Front. Biol. 2011, 6(1): 58–68
DOI 10.1007/s11515-011-0970-8

REVIEW

Tumor suppressor p53: new functions of an old protein

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Abstract **p53 was discovered 30 years ago. Extensive studies have been done on p53 since then, which makes *p53* one of the most extensively studied genes. p53 has long been recognized as a key tumor suppressor. Cell cycle arrest, apoptosis and senescence have been traditionally recognized as the main functions of p53 in tumor suppression. Recently, some novel functions of p53 have been identified, including the regulation of energy metabolism, antioxidant defense, and microRNA expression and maturation, which all contribute to the role of p53 in tumor suppression. Furthermore, the contribution of p53 to normal biologic processes (e.g. reproduction and aging) and some other aspects of diseases (e.g. neurodegenerative diseases) is only now being appreciated. Here we will review recent advances in the study of some new functions of p53.**

Keywords p53, tumor suppressor, energy metabolism, oxidative stress, microRNAs, reproduction

Introduction

p53 was discovered 30 years ago as a cellular partner of SV40 large T-antigen. During these 30 years, over 50,000 PubMed-listed publications have been published on p53, which makes p53 one of the most extensively studied genes. These studies have clearly established the role of p53 as a key tumor suppressor and the "guardian of the genome" (Levine et al., 2006; Levine and Oren, 2009; Vousden and Prives, 2009; Feng and Levine, 2010). However, the concept that p53 is a key tumor suppressor did not come easily. p53 had been regarded as a cellular oncogene for almost a decade since its discovery. Only in late 1980s, p53 was identified as a tumor suppressor which is frequently mutated in human tumors. The second decade of research leads to the concept that p53 is a transcription factor, which can be activated by stress signals and starts cell cycle arrest, apoptosis and senescence through the transcriptional regulation of a group of target genes to exert its role as a tumor suppressor (Levine and Oren, 2009). Only recently, studies have revealed some novel functions for p53 in tumor suppression, including the regulation of energy metabolism, antioxidant defense, and microRNA (miRNA) expression and maturation. In addition to its role in tumor suppression, emerging evidence has also demonstrated that p53 plays important roles in normal biologic processes, such as reproduction, development and aging, and some other aspects of diseases, such as neurodegenerative diseases and diabetes (Levine et al., 2006; Levine and Oren, 2009; Vousden and Prives, 2009; Feng and Levine, 2010).

Received September 30, 2010; accepted November 2, 2010
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p53 and its signaling pathway

As the "guardian of the genome," p53 plays a critical role in maintaining genomic stability and tumor suppression. *p53* is the most frequently-mutated gene in human tumors (Levine et al., 2006; Levine and Oren, 2009; Vousden and Prives, 2009; Feng and Levine, 2010). p53 mutations occur in almost every type of cancer and in over 50% of all tumors. It was estimated that over 80% of tumors have dysfunctional p53 signaling, including tumors with rare p53 mutations (Olivier et al., 2004; Levine et al., 2006). In cervical cancer with a low mutation rate of p53, p53 is often inactivated by human papillomavirus (HPV) oncoprotein E6, which binds to and degrades p53 protein (Scheffner et al., 1990). p53 can be inactivated by DNA amplification and/or overexpression of MDM2, a critical negative regulator of p53 which can bind to and degrade p53 protein through ubiquitiation, in many

tumors including sarcoma (Bond et al., 2005; Wade and Wahl, 2009). p53 mutations and MDM2 amplification and/or overexpression usually are mutually exclusive in cancers (Bond et al., 2005; Wade and Wahl, 2009). Disruption of normal p53 function is often a prerequisite for the development or progression of tumors (Donehower et al., 1992; Jacks et al., 1994). For example, *p53* null mice all succumb to various tumors within several months, and heterozygous *p53* mutant mice develop tumors over a period of a year or more (Donehower et al., 1992; Jacks et al., 1994). Li–Fraumeni syndrome patients with germline heterozygous *p53* gene display a 50% cancer incidence by the age of 30 (Strong, 2003). Furthermore, it has recently been shown that the naturally existing single nucleotide polymorphisms (SNPs) in p53 (codon 72) and in the promoter of *Mdm2* (SNP309), which can slightly decrease p53 activity, have significant impacts upon tumorigenesis in humans (Bond et al., 2004; Murphy, 2006; Hu et al., 2007a).

