REVIEW

Coordination and communication between the p53 and IGF-1–AKT–TOR signal transduction pathways

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Over the past 10 years the signal transduction networks for p53, IGF-1–AKT, and TOR pathways have been assembled in worms, flies, and mammals, and their functions elucidated. In the past 1–2 years a number of genes and their proteins have been identified that permit extensive communication and coordination between these pathways. These three pathways are involved in sensing and integrating signals arising from nutrient and growth factor availability, signals from sensory and sexual organs, and intrinsic and extrinsic stress signals. In turn these pathways regulate cell growth, proliferation, and death. These networks are central to our understanding of a variety of physiological and pathological conditions, including cancer, diabetes, and longevity.

The normal physiology of a multicellular organism depends on the cooperative functions of its constituent cells. Some diseases or physiological processes may arise as a result of abnormalities of a population of cells (e.g., aging or diabetes) or even a single cell (e.g., cancer). Thus the normal physiological status of individual cells; that is, the growth, proliferation, arrest, or death, is carefully regulated to ensure the well being and survival of the organism. This regulation is achieved, as a single cell in a multicellular organism responds to its environmental and internal cues, namely, growth factors, nutrients, oxygen, and stress. A large number of experimental results indicate that three major signal transduction pathways play critical roles in sensing and integrating these important signals: the p53 pathway, the IGF-1–AKT pathway, and the TOR pathway. In turn these pathways lead to cellular responses including the p53 transcriptional program and apoptosis, the forkhead transcriptional programs, autophagy, and translational controls, which determine cell growth or arrest, cell survival or death. A variety of experiments have demonstrated the extensive communication and coordination between all these pathways. The importance of the integrators between these signal transduction networks is demonstrated by the observation that these genes and their protein products (p53, PTEN, TSC2, PI3K, AKT-1, MDM2, AMP kinase, and mTOR) are among the most commonly altered tumor suppressor genes and oncogenes detected in cancers (Feng et al. 2005; Jones et al. 2005). The identification of these mediators between networks starts the process of understanding how these different pathways interact. Connecting the p53–AKT–TOR pathways to those reproductive and sensory signals as well as to factors responsible for longevity is a challenge for the future. As a more complete picture emerges we will have a clearer approach to treating cancer and diabetes. This review provides an early attempt to construct an integrated circuit between the p53–IGF-1–AKT–TOR pathways, and as such it contains speculative aspects. The real value of this approach is to provide testable ideas that derive from these concepts.

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The p53 pathway

The p53 pathway responds to a wide variety of cellular stress signals. These include DNA damage and telomere shortening, hypoxia, low nucleoside triphosphate pool sizes, spindle damage, heat and cold shock, inflammation and nitric oxide production, as well as oncogene activation by mutations (Levine 1997; Vogelstein et al. 2000; Jin and Levine 2001). These stresses all have the potential to decrease the fidelity of cell cycle progression and DNA replication and thus to increase the mutation rates in cells. These stress signals (Fig. 1) are communicated to the p53 protein in part by post-translational modifications: phosphorylation, acetylation, methylation, ubiquitination, sumoylation, and neddylation (Brooks and Gu 2003; Bode and Dong 2004; Chuikov et

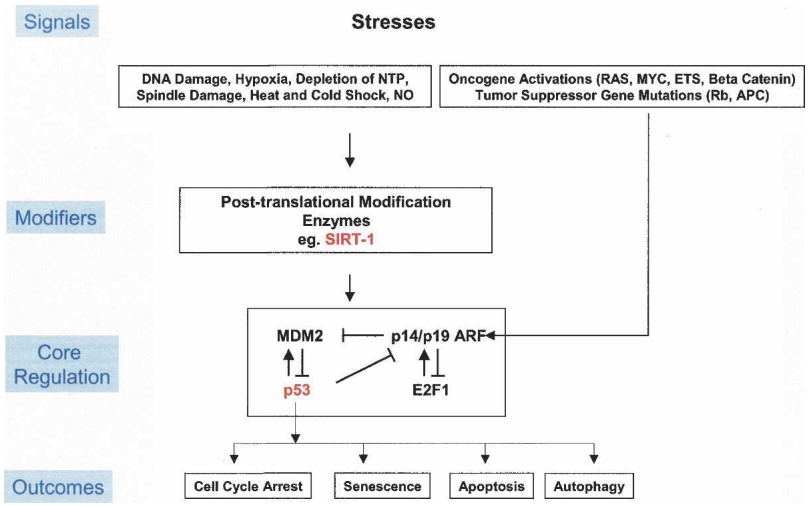

Figure 1. The p53 pathway. Several types of extrinsic or intrinsic stresses or signals activate the p53 pathway. The existence of a stress signal is mediated by modifications of several proteins in central nodes of the p53 pathway (p53, MDM2). The core regulation of the pathway is accomplished by changing the concentrations and activities of MDM2 and p53 by protein modifications, degradation (changes in half-life), or protein–protein interactions (ARF inhibits MDM2 activity). p53 acts as a transcription factor or more directly initiates one of several possible outcomes (cell cycle arrest, senescence, apoptosis, and autophagy). Which of these outcomes is employed often depends on interactions and inputs from other signal transduction pathways.

