

Review

New insights into p53 functions through its target microRNAs

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The tumor suppressor p53 pathway, whose alterations are highly associated with all types of human cancers, plays an essential role in preventing tumor development and progression mostly through its downstream target genes. Over the last decade, a growing list of p53 microRNA (miRNA) targets has been identified as additional downstream players of this pathway. Further studies of these miRNAs have revealed their more complicated regulations and functions in executing and/or regulating p53 activity. Here, we review the p53 miRNA targets identified thus far, and discuss how they fine-tune p53 stress responses, mediate the crosstalk between p53 and other signaling pathways, and expand the role of p53 in other human diseases in addition to cancers.

Keywords: miRNA, p53, tumorigenesis, cancer

Introduction

As a transcriptional factor, the p53 tumor suppressor drives the expression of a large number of genes in response to multiple stimuli, such as DNA damage (Sakaguchi et al., 1998), genome instability (Leonova et al., 2013), oncogenic stresses (Lowe, 1999), ribosomal stresses (Zhang and Lu, 2009; Zhou et al., 2012), nutrient deprivation (Boren and Brindle, 2012), and hypoxia (Sermeus and Michiels, 2011). Consequently, the proteins encoded by these target genes execute cellular functions of p53 by triggering biochemical pathways that lead to DNA repair, cell cycle arrest, apoptosis, autophagy, and senescence (Soria et al., 2010; Levine, 2011). However, it remains elusive how the various p53-dependent cellular events are coordinated and integrated in response to any singular type of stresses and how p53 exactly fine-tunes the expression of its target genes involved in various cell fates that might or might not occur simultaneously. Also, it still remains incompletely understood how p53 exactly interplays with other signaling pathways at a global level during cancer development and progression. Although p53 often acts as the ‘good’ fighter in cellular anti-cancer battles, it could also be the ‘bad’ pathogenic molecule for several non-cancer diseases, yet its exact roles in these diseases remain in questions. Apparently, more systematic and careful studies are necessary to address these issues. In attempts to do so, a number of miRNAs have been identified as new p53 targets over the past decade (Figures 1–3), which could offer some new insights into understanding at least some of these issues.

MicroRNAs (miRNAs) are small noncoding RNAs with 19–22 nucleotides, which usually bind to the 3′ untranslated region (3′UTR) of target mRNAs and posttranscriptionally inhibit their expression. In general, the biogenesis of miRNAs in cells includes two steps: transcription, during which pri-miRNAs are synthesized by RNA polymerases II or III in the nucleus, and maturation, during which pri-miRNAs are processed into mature miRNAs by a number of RNases and regulators. The latter mostly occurs in the cytoplasm. Briefly, hairpin pre-miRNAs are made from pri-miRNAs and then cleaved into double-stranded miRNAs. Only one strand of each miRNA is employed to form the final functional RNA-induced silencing complex (RISC) (Bartel, 2004). Interestingly, p53 has been shown to play a role in both of these two steps. On one hand, p53 could directly bind to the promoters of its target miRNA genes and control their transcription (He et al., 2007); On the other hand, p53 also regulated the maturation of miRNAs by affecting the RISC complex function (Suzuki et al., 2009). More recent studies have revealed that p53-regulated miRNAs regulate not only the p53 pathway, but also other pathways that are involved in different human diseases. In this review, we will discuss the functional importance of p53-regulated miRNAs, with emphasis on the interplay between these miRNAs and how p53 affects its crosstalk with other tumor-related pathways.

Roles of p53-responsive miRNAs in p53 signaling

Since miR-34 was identified in 2007 as the first p53 target miRNA to induce p53-dependent apoptosis, cell cycle arrest, and senescence (Bommer et al., 2007; Chang et al., 2007; Corney et al., 2007; He et al., 2007; Raver-Shapira et al., 2007; Tarasov et al., 2007; Tazawa et al., 2007), a number of p53-regulated miRNAs have been discovered to be involved in these classical p53