In normal unstressed cells, p53 is maintained at a low level by rapid degradation through the ubiquitin-proteasome pathway. As an E3 ubiquitin ligase, MDM2 is a most critical negative regulator for p53 (Harris and Levine, 2005; Brooks and Gu, 2006). p53 responds to a wide variety of stress signals, including various DNA damages, hypoxia, ribonucleotide and nutritional depletion, and oncogene activation (Fig. 1). These signals activate p53 primarily through post-translational modifications by a wide variety of enzymes, which lead to the increase of p53 protein half-life and therefore p53 protein accumulation in cells. These enzymes include kinases (e.g. ATM, ATR), phosphatases (e.g. PP2A, Wip1), acetyltransferases (e.g. p300, CBP), deacetylases (e.g. SIRT1, HDAC), ubiquitin Ligases (e.g. MDM2, Cop1, Pirh2), deubiquitinases (e.g. HAUSP), methylases (e.g. Set9), and sumoylases (e.g. PIAS-1, Ubc9) (Levine et al., 2006; Feng and Levine, 2010). For example, in response to gamma-irradiation, p53 and MDM2 are phosphorylated by the ATM kinase, which leads to the dissociation of p53 from MDM2 and resultant p53 protein accumulation in cells. As a transcription factor, p53 mainly exerts its functions through its transcriptional regulation of many target genes. Over a hundred of p53 target genes have been identified so far. Once activated, p53 protein binds to a specific DNA sequence, termed the p53-responsive element (RE) to regulate p53 target genes. A p53-RE is composed of RRRCWWGYYY (spacer of 0–21 nucleotides) RRRCWWGYYY, where R is a purine, W is A or T, and Y is a pyrimidine (el-Deiry et al., 1992). Depending on cell type, environmental context, and/or degree of stress, p53 selectively regulates a set of its target genes to start various cellular responses, including cell cycle arrest and senescence, or apoptosis. To date, regulating cell cycle arrest and apoptosis are most well-understood functions of p53 (Levine et al., 2006; Vousden and Prives, 2009).

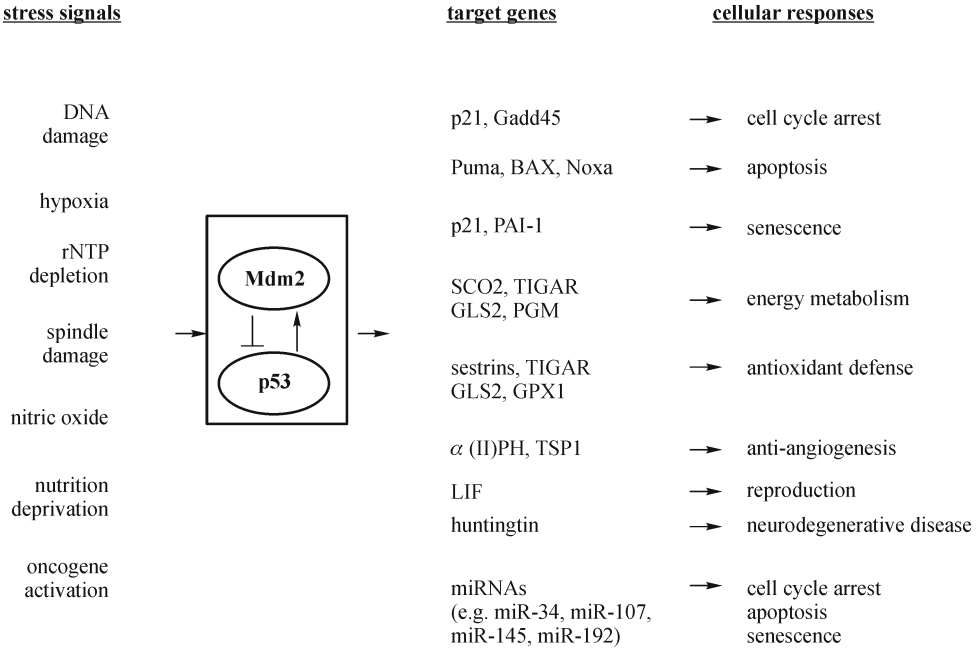

Figure 1 Tumor suppressor p53 and its signaling pathway. In response to a wide variety of intracellular and extracellular stress signals, p53 is activated, which leads to the accumulation of p53 in cells. Depending on cell type, environmental context, and the type and/or degree of stress, activated p53 selectively transcribes a set of its target genes (shown are representative genes) and initiates various cellular responses. p53 exerts its function in tumor suppression through its regulation of cell cycle arrest, apoptosis, senescence, energy metabolism, antioxidant, anti-angiogenesis, and miRNAs as well. In addition, p53 plays important roles in normal biologic processes, such as reproduction, and some other aspects of diseases, such as neurodegenerative diseases.

Through these responses, p53 functions to stop cells from dividing, thereby allowing for DNA repair to occur, or induce damaged cells to die, thereby eliminating severely damaged cells from the replicative pool. Regardless of whether the damaged cell is arrested and repaired, killed, or made senescent, the end result is the same: the organism is protected from propagation of mutation-bearing cells that could potentially become cancerous (Levine et al., 2006; Vousden and Prives, 2009; Feng and Levine, 2010).