al. 2004; Harper 2004). At the same time, the half-life of the p53 protein increases, raising its concentration in the cell. This is most immediately accomplished by the degradation of the p53 ubiquitin ligase, the MDM2 protein, in response to the stress signals (Perry 2004). The post-translational modifications of the p53 protein are reversible by a series of protein phosphatases, histone deacetylases (HDAC), and deubiquitinases. In particular SIRT-1 (the Sir2 ortholog), which is an NAD-dependent HDAC, acts on p53 to remove the acetyl-groups from the C-terminal lysines (Luo et al. 2001; Vaziri et al. 2001). This same activity acts on the acetylated forkhead transcription factors and has been shown to modify the expression of the IGF-1 pathway in worms (Tissenbaum and Guarente 2001). Sir2 and its orthologs play a role in enhancing longevity (Guarente and Kenyon 2000; Hekimi and Guarente 2003) in several organisms. The activated p53 protein in a stressed cell acquires the ability to bind to specific DNA sequences (RE or response elements) adjacent to genes and enhances the rate of transcription of those genes. Specific stress signals result in different protein modifications of the p53 protein and this in turn results in different transcriptional programs and different outcomes in a cell. p53 activation may result in cell cycle arrest (G1 or G2 arrest), senescence, or apoptosis. In addition, a variety of negative and positive feedback loops are activated that communicate with other signal transduction pathways, resulting in turning off p53-regulated functions or enhanced p53 activity and programmed cell death (Harris and Levine 2005). In the case of oncogene activation (myc, β-catenin, Ras, Ets) or a tumor suppressor gene mutation (Rb-E2F-1, APC-β-catenin), these transcription factors act to increase the level of p14/19 ARF protein, which in turn binds to the MDM2 protein and inhibits its action as a ubiquitin ligase, raising p53 levels and activity (Damalas et al. 2001; Zindy et al. 2003). These are good examples of p53 regulatory feedback loops that communicate with a variety of signal transduction pathways (Wnt, Ras, Myc, Rb) (Harris and Levine 2005).

The IGF-1–AKT and mTOR pathways

The IGF-1–AKT network is an evolutionarily conserved pathway that transmits survival signals in a cell in response to growth factor stimulation. In the absence of these survival signals, a programmed cell death can ensue. The binding of a growth factor (IGF-1) to its tyrosine kinase receptor (IGF-1R) results in the recruitment and activation of the PI3 kinase to the plasma membrane receptor, which in turn phosphorylates the phosphoinositides, increasing the local concentration of PIP_3 and PIP_2 at the plasma membrane. The PI3 kinase activity (an oncogene often found to be mutated in some cancers) is counteracted in the cell by PTEN, a lipid 3′ phosphatase and the second most common sporadically mutated tumor suppressor (behind p53) (Downward 2004; Vogelstein and Kinzler 2004). This increase in lipid second messengers recruits and activates the PDK and AKT protein kinases at the plasma membrane where AKT is then fully activated by phosphorylation of ser-473 and thr-308 (Blume-Jensen and Hunter 2001). AKT has several substrates that are antiapoptotic such as BAD (Datta et al. 1997; del Peso et al. 1997) and MDM2 (Zhou et al. 2001). In addition the activated AKT protein moves to the cell nucleus where it phosphorylates the forkhead transcription factors, resulting in their removal from the nucleus into the cytoplasm and producing a change in the forkhead transcriptional activity (Brunet et al. 1999). These events will result in a program leading to antiapoptotic signaling, preparation for entry into the cell cycle (turning off p27) and cell growth, and communication with the TOR kinase pathway, which senses nutrient levels (glucose and amino acids) in the environment. This is accomplished by the AKT-1 phosphorylation and inactivation of TSC2 (Inoki et al. 2002; Potter et al. 2002), which forms a TSC1–TSC2 protein complex that is a GAP for the RHEB G-protein. RHEB, in turn, activates the TOR kinase (Gao and Pan 2001; Inoki et al. 2003a; Zhang et al. 2003). Thus an active AKT-1 activates the TOR kinase, both of which are positive signals for cell