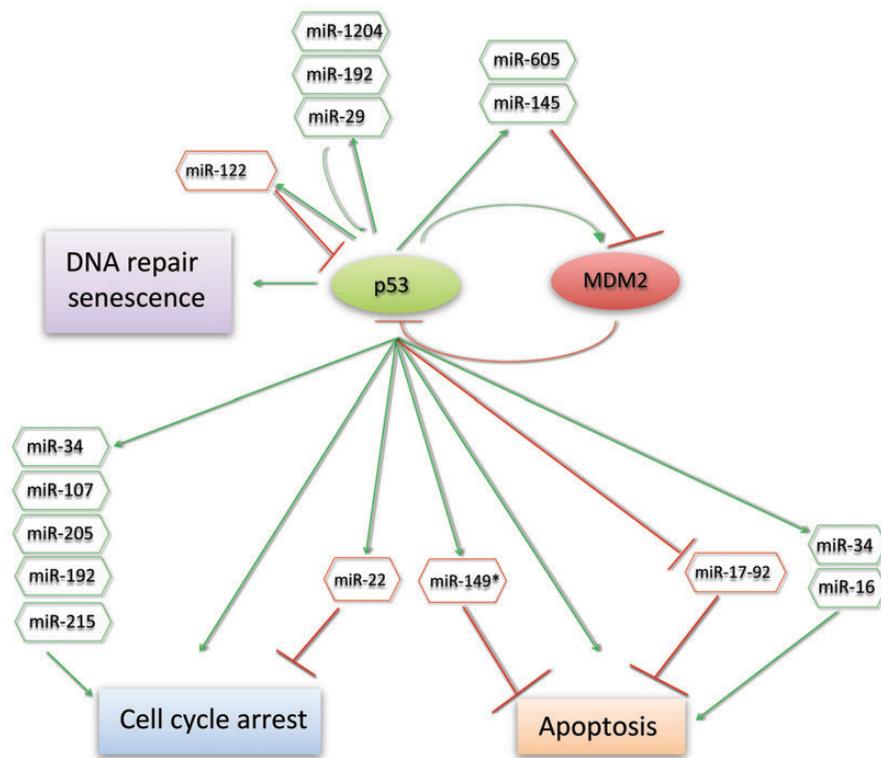


Figure 1 Role of miRNAs in p53 autoregulation, cell cycle arrest, and apoptosis. Square: cellular processes involving p53. Green hexagon: miRNAs that promote p53 activities. Red hexagon: miRNAs that suppress p53 activity.

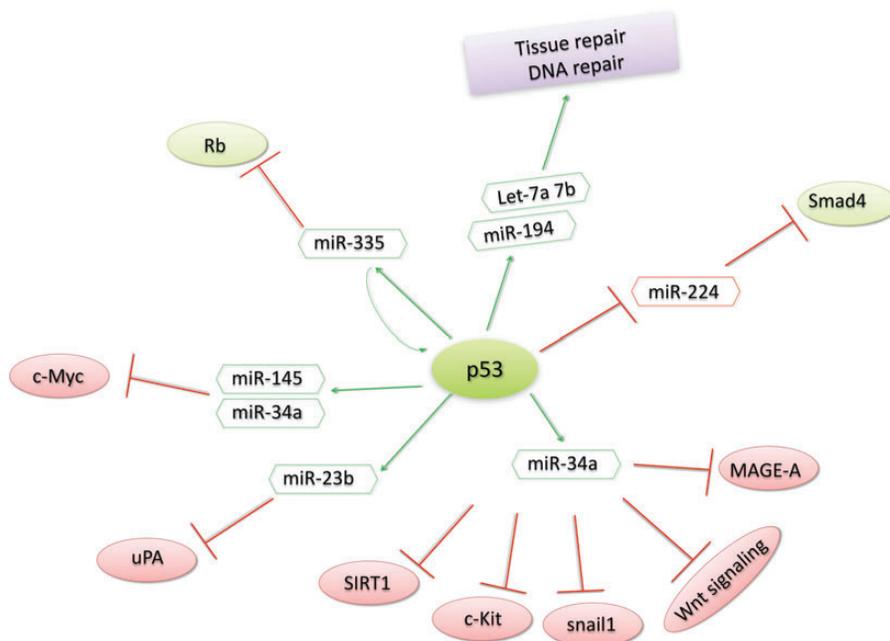


Figure 2 Role of miRNAs in crosstalk between p53 and other cancer-related pathways. Green oval: tumor suppressors. Red oval: oncoproteins. Green hexagon: miRNAs that promote p53 activities. Red hexagon: miRNAs that suppress p53 activity.

functions. However, some of these miRNAs have also been shown to finely tune p53 activation and adjust the sensitivity of cells to this signaling pathway. As discussed below, these new functions of p53 target miRNAs have become more greatly appreciated now.