Novel functions of p53

For almost a decade, cell cycle arrest, apoptosis and senescence were thought of as the major functions of p53 (Levine et al., 2006; Levine and Oren, 2009; Vousden and Prives, 2009). With the identification of a set of new p53 target genes, recent studies have revealed some additional novel functions for p53 (Fig. 1). For example, p53 regulates metabolic pathways, including IGF-1/AKT and AMPK/mTOR pathways, and prevents cell growth and division in response to stress (Feng et al., 2005; Feng et al., 2007a; Budanov and Karin, 2008; Feng, 2010). p53 regulates energy metabolism by promoting oxidative phosphorylation in mitochondria and reducing the activity of glycolysis (Bensaad et al., 2006; Matoba et al., 2006; Bensaad and Vousden, 2007; Hu et al., 2010b). p53 reduces ROS levels and exerts an antioxidant defense function, which has been shown to be a main component of p53's role as a tumor suppressor (Budanov et al., 2004; Sablina et al., 2005). Furthermore, p53 inhibits angiogenesis and metastasis, two critical steps in tumorigenesis (Teodoro et al., 2006; Teodoro et al., 2007). Recently, it has been reported that p53 regulates the expression and maturation of microRNAs (miRNAs) with tumor suppressive functions. All these functions of p53 contribute to p53's role in tumor suppression. In addition to its role in tumor suppression, p53 also functions in a number of important biologic processes. For example, p53 is required for the implantation of the embryo into the uterus (Hu et al., 2007b; Hu et al., 2008), as well as the normal development of embryo (Sah et al., 1995; Choi and Donehower, 1999). p53 function was also identified as an important checkpoint during the multifactor reprogramming process in which induced pluripotent stem (iPS) cells are derived from differentiated adult cells (Hong et al., 2009; Marión et al., 2009). Furthermore, an interesting, albeit confusing, role of p53 in aging has also come to our attention (Tyner et al., 2002; Mendrysa et al., 2006; Feng et al., 2007b; Feng et al., 2008). Recent studies also show that p53 is involved in some other aspects of diseases besides cancer. For example, p53 activation by mutant huntingtin contributes to Huntington's disease, a type of neurodegenerative disease (Bae et al., 2005). On the other hand, p53 induces the expression of Huntingtin in response to stress and may further exacerbate the progress of the disease (Feng et al., 2006). Interestingly, p53 is also involved in diabetes; p53-mediated senescence has been reported to contribute to the development of insulin resistance in mice (Minamino et al., 2009). In this review, we will focus on the roles of p53 in energy metabolism, antioxidant defense, miRNA regulation, and reproduction.

p53 and energy metabolism

Maintaining the proper energy metabolism is crucial to normal cell growth and division. One of the hallmarks of tumor cells is the metabolic alterations (Garber, 2006; Hsu and Sabatini, 2008). While majority normal differentiated cells utilize mitochondrial oxidative phosphorylation to provide energy, majority tumor cells utilize aerobic glycolysis for their energy needs, a switch known as the Warburg effect (Warburg, 1956). Because glycolysis produces ATP much less efficiently than aerobic respiration, tumor cells compensate by having a much higher rate of glucose uptake and utilization than normal cells. Based on the Warburg effect, Positron Emission Tomography (PET) has been established and widely used for tumor detection because tumors take much more glucose analog ^{18}flurodeoxyglucose than adjacent normal tissues (Gambhir, 2002). However, the physiologic significance of the Warburg effect has been elusive since its discovery over 70 years ago, and the underlying molecular mechanisms are not well understood. Recently, metabolic changes in tumors have been identified as a possible key contributor to malignant progression by conferring tumor cells advantages of proliferation and survival. Emerging evidence has shown that reversing the Warburg effect in tumor cells greatly compromises the tumorigenicity of cancer cells, which suggests that targeting the metabolic changes could be an effective strategy for cancer treatment (Garber, 2006; Hsu and Sabatini, 2008). The activation of several oncogenes in cancer cells has been shown to contribute to the Warburg effect, including *Myc* and *Akt*, and *HIF-1* (Bensaad and Vousden, 2007; Hsu and Sabatini, 2008).

Recent studies have revealed a new function for p53 in the regulation of energy metabolism. p53 was shown to respond to nutritional starvation (e.g. glucose starvation), and prevent cell proliferation and division in response to stress (Feng et al., 2005; Jones et al., 2005). This effect of p53 can be achieved through the inhibition of PI3K/AKT and AMPK/mTOR pathways, two critical signaling pathways in regulating energy metabolism and promoting cell growth and proliferation. p53 upregulates several target genes in these 2 pathways, including *PTEN*, *IGF-BP3*, *AMPK* beta subunits, *TSC2* and *Sestrins 1* and *2*, whose products can all lead to the downregulation of these 2 pathways (Feng et al., 2005; Feng et al., 2007a; Budanov and Karin, 2008;). This activity of p53 can also enhance autophagy (Feng et al., 2005), a mechanism to allow cell survival by consuming organelles in cells under the condition of nutritional starvation, through the upregulation of p53 target genes *DRAM*, *Bax* and *Puma* (Crighton et

al., 2006; Yee et al., 2009).

Recent studies further show that p53 can reduce glycolysis and promote mitochondrial oxidative phosphorylation in cells (Fig. 2). Loss of p53 results in decreased oxygen consumption and impaired mitochondrial respiration, and promotes a switch to high glucose utilization in aerobic glycolysis in cultured cells and mouse tissues (Matoba et al., 2006). p53 can induce the expression of several genes to regulate energy metabolism, including SCO2 (synthesis of cytochrome c oxidase 2), TIGAR (TP53-induced glycolysis and apoptosis regulator), and GLS2 (glutaminase 2). The SCO2 gene is a key regulator of the cytochrome c oxidase complex that is essential for mitochondrial respiration. p53 induces the expression of SCO2 to ensure the maintenance of the cytochrome c oxidase complex, thereby enhancing mitochondrial respiration (Matoba et al., 2006). TIGAR functions to lower the intracellular levels of fructose-2, 6-bisphosphate, thereby slowing glycolysis and directing glucose to an alternative pathway, the pentose phosphate pathway (PPP) (Bensaad et al., 2006). Recently, we identified *GLS2* as a novel p53-regulated gene (Hu et al., 2010b). *GLS2* encodes a mitochondrial glutaminase that catalyzes the hydrolysis of glutamine to glutamate. GLS2 regulates cellular energy metabolism by increasing the production of glutamate and α-ketoglutarate, which in turn results in enhanced mitochondrial respiration and ATP generation in cells. In addition, p53 may regulate mitochondrial respiration through its regulation of the expression of the ribonucleotide reductase subunit p53R2, which is important to maintain mitochondrial DNA levels (Kulawiec et al., 2009). Loss of p53R2 can result in decreased mitochondrial DNA levels, and therefore impairs mitochondrial function in cells (Bourdon et al., 2007).