growth (an increase in cell mass) and division. The mammalian TOR (mTOR) forms two complexes in cells, the Raptor complex (Hara et al. 2002; Kim et al. 2002) and the Rictor complex (Jacinto et al. 2004; Sarbassov et al. 2004). The Raptor mTOR complex, which contains mTOR, GβL, and Raptor, is rapamycin sensitive, while the Rictor mTOR complex, which contains mTOR, GβL, and Rictor, is rapamycin insensitive. It appears that while the Rictor mTOR complex is involved in cytoskeleton reorganization, the Raptor mTOR complex is the one that is involved in cell growth regulation. In addition to sensing the growth factor signals, the Raptor mTOR complex is regulated by a second input from the signaling pathway sensing nutrient levels in the medium. The absence of glucose activates the LKB1 kinase (employing intermediates that are unknown), which in turn phosphorylates the AMPK (Yoo et al. 2002; Shaw et al. 2004). The absence of glucose in the cell also increases the levels of AMP, a coactivator of the AMPK. The active AMPK positively regulates the activity of the TSC1–TSC2 complex by phosphorylating the TSC2 protein (resulting in the opposite activity of the AKT-1 phosphorylation of TSC2), which then turns off the RHEB G-protein and modulates down TOR activity (Inoki et al. 2003b; Corradetti et al. 2004). Apparently the TSC1–TSC2 complex serves as the converging point for the AMPK (nutrient) and AKT (growth factor) signaling in higher eukaryotes, even though the counterpart does not exist in budding yeast. The TOR kinase reciprocally regulates two major processes (along with a number of other cellular processes): translation of selected mRNAs in the cell and autophagy. In the presence of high nutrient levels TOR is active and phosphorylates the 4EBP protein releasing the eukaryotic initiation factor 4E (eIF4E), which is essential for cap-dependent initiation of translation and promoting growth of the cell (Hay and Sonenberg 2004). TOR also phosphorylates the S6 kinase and this kinase has been implicated in ribosome biogenesis (Hannan et al. 2003) as well as the modification of a ribosomal protein, S6. The levels of 4EBP are modulated down in a number of cancers. Rapamycin derivatives that inhibit TOR and result in sustained higher 4EBP activity are in clinical trials as anti-cancer drugs (Hay and Sonenberg 2004). During nutrient deprivation, when translation is modulated down, autophagy is activated. This entails the formation of double-membrane vesicles in the cell cytoplasm that engulf cytoplasmic components, including defective mitochondria, and move them to the lysosomal compartment where they are degraded. Autophagy thus accomplishes two important survival functions; through a catabolic breakdown of cellular components, it provides nutrients from protein, lipid, and carbohydrate turnover, supplying the nutrient-deprived cell, which gets smaller in mass and volume (Lum et al. 2005). Autophagy is also the normal cellular process (occurring all the time) that destroys defective mitochondria that are uncoupled and produce dangerous reactive oxygen species. Thus autophagy reduces ROS and lowers the potential mutation rate (Shintani and Klionsky 2004). Mice with a reduced capacity for autophagy (due to a haplo-insufficient level of the Beclin gene product that is required for autophagy) develop tumors at an elevated frequency over their lifetimes (Qu et al. 2003; Yue et al. 2003). Thus autophagy is both a survival and a fidelity mechanism. In summary, TOR is positively regulated by the active AKT-1 kinase in response to PI3 kinase signaling and negatively regulated by the LKB1 kinase–AMPK pathway in response to nutrient deprivation, acting through the TSC1–TSC2 complex (both of these genes are tumor suppressor genes that produce hamartomas when mutated in humans and cancers when mutated in rodents). Recently (Sarbassov et al. 2005) the Rictor mTOR complex was shown to be a portion of the PDK activity that phosphorylates and activates AKT-1. This creates an autoregulatory feedback loop, where the Rictor mTOR complex activates AKT-1, which in turn communicates with the Raptor mTOR complex.

