process that ultimately involves both the activation of intracellular signaling pathways and the rapid communication of these signals to neighboring cells. The secretion of and response to type 1 IFNs is a specific example of such intercellular communication. Several independent developments suggest potential roles for p53 in non-cell autonomous signaling pathways, which could conceivably relate to a common role in innate immunity (Figure 3). For example, p53 causes some types of cells to become senescent (16, 85). This distinctive phenotype was long believed to represent an additional mechanism for cell-autonomous proliferative arrest. Recent studies indicate that senescent cells have an important paracrine effect as well. In the liver, p53 enables senescent stellate cells to release extracellular factors that cause neighboring macrophages to become active against the cells of a nearby tumor (86). By employing this secretory program (known as the senescence-associated secretory phenotype, or SASP), p53 thus acts in a non-cell autonomous manner to modulate the immune system and thereby maintains tissue homeostasis.

A distinct homeostatic mechanism has recently been attributed to the transmembrane receptor Death Domain 1 α (DD1 α). This surface receptor is encoded by a transcriptional target of p53 and expressed on the cell surface (87). Induced by p53 in cells as they undergo apoptosis, DD1 α marks the resultant cell corpses for engulfment by macrophages. Like the p53-dependent maintenance of oxidative phosphorylation in the mitochondrion, this p53-dependent corpse disposal mechanism has been proposed to represent a barrier to tumorigenesis (87). It is interesting to consider that the enhanced engulfment of virion-packed cell corpses would presumably be an effective means of limiting the extent of a viral infection.

6. Innate immunity and tumorigenesis

TP53 is arguably the most important tumor suppressor gene. Mutations in *TP53* contribute to a majority of all malignant tumors, and the study of p53 in normal and diseased cells has provided innumerable insights into the molecular basis of cancer. In accordance with its unique position as a central node in a vast signaling network of DDR and tumor suppressor proteins, p53 has a multitude of functions. Considered individually, most of these functions make sense. For example, it is intuitive that the activation of apoptosis and the imposition of cell cycle arrest would prevent neoplastic growth. However, considered as a collective array of distinct cellular functions, the phenotypes of *TP53* present something of an intellectual puzzle. What sort of selective pressures could possibly have resulted in the evolution of such an interconnected and multifunctional anti-cancer gene? Would the eventual death of an organism caused by rampant neoplasia provide sufficient selection for such molecular traits? These remain fascinating questions.

A satisfying answer, not yet proven, is that *TP53* is an evolutionary product of the continuous interaction of cells with environmental pathogens. p53 was discovered on the basis of its interactions with viruses, and this was most certainly not an incidental finding. Oncogenic viruses provided us with our first real opportunities to learn about cancer. As a narrative formed and developed – pushed forward by the explosive development of biotechnology and development of increasingly sophisticated model systems - we began to

rely less upon these simple viruses. Our focus was naturally drawn away from viruses and into the cancer cell itself, to the mutated genes and dysfunctional pathways that underlie its malignant nature. Amid the excitement over the digital characterization of complex cancer transcriptomes and genomes, it becomes easy to forget that viruses are much more than convenient model systems. They are a real and omnipresent threat to our viability. Recent studies serve as a reminder that just as viruses evolve to evade host defenses, host genes also evolve to meet the challenges to genetic integrity and stability posed by viruses.

Cancer is not all about cell growth. Recent years have seen an increased appreciation of the immune system and its primacy as a barrier to cancer (88, 89). We propose that the antiviral effects of p53 are not incidental to its function as a tumor suppressor, and that in fact the inverse is true: the loss of innate immunity caused by p53 inactivation incidentally confers an immune-privileged status that allows tumors to grow. It is imperative that we view p53 from this new perspective. In the most practical sense, considering p53 primarily as a component of the innate immune response simplifies the interpretation of its many cell-autonomous phenotypes, and allows us to integrate newly discovered non-cell autonomous activities into a unified model. More importantly, strategies to preventively and therapeutically enhance the innate and adaptive immune responses against evolving tumors will likely be based on an improved understanding of the factors that mediate neoplastic anergy. A causative link between *TP53* mutations and the most basic responses to non-self DNA would constitute a truly dramatic conclusion to a story that has thus far been epic in scope.

Acknowledgments

The authors of this review are supported by grants from the National Institutes of Health (CA157535, CA158428).

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Figure 1. The E1 and E4 genes of Adenovirus 5

The Adenovirus 5 genes expressed at the early-intermediate early stages of infection express small proteins that modify the DNA damage and interferon responses. E1B-55K acts directly on p53. This multifunctional viral protein alters p53 abundance via a cooperative interaction with E4-ORF6 and also alters its intracellular location. The E4-ORF3 protein acts in an indirect manner by assembling a nuclear scaffold that blocks transcription by p53 and activation of the MRN complex. E4-ORF3 and E1B-55K also promote the redistribution of PML bodies, which is required for the nuclear export of p53.

Figure 2. Regulation of p53 by adenovirus proteins

E1B-55K (red) and E4-ORF6 (green) recruit host proteins that all together form an E3 ubiquitin ligase, which marks nuclear p53 for proteasomal degradation. The nuclear scaffold formed by E4-ORF3 (purple) prevents the engagement of nuclear p53 (denoted as p53ⁿ) with the promoters of its target genes. In addition, E4-ORF3 and E1B-55K redistribute PML bodies (yellow), a requirement for the translocation of p53 to the cytoplasm (p53^c).

E1B-55K is similarly transported, and the two proteins reside together in a cytoplasmic body. In the absence of infection, p53^c can promote transcription-independent apoptosis. E1B-55K blocks this function. Recent studies have shown a direct pro-apoptotic effect of cytoplasmic p53 on the Ca²⁺ transport pump SERCA.

Figure 3. A model for intra- and inter-cellular antiviral signaling by p53

Several lines of evidence suggest that p53 promotes the induction of interferon-stimulated genes (ISGs) by a non-transcriptional mechanism. We propose that this activity may reside in the cytoplasmic pool of p53 ($p53^c$), which has previously been implicated in apoptosis. In response to DNA damage or foreign DNA, the nuclear pool of p53 ($p53^n$) is stabilized and mediates a complex transcriptional program. Many and perhaps all of the p53-dependent, cell-autonomous phenotypes are anti-viral in nature. Two recently described modes of intercellular communication, involving the senescence-associated secretory phenotype (SASP) and the death domain receptor DD1a, have been shown to be involved in the paracrine activation of innate immune cells that secrete interferons (IFN) and engulf cell corpses. The release of IFN into the cellular environment induces the activation of the *TP53* gene, which is itself an ISG, thus completing a cycle that results in the amplification of an immune "danger" signal.