The p53–miRNA feedback loop

The negative feedback regulation of p53 has been biochemically and genetically demonstrated, through two of its antagonists called MDM2 (also called HDM2 in human) and MDMX (also

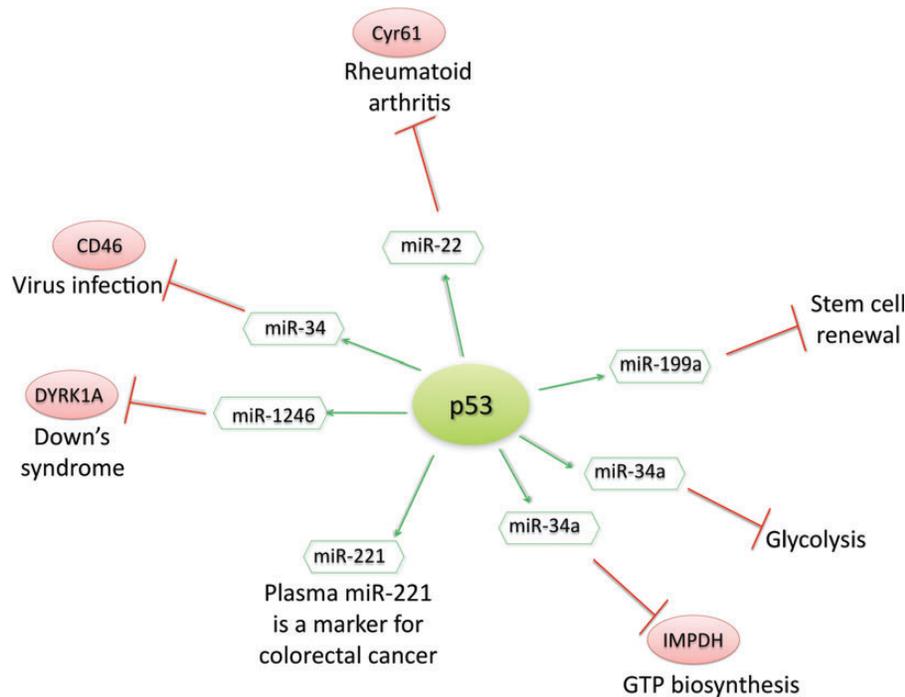


Figure 3 Role of miRNAs in mediating p53 functions in other cellular events. Green oval: tumor suppressors. Red oval: oncoproteins. Green hexagon: miRNAs that promote p53 activities.

called MDM4), as one of the most important feedback regulations in cell survival and proliferations (Shvarts et al., 1996). Interestingly, the feedback regulation can also be implemented by some p53 target miRNAs positively or negatively (Figure 1) (Suh et al., 2011; Xiao et al., 2011; Zhang et al., 2013a). One mechanism involves the control of MDM2 expression, which is the major p53 suppressor that possesses E3 ubiquitin ligase activity and mediates ubiquitin-dependent proteosomal turnover of p53 in cells (Oliner et al., 1992, 1993; Honda et al., 1997). For instance, miR-605 (Xiao et al., 2011) and miR-145 (Suh et al., 2011; Zhang et al., 2013a) are transcriptionally induced by p53 to suppress Mdm2 expression via directly binding to its mRNA and thus protecting p53 from degradation. These two miRNAs show a positive feedback regulation on p53. Some p53-regulated miRNAs can also modulate the p53 level by controlling other regulators of p53. For example, miR-29 induced transcriptionally by p53 in response to DNA damage can positively influence p53 level by repressing the expression of Ppm1d phosphatase (Ugalde et al., 2011) that dephosphorylates p53 at Ser 15 and destabilizes p53 (Lu et al., 2005), thus forming another positive regulatory circuit in response to DNA damage. Similarly, miR-1204 that is activated transcriptionally by p53 also positively regulates the p53 level (Barsotti et al., 2012), and so does miR-192 (Georges et al., 2008), which was shown to induce p53 expression in the renal cortex in response to TGF- β signaling (Deshpande et al., 2013). Besides, a negative feedback regulation between miR-122 and p53 has also been reported recently. miR-122 had been reported to promote p53 activity via targeting cytoplasmic polyadenylation element binding protein (Burns et al., 2011) and cyclin G1 (Fornari et al., 2009). Intriguingly, it was found to be posttranscriptionally upregulated by p53 and in turn to suppress p53 activity via activation of Akt in

cutaneous T-cell lymphoma, thus forming a negative feedback loop (Manfe et al., 2012). These studies demonstrate that some of the p53 target miRNAs can modulate p53 level and activity in a feedback fashion.