The interconnections between the p53, IGF-1–AKT, and TOR pathways

Figure 2 summarizes the integrated circuits that connect these three pathways and predict the level of coordinated regulation that has been and can be tested in cells. There are two major connections between the proteins of these three pathways that form a rapid and a slower response to stress signals after activation of p53. First, the rapid signal transduction pathway responds to DNA damage by the activation of p53 and the AMPK, which in turn activates TSC2 via phosphorylation (Feng et al. 2005). This inactivates RHEB and then mTOR and shuts down translation while turning on autophagy. These events are p53 dependent in a cell, as well as TSC1–TSC2 dependent after DNA damage, as demonstrated by using cells that had no p53, TSC1, or TSC2 gene (from knockout mice). The dependence on an active AMPK was demonstrated by using an inhibitor of this kinase. This establishes the rapid (in time) pathway between DNA damage–p53 activation–AMPK–TSC2–RHEB and TOR and the downstream activities of TOR, translation (off) and autophagy (on). A different stress signal also rapidly activates this pathway involving p53 and the AMPK. Glucose starvation rapidly results in ser-15 phosphorylation of the p53 protein, and the AMPK can carry out this reaction (Feng et al. 2005; Jones et al. 2005). This has been demonstrated by employing an inhibitor of AMPK, Compound C, which blocks this phosphorylation, or an activator of the AMPK, AICAR (5-aminoimidazole-4-carboxamide-β-ribofuranoside), which enhances ser-15 phosphorylation of p53 (Imamura et al. 2001). This occurs rapidly, within 15 min after removal of glucose and under these conditions, in normal cells, this signal is reversed within 30–60 min by removal of this phosphate. A ser-15 p53 phosphatase, composed of an α-4 subunit and the PP2A catalytic subunit, is activated via phosphorylation by the mTOR kinase (Kong et al. 2004). Thus TOR forms a negative feedback loop for the phosphorylation of ser-15, while the AMPK positively acts on the ser-15–p53 protein and negatively acts on TOR. This

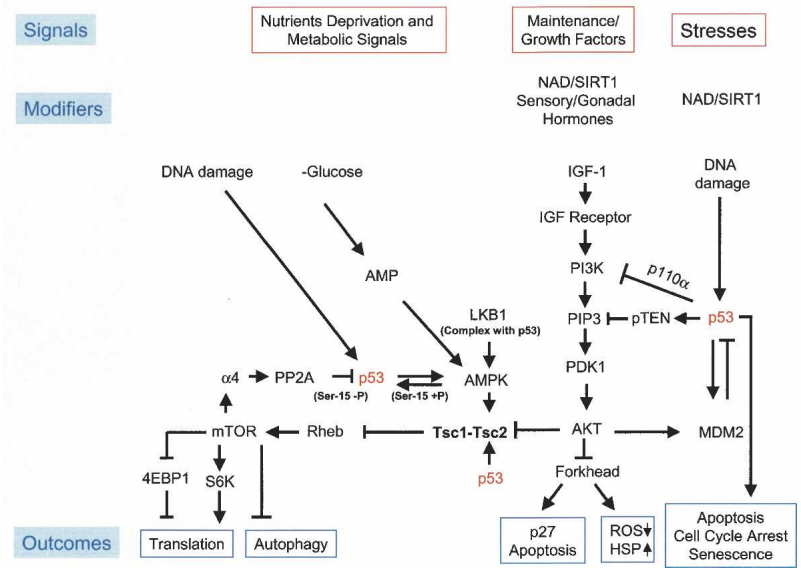

Figure 2. The interconnections between the p53 pathway and the IGF-1–AKT-1–mTOR pathways. The signals that activate the p53, IGF-1 and mTOR pathways can be modified by many diverse inputs (metabolic state-SIRT-1, gonadal hormones, sensory signals, cytokines and interleukins). Each of these pathways activates a central node (p53–MDM2; IGF-1–AKT-1; TSC1–TSC2–mTOR), which results in several alternative outcomes. These outcomes are coordinately controlled by protein functions that communicate the activity of a pathway (PTEN, TSC2, p53–AMPK, p53–LKB1 complex, p53–IGFBP-3–IGF-1) to the function and output of another pathway. These types of interconnections at the molecular and physiological levels translate to contributions of all three pathways to longevity, cancer, and diabetes.

feedback loop, as drawn (Fig. 2), predicts a continual phosphorylation of p53 ser-15, not the transient phosphorylation that was observed experimentally (Feng et al. 2005). This suggests that we are either missing a feedback connection in this loop and/or that this circuit will be controlled by the kinetics and reaction constants of this process. It is likely that a particular length of time without glucose is required for TOR to become inactive and then PP2A will fail to remove ser-15 phosphorylation from p53, resulting in the engagement of the positive feedback loop. The phosphorylation of ser-15 on the p53 protein by itself does not activate or stabilize p53. Rather it is an early step in activation of p53 that requires further phosphorylation events, at ser-20, ser-33, and ser-46, and thr-18, which then appear to favor a p53 apoptotic response. Thus glucose starvation that results in ser-15 phosphorylation in a transient manner in normal MEFs does not result in apoptosis. However, if those cells contain an activated oncogene, such as E1A, which binds the Rb protein and liberates E2F-1 to activate p19 ARF, then glucose starvation results in a full p53 response and cellular apoptosis (S. Lowe, pers. comm.). Thus glucose starvation sensitizes the p53 protein (ser-15 transiently by AMPK) and, if other signal transduction pathways are engaged, the second steps (MDM2 is degraded and other serines and threonines on p53 are phosphorylated by the ATM, ATR, p38 mitogen-activated protein kinase [MAPK], casein kinase) result in an activated p53, and consequently cell cycle arrest or apoptosis.