In addition to the upregulation of gene products that promote mitochondrial respiration and/or decrease glycolysis, p53 also reduces gene products that enhance glucose uptake and glycolysis in cells (Fig. 2). p53 decreases the glucose uptake through repressing the expression of glucose transporters (GLUTs) in cells. For example, p53 directly represses the transcriptional expression of GLUT1 and GLUT4 (Schwartzenberg-Bar-Yoseph et al., 2004). At the same time, p53 inhibits the activity of IκB kinase alpha and beta (IKKα and IKKβ), resulting in the reduced activity of NF-κB, which in turn reduces the expression of GLUT3 (Kawauchi et al., 2008). Furthermore, p53 promotes the ubiquitiation and degradation of PGM protein (phosphoglycerate mutase), an important enzyme acting at the later stage of glycolysis pathway, through an unknown mechanism. Loss of p53 results in the increased PGM expression, thereby enhancing glycolysis (Kondoh et al., 2005). These findings together link the p53 protein with energy metabolism, and strongly suggest that loss of p53 function is a novel mechanism that contributes to the Warburg effect in tumors.

p53 and oxidative stress

Cells of the organisms living in aerobic conditions are constantly subjected to reactive oxygen species (ROS), the natural byproducts of the metabolism of oxygen generated to a large extent in mitochondria (Dröge, 2002). ROS are highly

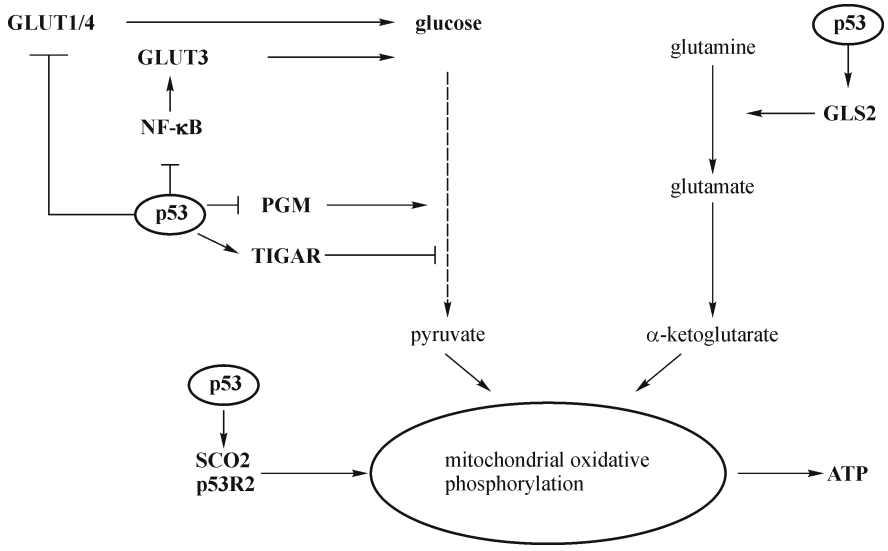

Figure 2 The regulation of energy metabolism by p53. p53 induces the expression of TIGAR, and inhibits the expression of PGM, resulting in the inhibition of glycolysis. p53 induces SCO2, GLS2 and p53R2 to enhance mitochondrial oxidative phosphorylation. Furthermore, p53 represses the expression of GLUT1 and GLUT4, and inhibits the NF-κB pathway to reduce the expression of GLUT3, which can all reduce glucose uptake and glycolysis. Thus, p53 regulates energy metabolism through inhibiting glycolysis and enhancing mitochondrial oxidative phosphorylation in cells.

reactive intermediates capable of modifying numerous biologic substrates. Endogenous ROS levels are the major source of DNA damage, and contribute significantly to DNA mutations and chromosome instability. It is estimated that the endogenous ROS modify 20,000 bases of DNA per day in a single cell. Increased intracellular ROS levels have been shown to contribute to cancer initiation and progression (Benhar et al., 2002; Nicholls, 2002). Several antioxidant defense pathways exist to keep ROS levels low in cells. They include superoxide dismutase (which converts superoxide to hydrogen peroxide), catalase and glutathione peroxidase (which convert hydrogen peroxide to water), peroxiredoxins (Prdxs) (which use thioredoxin as an electron donor, and scavenge peroxide), as well as non-enzymatic scavengers such as glutathione, ascorbic acid and carotenoids (Benhar et al., 2002; Halliwell, 2007). Loss of these mechanisms has been shown to result in genetic instability and cancer initiation and progression (Benhar et al., 2002; Ho et al., 2004; Lu et al., 2007). For example, knockout of CuZnSOD (copper and zinc-containing superoxide dismutase) or loss of one allele of MnSOD (manganese-containing superoxide dismutase) increased rates of various tumors in mice (Van Remmen et al., 2003; Elchuri et al., 2005). Simultaneous knockout of two glutathione peroxidases, GPx1 and GPx2, promoted the development of intestinal cancers in mice (Chu et al., 2004), whereas *prdx1* knockout promoted the development of lymphomas, sarcoma and adenoma in mice (Neumann et al., 2003).