Roles of miRNAs in p53-dependent cell cycle arrest and apoptosis

In addition to the aforementioned feedback regulation of p53 level and activity, one of the mostly characterized functions of p53 target miRNAs is to mediate p53-dependent cell growth control (He et al., 2007; Georges et al., 2008; Bohlig et al., 2011; Piovani et al., 2012). In response to cellular stressors, p53 often induces cell cycle arrest and apoptosis, consequently eliminating cancer cells (Soria et al., 2010; Levine, 2011). These cellular phenotypes are often determined by subtle changes of p53 target gene expression in a dose-dependent fashion (Kruse and Gu, 2009). By facilitating p53-dependent cell growth arrest and apoptosis, some miRNAs have also been shown to play a role in determination of cell fates.

miRNAs that mediate p53-induced cell cycle arrest include miR-34, miR-107, miR-205, miR-192, and miR-215 (Figure 1). Among them, miR-34 is the first miRNA identified as a p53 target, and acts to induce cell cycle arrest by suppressing the expression of a batch of cell cycle-associated proteins, including CCNE2, CKD4, and MET (He et al., 2007). Soon after the discovery of miR-34, miR-192/215 were also found to promote cell cycle arrest in response to p53 activation by targeting a series of proteins that regulate G1 and G2 checkpoints, including CDC7, LMNB2, MAD2L1, and CUL5 (Georges et al., 2008). Later on, miR-107 was shown to inhibit the expression of two cell cycle regulators, CDK6 and RBL2, and thus arrest cells at G1 as well (Bohlig et al., 2011), whereas miR-205 was shown to reduce cell cycle progression by

targeting E2F1 in response to p53 activation (Piovan et al., 2012). These miRNAs affect different downstream targets to achieve the same cellular outcomes, i.e. halting cell growth and proliferation triggered by p53.

In addition, several p53-activated miRNAs have been reported to mediate p53-induced apoptosis. For example, miR-34 can promote apoptosis in response to p53 activation by DNA damage stress (Chang et al., 2007; Raver-Shapira et al., 2007). By gene ontology classifications of the miR-34-regulated genes, it was found that miR-34a inhibits the expression of a number of anti-apoptotic proteins, including BCL2, BIRC3, and DcR3 (Chang et al., 2007), and functions as a pro-apoptotic miRNA. Also, miR-16 has been identified as a p53 target to induce apoptosis by inhibiting Survivin expression (Ma et al., 2013). Interestingly, p53 also suppresses the expression of some anti-apoptotic miRNAs. For instance, miR-17–92 can be transcriptionally suppressed by p53 in response to hypoxia, consequently sensitizing cells to hypoxia-induced apoptosis, as overexpression of miR-17-92 inhibits hypoxia-induced apoptosis (Yan et al., 2009).

More interestingly, other p53-regulated miRNAs play a role in cell fate determination. For instance, miR-22 and miR-149*, which are both p53-activated miRNAs, have been reported to suppress cell cycle arrest and apoptosis, respectively. Specifically, miR-22 as a p53 target gene can promote apoptosis by suppressing the expression of one important p53 target gene *p21*, which inhibits apoptosis once activated, instead of executing p53-induced cell growth arrest (Tsuchiya et al., 2011). Oppositely, the anti-apoptotic miR-149* has, however, been shown to suppress the expression of GSK2 α in response to p53 activation, leading to the induction of Mcl-1 and consequent resistance of melanoma cells to apoptosis (Jin et al., 2011). These intriguing studies, once proved to be true in animals, suggest that miRNAs can orient p53-dependent cell fates by finely controlling the expression of different protein molecules important for cell growth and/or apoptosis (Figure 1).

Roles of miRNAs in bridging p53 with other cancer-pertinent pathways

The level and activity of p53 are often inversely related to that of some important oncogenes, such as *c-Myc* or *Kit1*. Remarkably, emerging evidence has revealed that this inverse relationship can also be established through the action of several p53 target miRNAs as further described below.

Suppression of oncogene expression by p53-regulated miRNAs

It has been known for a while that p53 activation is often highly associated with the decreased expression of a number of oncogenes in response to various stresses. However, the underlying mechanisms had been elusive until miRNAs were found as p53 targets. A typical example is the inverse relationship between p53 and *c-Myc* levels. The mystery of this relationship was unveiled when miR-145 and miR-34, both being identified as p53 targets, were shown to suppress *c-Myc* expression in response to p53 activation (Sachdeva et al., 2009; Christoffersen et al., 2010).