After a DNA damage signal, p53 is activated and positively regulates the activation of the AMP kinase in an as yet unknown fashion (Feng et al. 2005). It has been reported that a p53–LKB1 complex forms that enhances the p53 mediated program for apoptosis (Karuman et al. 2001). This result has not been independently confirmed in the literature and so the role of this putative protein complex remains unclear. What is clear is that one branch of the p53 response to DNA damage in the cell results in the modulation down of mTOR, a decreased translational efficiency, and an increased level of autophagy (Feng et al. 2005). In normal cells in culture the p53 response to DNA damage is commonly a cell cycle arrest and in cells containing constitutively activated oncogenes one observes a p53-mediated apoptosis (Kastan et al. 1992; Lowe et al. 1993).

These rapid events occur within minutes to a few hours after p53 signaling and DNA damage. The timing of these events is in minutes for phosphorylation and a few hours for the rise in p53 levels caused by an inactivation of MDM2, the p53 ubiquitin ligase, and an accumulation of p53 by extending its half-life to hours from 20–40 min. There is a second wave of communication between p53 and the IGF-1–AKT and TOR pathways, that interestingly accomplishes the same end points but does it by activating p53-responsive genes via a slower (12–24 h) transcriptional mechanism (see Fig. 2). Mak and colleagues (Stambolic et al. 2001) first demonstrated that the PTEN gene contained a p53 RE and was induced by the activation of p53. This observation could only be reproduced in a subset of cell lines or cell types and it is now commonly believed that many p53-regulated genes and p53 outputs (apoptosis, senescence, cell cycle arrest) are regulated differently in different cell or tissue types. Hoh et al. (2002) developed an algorithm that detected p53 REs adjacent to genes in the human or mouse genome and predicted which genes are p53-regulated with ~75% success rate. This algorithm detected p53 REs in the PTEN and TSC2 genes (Feng et al. 2005). Cell lines or mouse tissues that transcriptionally activated the PTEN gene after DNA damage also induced TSC2 gene transcription, while cell lines or murine tissues that failed to regulate PTEN in a p53-dependent fashion also failed to regulate TSC2 (Z. Feng and A.J. Levine, unpubl.). The p53-mediated induction of PTEN levels and TSC2 levels by p53 acts in the same way as the faster p53–AMPK

pathway (Fig. 2). Increasing PTEN levels shuts down AKT activity and relieves its inhibition on TSC2, resulting in the inactivation of TOR, loss of phosphorylation of S6 kinase, and activation of autophagy (Noda and Ohsumi 1998; Feng et al. 2005). Similarly, the increased levels of TSC2 mRNA by p53 enhance and accomplish the same result. Measuring the TOR-regulated S6 kinase phosphorylation and autophagy, it could be demonstrated that p53 and both the TSC1 and TSC2 gene products were all required for the DNA damage response to be communicated to the TOR kinase (Feng et al. 2005). It has proven difficult to test the impact of PTEN loss upon p53-dependent down-regulation of S6 kinase phosphorylation or activation of autophagy, because the loss of PTEN alters (in an unknown way) p53 activity. Thus it is not possible to obtain a normal mouse embryonic fibroblast cell line that is PTEN negative and also has normal levels of the wild-type p53 protein. Similarly, TSC2 negative cells have proven impossible to grow and immortalize in culture because the absence of the TSC2 protein appearently leads to the activation of p53 and cellular senescence. Thus $PTEN^{-/-}$ cells and $TSC2^{-/-}$ cells (knockouts of both alleles) both create immortalized cell lines that have altered p53 activity. These observations still require clear mechanisms to understand them, but they relate the p53–IGF-1–AKT–TSC2 and TOR pathways in ways that make it clear we do not yet have a complete picture of these interactions between pathways.