Interestingly, p53 has both pro-oxidant and antioxidant activities, each of which contributes to tumor suppression (Fig. 3). It has been demonstrated that high levels of ROS lead to oxidative stress, which results in p53-mediated cell cycle arrest and apoptosis (Martindale and Holbrook, 2002; Bensaad and Vousden, 2007). At the same time, p53 can induce the expression of several pro-oxidant genes, including *PIG3*, *PIG6*, and *FDXR*, *Bax* and *Puma*, whose products can all increase intracellular ROS levels and sensitize the cells to oxidative stress (Liu and Chen, 2002; Martindale and Holbrook, 2002; Rivera and Maxwell, 2005; Lyakhov et al., 2008). Thus, under severe oxidative stress conditions, p53 can further induce ROS levels in cells, which in turn induces apoptosis and senescence to eliminate damaged cells and thereby preventing DNA mutations and tumorigenesis.

In contrast to this role of p53 in the induction of ROS in cells, recent studies have revealed a new role of p53 in modulating up cellular antioxidant defense mechanisms, especially under conditions of nonstress or low stress. This antioxidant activity plays an important role in the overall tumor suppressor function of p53. It has been demonstrated that ROS elevation, connected with the deficiency in p53, dramatically increases DNA oxidation and the rate of mutagenesis. These effects could be substantially reversed by overexpression of Sestrins, p53-regulated antioxidant genes. Furthermore, dietary supplementation with antioxidant N-acetylcysteine substantially improves karyotype stability and prevents malignant lymphomas in $p53^{-/-}$ mice, demonstrating that the antioxidant role is an important component of p53 as a tumor suppressor (Budanov et al., 2004; Sablina et al., 2005).

To exert its function in antioxidant defense, p53 promotes the expression of a group of antioxidant proteins that function to lower ROS levels in addition to Sestrins (Budanov et al.,

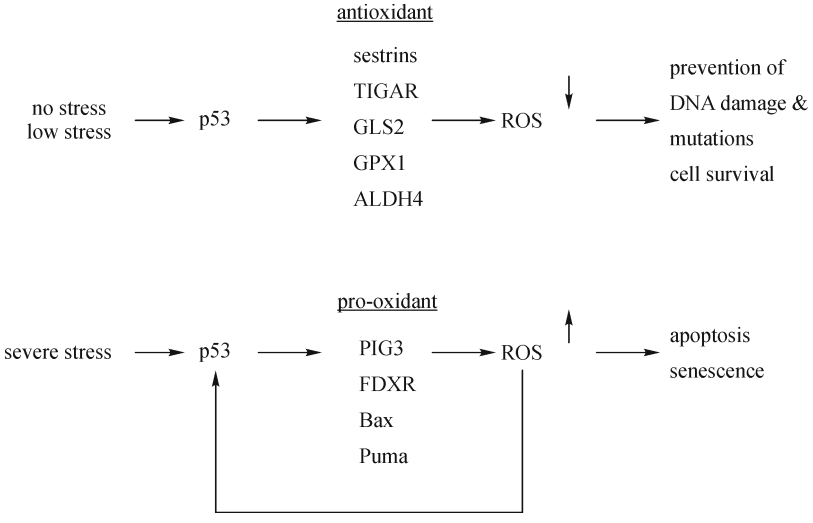

Figure 3 The regulation of oxidative stress by p53. p53 has both pro-oxidant and antioxidant activities, each of which contributes to tumor suppression. It appears that p53 selectively regulates a group of antioxidant genes, such as *Sestrins*, *TIGAR*, *GLS2*, *GPX1* and *ALDH4*, to downregulate ROS levels in cells under the conditions of nonstress or low stress. This antioxidant function protects cells from oxidative stress-induced DNA damage and mutations, and allows cell survival. In response to severe stress, p53 selectively regulates a group of pro-oxidant genes, including *PIG3*, *FDRX*, *Bax* and *Puma*, to further increase ROS levels in cells. This pro-oxidant function will in turn lead to the further activation of p53, resulting in the p53-mediated apoptosis and senescence to eliminate damaged cells.

2004) (Fig. 3). They include TIGAR (Bensaad et al., 2006), GLS2 (Hu et al., 2010b; Suzuki et al., 2010), GPX1 (glutathione peroxidase) (Tan et al., 1999), and ALDH4 (aldehyde dehydrogenase 4) (Yoon et al., 2004). Sestrins are a family of proteins required for regeneration of peroxiredoxins, which are a family of thiol-containing peroxidases and major reductants of endogenously produced peroxides in eukaryotes (Budanov et al., 2004). GPX1 is a primary antioxidant enzyme that scavenges hydrogen peroxide or organic hydroperoxides in cells. As a mitochondrial-matrix protein, ALDH4 is a NAD^+ -dependent enzyme catalyzing the proline degradation pathway, which lowers intracellular ROS levels through the regulation of proline metabolism (Yoon et al., 2004). TIGAR diverts glucose through the pentose phosphate pathway that produces more NADPH to help lower ROS levels (Bensaad et al., 2006). Recently, our laboratory and another laboratory independently identified *GLS2* as a p53-regulated gene involved in the regulation of intracellular ROS levels, which provides further direct evidence to support the important role of p53 in antioxidant defense in cells (Hu et al., 2010b; Suzuki et al., 2010). GLS2 increases the GSH levels and reduces ROS levels in cells through the increased levels of intercellular glutamate, a precursor of GSH. As one of the most important antioxidant molecules and a scavenger for ROS, increased GSH levels in cells decrease intracellular ROS levels. Furthermore, GLS2 increases the intracellular levels of NADH, which is another important antioxidant in cells (Hu et al., 2010b). These effects contribute to GLS2's function in lowering ROS levels in cells. Ectopic expression of *GLS2* protects cells from oxidative stress (H_2O_2)-induced cell death, whereas knockdown of endogenous *GLS2* promotes H_2O_2-induced cell death. Thus, under conditions of nonstress or low stress, p53 regulates the expression of these anti-oxidant genes and lowers the ROS levels in cells, which prevents oxidative stress-induced DNA damage and mutations, and promotes cell survival as well.

p53 and microRNAs

As a transcription factor, p53 mainly exerts its function through its transcriptional regulation of protein-coding target genes to initiate cellular responses. Recent studies have shown that p53 can exert its function through inducing the expression of miRNAs, the noncoding genes, as a new mechanism for p53 in tumor suppression (Chang et al., 2007; He et al., 2007; Raver-Shapira et al., 2007). microRNAs (miRNAs) are a class of endogenously expressed, small (20–25 nucleotide) noncoding regulatory RNA molecules, which play a key role in the post-transcriptional regulation of gene products (Pillai et al., 2007; Bartel, 2009). miRNAs pair with partially complementary sites in the 3′-untranslated regions (3′-UTRs) of target mRNAs, leading to translational repression and mRNA degradation. miRNAs are a class of most abundant regulatory genes in humans; over 700 human miRNAs have been identified so far, and the total number of miRNAs is estimated to be close to 1000. However, relatively few miRNA-target interactions have been experimentally validated, and the biologic functions of most miRNAs remain to be discovered (Lim et al., 2005; Bartel, 2009). miRNAs have been suggested to play important roles in a wide array of biologic processes including development and differentiation, cell proliferation, apoptosis and metabolism, all of which are often perturbed in tumors. Emerging evidence has demonstrated that miRNAs play important roles in tumorigenesis as either tumor suppressor genes or oncogenes (Calin and Croce, 2006; Kent and Mendell, 2006).

The interaction between p53 and miRNAs was first demonstrated through the identification of miR-34 family members as direct p53 target genes (Fig. 4). In 2007, several groups independently reported that p53 can regulate the expression of the miR-34 family members, miR34a/b/c, through direct binding to the p53 responsive elements in the promoters of miRNAs (Chang et al., 2007; He et al., 2007; Raver-Shapira et al., 2007; Tazawa et al., 2007). miR-34 family members repress the expression of several targets involved in the regulation of cell cycle and in the promotion of cell proliferation and survival, including cyclin E2, cyclin-dependent kinases 4 and 6 (CDK4 and CDK6), and anti-apoptotic BCL2 protein. Ectopic expression of miR-34 promotes p53-mediated apoptosis, cell cycle arrest, and senescence, whereas inactivation of endogenous miR-34 strongly inhibits p53-dependent apoptosis in cells. Decreased expression of miR-34 has been frequently observed in various tumors (Chang et al., 2007; Tazawa et al., 2007), suggesting that loss of miR-34 could promote tumorigenesis. In addition to miR-34 family, p53 also directly induces the transcriptional expression of several additional miRNAs, including miR-145, miR-107, miR-192 and miR-215. Decreased expression of these miRNAs has been observed in different tumors. The induction of these miRNAs by p53 in response to stress signals has been shown to contribute to the functions of p53 in tumor suppression, including inducing apoptosis and cell cycle arrest. These findings demonstrated that in addition to many protein-encoding genes, miRNAs, the non-coding genes, can also be regulated by p53 as a new group of p53 target genes. Thus, miRNAs are involved in the composition of the complex p53 signaling pathway and contribute to the role of p53 in tumor suppression.

In addition to the transcription regulation of specific miRNAs, p53 also promotes the post-transcriptional maturation of specific miRNAs (Suzuki et al., 2009) (Fig. 4). RNase III endonuclease Drosha plays a critical role in the processing of primary miRNA (pri-miRNA) transcripts into pre-miRNAs. For the maturation of some miRNAs (but not all), Drosha requires the involvement of RNA-associated proteins such as the DEAD box RNA helicases p68 and p72 to carry out its function (Bartel, 2009; Pillai et al., 2007). It was recently reported that p53 promotes the Drosha-mediated