Cell lines or tissues (such as prostate cancers) that mutationally inactivate PTEN have high AKT-1 activity, which activates mTOR and S6 kinase through a TSC1/2 complex. This is p53 independent as indicated in Figure 2. In cells and tissues (liver, muscle, white fat, kidney) where p53 can transcriptionally activate TSC2 and raise its levels in the cell, one would expect that the ratios of AKT-1 and TSC2 activities would determine whether or not mTOR and S6 kinase would be active. Thus tissue type may determine the sensitivity of cancers to chemotherapeutic agents, and the nature of the mutational spectrum in a tumor (PTEN or p53) would add further heterogeneity to the response to therapy.

Recently Kaelin and colleagues (Brugarolas et al. 2004) reported that hypoxia (a p53-inducible signal) inhibits mTOR function in a TSC2/TSC1-dependent fashion through the hypoxia-inducible REDD1 gene product. In this case the inhibition of mTOR by hypoxia did not require the AMP kinase (as it did with DNA damage) but the REDD1 gene, a p53-regulated gene after the exposure of cells to reactive oxygen species (Ellisen et al. 2002). Clearly then there are additional signaling pathways between other stress signals to p53 through REDD1 to TSC1 and TSC2 and mTOR that result in similar outcomes (apoptosis and autophagy).

Thus there is a rapid communication after p53 activation by a stress signal with the AKT-1 and mTOR pathways mediated by the AMPK, TSC1 and TSC2 proteins employing phosphorylation and RHEB G-protein inactivation and a slower process mediated by the transcription of the PTEN and TSC2 genes by p53. Both processes lead to p53 activation and AKT-1 and mTOR inactivation. Similarly, the activation of AKT-1 and mTOR by the presence of nutrients and growth factors leads to the AKT-1-dependent activation of MDM2 by phosphorylation, which enhances its activity as a ubiqutin ligase and moves it into the nucleus so that it more effectively degrades and inactivates p53 (Gottlieb et al. 2002) (Fig. 2). The Chk-1 kinase is also a target of the AKT-1 kinase, and Chk-1 has been implicated in the p53 DNA damage response pathway by phosphorylating the p53 protein under certain circumstances. This could create another feedback loop in the p53–IGF-1 pathways (Harris and Levine 2005).

Additional connections in the p53 and forkhead transcription factor pathways

The activation or deactivation and/or the selection of genes to be transcribed or repressed by the p53 and forkhead transcription factors are accomplished at least in part by protein modifications. Phosphorylation of forkhead proteins by the AKT and the SGK-1 (serum-glucocorticoid kinase) appears to occur on different amino acid residues and may result in some differences in gene regulation, but both kinases, when activated, remove forkhead proteins from the nucleus and presumably derepress a number of genes (Brunet et al. 1999, 2001). After DNA damage, p53 is activated, the AKT kinase is shut down in those cell types that transcribe the PTEN gene, but the SGK-1 kinase is induced and removes FOXO-3a from the nucleus. This induction of SGK-1 is mediated by the ERK-1/ERK-2 kinases in a p53-dependent fashion (You et al. 2004). In this case p53 regulates the removal of Foxo-3a from the nucleus. A second common feature in the p53 and Foxo pathways is the ability of the same HDAC, Sir2/Sirt-1 to deacetylate both p53 and forkhead (Luo et al. 2001; Vaziri et al. 2001; Brunet et al. 2004; Motta et al. 2004). It is thought that the acetylation of lysine residues on the p53 and forkhead transcription factors may alter their transcriptional activity or the genes they may transcribe. High levels of SIRT1/Sir2 increase longevity of the organism (yeast and worms), modulate (down) the activity of the IGF-1 pathway (worms), and impact the p53 pathway in ways that need to be further explored. Because Sirt-1 is an NAD-dependant HDAC, these activities are tied to cellular metabolic functions as shown in studies with yeast (Imai et al. 2000). These observations may well relate the role of caloric restriction to longevity and the response of p53 to glucose starvation. Sir2/SIRT1 is yet another connection in the abilities of both the IGF-1 pathway and the p53 pathway to impact on longevity of cells or organisms.

This review has outlined the many interconnections between the p53–IGF-1–AKT–TSC2–mTOR pathways. These connections fundamentally take four forms: (1) major connections that act rapidly (DNA damage–p53–AMPK–TSC2–TOR) in minutes or slowly (p53–PTEN–AKT; p53–TSC2–mTOR) in hours; (2) the formation of positive feedback loops that integrate these path-

ways (p53–PTEN AKT–MDM2–p53) and enhance p53-mediated apoptosis, and the formation of feedback loops that sense nutrient deprivation and integrate these signals with other pathways (minus glucose-AMPK–[p53 ser-15]–TSC2–mTOR–α-4–PP2A–p53–ser-15); (3) the possible formation of protein complexes that may well alter the activity of these protein subunits in these pathways; and (4) the possible formation of more transient protein complexes that act to enzymatically modify proteins and the outputs of these pathways (p300–FOXO3a and p300–p53), (SIRT1–p53 and SIRT1–FOXO3a). These are a few examples of how the cell mediates the interconnections and communication between pathways.