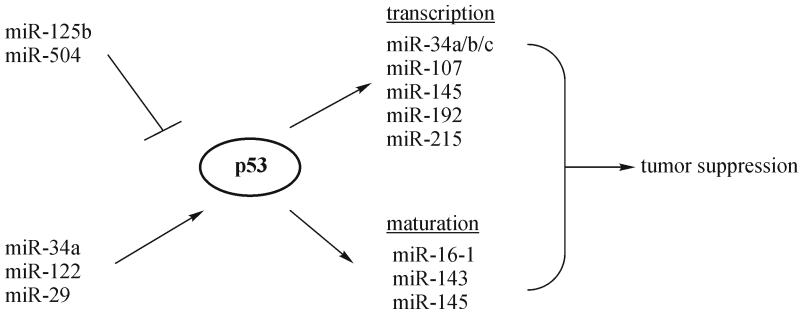

Figure 4 The interaction between p53 and miRNAs. p53 induces the expression of a set of miRNAs, including miR-34a/b/c, miR-145, miR-107, miR-192 and miR-215, which can all contribute to p53's role in tumor suppression as a new group of p53 target genes. p53 also promotes the maturation of a set of miRNAs with tumor suppressive functions, including miR-16-1, miR-143, miR-145. On the other hand, multiple miRNAs directly or indirectly regulate the activity and function of p53. For example, miR-125b and miR-504 directly downregulate p53 protein levels and functions in apoptosis and cell cycle arrest. miR-34a, miR-122 and miR-29 upregulate p53 activity and function through their repression of negative regulators of p53.

processing of certain miRNAs with growth-suppressive function in cells in response to stress signals (e.g. DNA damage) (Suzuki et al., 2009). This function of p53 is mediated by the interaction of p53 with Drosha, and furthermore, this interaction requires p68 and p72. These miRNAs regulated by p53 include miR-16-1, miR-143 and miR-145, which are decreased in various human cancers. Ectopic expression of these miRNAs in cells reduces tumor cell proliferation. These miRNAs negatively regulate some important regulators of the cell cycle and cell proliferation, such as K-Ras (as a target of miR-143) and CDK6 (as a target of miR-16 and miR-145). DNA mutations in p53 gene, such as R175H and R273H which are frequently observed in tumors, can lead to the decreased processing of pri-miRNAs by Drosha and decreased levels of mature miRNAs in cells, including miR-16-1, miR-143 and miR-145. These findings suggest that transcription-independent modulation of miRNA biogenesis is intrinsically embedded in a tumor suppressive program governed by p53, which provides a new mechanism by which p53 mutation contributes to cancer.

The interaction between p53 and miRNAs has been further demonstrated by the regulation of p53 and its pathway by miRNAs (Fig. 4). miR-125b has been recently reported to be a miRNA targeting p53 in both zebrafish and humans (Le et al., 2009). Overexpression of miR-125b decreases p53 protein levels and reduces apoptosis in cells, whereas knockdown of miR-125b increases p53 protein levels and induces apoptosis in human cells and zebrafish brain. Our recent study identified miR-504 as a novel miRNA which can negatively regulate p53 expression through its binding to human p53 3′-UTR (Hu et al., 2010a). Ectopic expression of miR-504 in colon cancer cells reduces p53 protein levels and impairs p53 functions, especially p53-mediated apoptosis and G1 cell cycle arrest. Furthermore, miR-504 overexpression promotes tumorigenicity of colon cancer cells in vivo (Hu et al., 2010a). These findings demonstrate that p53 is subjected to the negative regulation of specific miRNAs, which is a novel mechanism for cells to regulate p53 protein levels and functions.

In addition to the direct negative regulation of p53 protein by miRNAs, recent studies also show that p53 can be indirectly regulated by several miRNAs through their regulation of regulators for p53 in cells, including miR-34a, miR-29 and miR-122 (Fig. 4). miR-34a, a transcription target of p53 protein, was found to positively regulate p53 activity and function in apoptosis through its direct repression of SIRT1, which physically interacts with p53 and deacetylates p53 and thereby reduces p53 activity (Yamakuchi et al., 2008). miR-29 family members (miR-29a, miR-29b and miR-29c) were reported to upregulate p53 protein levels and induce p53-mediated apoptosis through their direct suppression of p85α, a regulatory subunit of PI3 kinase (PI3K) (Park et al., 2009). The PI3K/AKT pathway negatively regulates p53 activity through the direct phosphorylation and activation of MDM2 by AKT (Zhou et al., 2001). miR-122 increases p53 protein levels and activity through its negative regulation of cyclin G1, which forms complex with PP2A to enhance MDM2 activity and inhibit p53 (Fornari et al., 2009). These findings demonstrate that miRNAs are a new group of regulators for p53, joining a panel of kinases (e.g. ATM, ATR), ubiquitin ligases (e.g. MDM2, Cop1, Pirh2), and phosphotases (e.g. Wip1, PP2A) to tightly regulate the levels and activity of p53 in cells (Harris and Levine, 2005; Levine et al., 2006).

p53 and reproduction

In addition to p53's role in tumor suppression, recently, we have shown that p53 has an unanticipated, but essential, role in regulating maternal reproduction (Hu et al., 2007b). p53 is conserved from invertebrates to vertebrates. Orthologs of p53 have been identified in *C. elegans*, *Drosophila*, zebrafish and frogs. The existence of p53 in short-lived organisms with no occurring of cancers in the adult, such as flies and worms,

suggests that tumor suppression is not the original function for p53 and its pathway (Hu, 2009). It has been suggested that the evolutionary origin of the p53 protein in lower organisms utilizes its functions to protect the germ line from DNA damage and mutations. In *Drosophila* and *C. elegans*, p53 is most commonly expressed in the germ cells, and functions in the surveillance of damaged germ cells to eliminate defective offspring. In mouse embryos, p53 is expressed at a high level until the midgestation stage, and the p53-dependent DNA damage responses (e.g. apoptosis) are highly active throughout this period of development. p53 wild type embryos treated with ionizing irradiation show a p53-dependent apoptosis, resulting in a high percentage of death to efficiently eliminate the damaged offspring, whereas $p53^{-/-}$ embryos have a very small percentage of death and a high percentage of developmental abnormalities (Norimura et al., 1996).

Our recent work has shown that p53 deficiency results in a significant decrease in the fertility rate in female mice, but not male mice, with a poor pregnancy rate and a small litter size (Hu et al., 2007b). This is mainly caused by the impaired blastocyst implantation in the uterus in $p53^{-/-}$ mice. This function of p53 is mediated by leukemia inhibitory factor (LIF), a novel p53 target gene. LIF is a multi-functional cytokine, which plays an essential role in blastocyst implantation. Implantation is a stage critical in mammalian embryonic development during which the blastocyst establishes a close interaction with the uterine tissues, leading to the formation of the placenta to support the growth and development of the fetus. $LIF^{-/-}$ female mice are infertile due to the defect in implantation, which can be rescued by injection of exogenous LIF to these mice at day 4 of pregnancy, the onset stage of implantation in mice. p53 upregulates the expression of LIF under both stress and nonstress conditions. $p53^{-/-}$ female mice have decreased uterine LIF expression levels, especially at the onset stage of implantation, which may contribute to the impaired implantation. Indeed, the impaired blastocyst implantation and the following poor pregnancy rate and a small litter size in $p53^{-/-}$ female mice can be rescued by injection of exogenous LIF at implantation stage (Hu et al., 2007b). These observations clearly demonstrate an important role of p53 in maternal reproduction in mice.

Besides a decreased fertility rate in $p53^{-/-}$ female mice, a substantial fraction of the female $p53^{-/-}$ embryos also exhibit embryonic defects, including a neural tube closure defect called exencephaly, upper incisor fusion, ocular abnormalities and polydactyly of the hindlimbs. The mechanisms underlying these fatal defects in a significant fraction of $p53^{-/-}$ embryos are unclear but they are reminiscent of the function of p53 in fetal development in other organisms. While the LIF injection improved embryonic implantation in $p53^{-/-}$ female mice, similar birth defects were still observed in a substantial fraction (~30%) of mice born from $p53^{-/-}$ females with the LIF injection, indicating that p53 plays a role in post-implantation development as well (Hu et al., 2007b).

Interestingly, p53 also affects the efficiency of human reproduction. Implantation is a pivotal event in pregnancy in humans, and LIF also plays an important role in implantation. Recent studies from our laboratory and some other laboratories show that the naturally existing SNPs in the p53 and p53 signaling pathway which affect the activity and function of p53 impact significantly upon the efficiency of human reproduction (Hu, 2009; Kang et al., 2009). For example, the p53 allele encoding proline at codon 72 (P72) was found to be significantly enriched over the allele encoding arginine (R72) among *in vitro* fertilization (IVF) patients. The P72 allele serves as a risk factor for implantation failure. LIF levels are significantly lower in cells with the P72 allele than in cells with the R72 allele, which could contribute to the decreased implantation and fertility associated with the P72 allele (Kang et al., 2009; Kay et al., 2006). Selected alleles in SNPs in other genes in p53 pathway which regulate p53 levels in cells, including *Mdm2*, *Mdm4*, and *Hausp* genes, are also enriched in IVF patients. The association of SNPs in the p53 pathway with human fertility suggests that p53 regulates the efficiency of human reproduction. These results also provide a plausible explanation for the evolutionary positive selection of some alleles in the p53 pathway.

Perspectives

Recent studies have revealed that p53 induces various cellular responses in addition to cell cycle arrest, apoptosis and senescence to exert its function in tumor suppression. Furthermore, p53 plays important roles in normal biologic processes (e.g. reproduction and longevity) as well as various pathological processes (e.g. neurodegenerative diseases and diabetes) in addition to its role in cancers. At the same time, these studies have led to the growing complexity of p53. Future studies on p53 should see more detailed description of the roles of p53 in above-mentioned biologic processes, and some additional novel roles of p53 as well.

Since p53 is dysfunctional in most human cancers, it seems clear that manipulating p53 signaling will bring therapeutic benefits. This concept is strongly supported by recent studies showing that reactivation of p53 in tumors leads to the tumor regression in animal models (Ventura et al., 2007; Xue et al., 2007). Our growing understanding of p53 and its signaling pathway has led to the development of a number of small molecule drugs that directly or indirectly activate p53 protein and p53 responses (Lane et al., 2010). One of the best examples is Nutlins (Vassilev et al., 2004; Vassilev, 2007). Nutlins interact with MDM2 to release p53 from its interaction with MDM2, thereby leading to the p53 activation and p53 responses. Nutlins have been shown to induce tumor regression in animals. At the same time, the potential application of p53 in therapy should not be limited to cancer. It has been reported that inhibition of p53 functions, especially p53-mediated apoptosis, could benefit some diseases. For example, p53-inhibitory drugs have been

applied with some success to animal models of Parkingson's disease (Duan et al., 2002), which is characterized with chronic neuronal cell death. Future studies on p53 should lead to the application of p53-based drugs to cancer as well as other diseases.

Acknowledgements

Z. Feng is supported by the grant from National Institutes of Health (1R01CA143204-01) and New Jersey Commission on Cancer Research (09-1970-CCR-EO). W. Hu is supported by the grant from National Institutes of Health (1P30CA147892-01).

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