Conclusions, speculations, and tests of this proposal

Figure 2 and the text that reviews and amplifies it are constructed from a wide variety of observations in the literature. Because experimental systems and conditions differ between the observations made by many different research groups, it may not always be correct to connect processes and pathways in the way that is done here. In spite of that, this review creates an internally consistent network whose steps are supported by experimental observations, and this scheme has the virtue of predicting additional experiments that will surely result in its modification in the future. The scheme also points to critical places where we simply do not know enough. For example, it is not yet clear how p53 activates the AMPK activity. In addition, this network surely differs depending on the cell or tissue type under study or even the nature of the stress signal that initiates a p53 response. The concentrations of critical gene products and mediators of these pathways may well differ in different cell types and so the binding constants of complexes and the concentrations of the components could result in different outcomes in different tissues. In addition, redundant features in a pathway may well be different in different cell types leading to differences in which step is rate limiting or subject to mutational impact. These ideas could help to explain why very different genes in this integrated scheme are selected for mutation in cancers from different tissue types. If this notion is correct then as the types of mutations that occur in cancers from different tissues are classified and characterized, they will indicate which of these functions in a signal transduction pathway act in parallel (and are therefore backed up in function) and which functions act in series. It is already clear that different oncogenes and tumor suppressor genes are mutated in a tissue preferred fashion in the different types of cancers but we need more systematic studies to understand the meaning of this. Clearly, p53 regulates some genes in a tissue-specific fashion (PTEN and TSC2) and, therefore, communicates in novel ways with different pathways in different cell types. This scheme also points out that we need to know more about the transcriptional programs and the regulatory overlap of p53 and the forkhead transcription factors. We need to understand why the transcriptional machinery has some rate-limiting components shared by competing pathways. Finally, we need to understand the role of autophagy in cancer and its role during apoptosis.

The integration of the p53 pathway with the IGF-1 and TOR pathways brings together a number of overlapping concepts that play a central role in life processes. Selective mutations in the IGF receptor, AKT, and forkhead genes have been shown to enhance/reduce longevity in worms (Lin et al. 2001), flies (Giannakou et al. 2004; Hwangbo et al. 2004), and mice (Holzenberger et al. 2003). Mutations in the p53 gene enhance the life span of cells in culture by ignoring the signals of telomere shortening (loss of this checkpoint). Mice with hyperactive p53 proteins are cancer resistant but have short life spans and contain stem cells that show an early senescence phenotype (Tyner et al. 2002; Maier et al. 2004). In humans, higher MDM-2 levels can lead to cancers at earlier ages (Bond et al. 2004). On the other hand, mice with a hypomorphic MDM-2 gene product, and a more active p53 protein, were also resistant to tumor formation but had a normal longevity (Mendrysa et al. 2006). Clearly the relative levels of p53 and MDM-2 are critical to the phenotype under study and are themselves regulated by a variety of other gene products that can influence that phenotype (Poyurovsky and Prives 2006). Several of the positive and negative regulatory functions that influence p53 activity are part of the IGF–AKT and mTOR pathways (Fig. 2), and so it is not surprising that apparent contradictions in the literature about the role of the p53 pathway in longevity may be reconciled only when we have a better understanding of the interconnections between these networks. The role of Sir2/Sirt-1 in modulating the IGF-1 pathway in worms and in impacting the regulation of both the forkhead and p53 transcription factors is consistent with their roles in longevity. Longevity is always coupled with the age of attaining reproductive maturity in animals. The later in life that reproductive maturity occurs the longer the longevity of an animal. Animals will most often not reproduce in times of stress and starvation of nutrients (caloric restriction) and will shut down their reproductive processes. In worms starvation of bacterial food sources results in p53-mediated apoptosis in germ cells, again connecting these pathways (Derry et al. 2001). Indeed, p53 in adult worms and flies is predominantly localized in the germline where it is employed in the prevention of reproduction in response to stress signals such as DNA damage and starvation. Thus p53 has its origins, in an evolutionary sense, as a function for germline surveillance of starvation or DNA damage. It is only in vertebrates, where the body plan requires self renewal of tissues (flies and worms are largely post-mitotic as adults, except for the germline), where the p53 protein is found in somatic tissues and it takes on the function of a tumor suppressor. Yet there is some evidence for germ cell communication (in worms and flies signals from both sensory neurons and gonads impact on the IGF pathway) with the p53 pathway in vertebrates. This may be seen in observations of sexual dimorphism in the p53 pathway. Female p53 knockout mice can be born with an ex-encephalic condition (with a frequency that is strain-specific)

and die at birth. This does not happen in male mice and so there is a sex ratio distortion after birth (Armstrong et al. 1995; Sah et al. 1995). In addition female mice that are heterozygous for the p53 allele develop more osteosarcomas than do male mice of the same inbred strain. In Li-Fraumeni families of humans born with a p53 mutation in one allele, the females obtain tumors at an earlier age of onset than do the males in the same family, even excluding breast tumors (Hwang et al. 2003). In humans the MDM2 gene is at least in some circumstances under the regulation of the estrogen receptor. There is clearly some communication (hormonal) between the germline and the p53–IGF-1–TOR pathways as reflected in a wide variety of sexual dimorphisms in these pathways. These include differences in imprinting of the IGF-2 gene in the germline, the differences in longevity of males and females, and differences in the frequency of diabetes and specific types of cancer in males and females. We need to understand how the signals sent from male or female gonads, as well as sensory inputs, influence these combined signaling pathways and the outcomes of these networks.

In addition to longevity and communication with reproductive processes, the p53–IGF-1–TOR pathways sense nutrient levels in the environment and couple this to metabolic processes and mitochondrial function and dysfunction. The efficiency of converting 1 mol of glucose to 36 ATP molecules in oxidative phosphorylation declines with age and entropic forces. Our responses to a wide variety of intrinsic and extrinsic stresses are processed by the p53–IGF-1–TOR pathways and the efficiency of these responses are impacted by aging often with a resulting exponential rise in type 2 diabetes and cancers as a function of age. Our abilities to develop and rejuvenate our cell and tissue mass through the initiation and regulation of cell growth as well as the initiation and checkpoint regulation of cell division are regulated in part by the p53–IGF-1–TOR pathways, and these processes also decay with age. Individuals in a population that live longer often do so by slowing the rate of loss of all of the functions of these three pathways and largely living disease-free lives with longer reproductive capabilities as well as retaining somatic stem cell capabilities. Many of these life processes appear to be quite integrated. It will be of some interest to identify those polymorphisms in the genes that populate these three pathways in Figure 2 that make these pathways very efficient or function poorly. The sum of these polymorphisms interacting with our environment may well permit us to understand individual variations in longevity, reproductive capabilities, the development of cancers or diabetes, response to stress and therapies, and even the regeneration of some cell or tissue types.

Finally, as we begin to understand the interconnections between these pathways we will get better at rationally designing compounds to treat diseases that are caused by genetic mutations in these pathways. Replacing the p53 cDNA in a head and neck cancer cell using adenovirus gene therapy is already an approved use in China. Designing drugs that break the p53–MDM2 complexes in cells and activate p53-mediated apoptosis in these cancer cells are awaiting a clinical trial (Vassilev et al. 2004). Inhibitors of TOR and HDACs are presently being tested in humans as cancer therapies. As we examine the many negative feedback loops in the p53 pathway (Harris and Levine 2005), we can gain new insights for a rational approach to drug development. Can we inhibit cyclin G-PP2A phosphatase? Cyclin G is a p53-inducible gene that combines with PP2A to remove a phosphate residue from the MDM2 protein and this increases the MDM2 activity and decreases p53 levels in the cell (Okamoto et al. 2002). This is a negative feedback loop for p53, and blocking it with a drug would activate p53 and possibly kill cancer cells. Can we design a drug against the WIP1 phosphatase? The WIP1 gene is amplified in 11% of breast cancers and it is a p53-regulated gene (Bulavin et al. 2004). It can remove a phosphate residue from the p38 MAPK, which results in its inactivation. The p38 MAPK acts to phosphorylate p53 at serine residues 33 and 46 resulting in a proapoptotic p53 response. Thus inhibition of WIP1 would activate p53 in cancer cells. Can we inhibit the MDM2 ubiquitin ligase activity and block p53 degradation in cancer cells with amplifications of the MDM2 gene? The pathway outlined in Figure 2 provides many targets. If drugs are produced against these target proteins and they work as predicted, this process will validate the pathway as drawn. When these drugs, inevitably, do unexpected things, we will have to revise this scheme and learn from our mistakes.

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Coordination and communication between the p53 and IGF-1–AKT–TOR signal transduction pathways

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