Besides *c-Myc*, miR-34 has also been shown to inactivate other oncoproteins that are involved in various signaling pathways (Figure 2). For example, miR-34 targets Sirt1 that controls cell senescence and limits longevity (Guarente, 2000). Because Sirt1

deacetylates p53 and regulates its stability (Luo et al., 2001; Vaziri et al., 2001), it was proposed that miR-34 might act as a positive feedback regulator of p53 (Yamakuchi and Lowenstein, 2009). In addition, miR-34 can repress *c-Kit* expression, consequently impeding Erk signaling and transformation (Siemens et al., 2013). Furthermore, miR-34 directly targets Snail1 and inhibits Snail1-dependent epithelial-mesenchymal transition (EMT) in colon, breast, and lung carcinoma cells (Kim et al., 2011b). Likewise, this miRNA can interact with 3'UTR of β -catenin mRNA and inhibit Wnt signaling in cancer cells (Kim et al., 2011a). Finally, it represses the oncogenic *MAGE-A* gene family in medulloblastoma (Weeraratne et al., 2011). Although it is not surprising that almost every single miRNA should be able to target more than one mRNA target, these studies demonstrate that miR-34 can mediate p53 functions by targeting a broad panel of oncogenes involved in diverse signaling pathways.

Another miRNA, miR-23b, was also identified to potentially target an oncogene upon transcriptional activation by p53. The urokinase-type plasminogen activator (uPA) is a serine protease that highly expresses in cervical cancer in response to human papillomavirus (HPV)-16-induced transformation. Its overexpression is associated with poor patient prognosis. uPA is believed to be an important player in the development of cervical cancer and to promote cell invasion and cancer metastasis (Turner and Palefsky, 1995; Andreasen et al., 1997; Riethdorf et al., 1999; Dass et al., 2008). The expression of miR-23b appeared to be reversely correlated with that of uPA in HPV-associated cervical carcinomas. Intriguingly, miR-23b inhibits uPA expression via direct binding to its mRNA in a p53-dependent manner (Au Yeung et al., 2011). This suggests that the anti-cancer functions of p53 in preventing HPV-16 E6-associated cervical cancer development may partly be attributed to the negation of uPA activity by miR-23b.

Therefore, one of the strategies that p53 'utilizes' to eliminate cancer cells is to indirectly downregulate the expression of important oncogenes, such as *c-Myc*, via its own target miRNAs, such as miR-34 (Figure 2). Also, p53 can recruit its allies, such as other tumor suppressors, to kill cancer cells through its target miRNAs as described below.

miRNAs connect p53 with other tumor suppressors

In mammalian cells, there are more than dozens of protein molecules that function similarly to p53 as tumor suppressors. p53 also employs a partnership strategy with other tumor suppressors via its target miRNAs (Figure 2). For instance, p53 has been reported to protect the activity of Smad4, another tumor suppressor, in granulosa cells. This study showed that miR-224 inhibits Smad4 expression in response to TGF- β signaling, whereas p53 suppresses miR-224 expression by binding to the promoter of the miR-224-encoding gene. As a result of miR-224 downregulation, p53 activation leads to the induction of Smad4 (Liang et al., 2013), indicating that miR-224 plays a role in uniting the two important tumor suppressors to cooperatively prevent cancer cell growth.

Another example is the connection between p53 and Rb via miR-335. In response to DNA damage signaling, p53 becomes active to induce the expression of miR-335, which in turn targets Rb1 mRNA and inhibits its expression in a p53-dependent fashion, suggesting a novel link between these two tumor suppressors

(Scarola et al., 2010). However, it remains quite puzzling why and under what physiological and/or pathological circumstance p53 really needs to turn off Rb1 via miR-335, as Rb1 would otherwise synergize the anti-cancer effect of p53. Regardless of the remaining questions, these studies demonstrate that miRNAs do play a role in linking p53 with other oncoproteins or tumor suppressors in the regulation of cell proliferation and cell growth.

Roles of miRNAs in association of p53 with non-cancer-related pathways

In addition to tumor suppression, p53 also functions in regulation of normal cellular processes or events, such as metabolism, inflammation, and stem cell renewal, which can be mediated through the action of miRNAs. Some p53-activated miRNAs have been reported to target genes that are highly related to human non-cancer diseases. As detailed below, these miRNAs expand functions or roles of p53 to a broader spectrum and a new layer of regulation of biology (Figure 3).

Roles of miRNAs in p53 regulation of metabolism

In spite of increasing evidence that shows the regulatory role or function of p53 in cellular homeostasis and metabolism (Bensaad et al., 2006; Matoba et al., 2006; Ide et al., 2009; Hu et al., 2010b; Maddocks and Vousden, 2011; Wang and Gu, 2014), only few studies have explored the role of miRNAs in p53-regulated metabolism. One report showed that p53 targets IMPDH, a regulator of *de novo* GTP biosynthesis, via miR-34 (Kim et al., 2012). This was found when screening p53-responsive genes involved in the purine metabolic pathway in human lung non-small cell carcinoma H1299 cells with adenoviral expression of p53. Specifically, p53 was shown to reduce the expression of IMPDH1 and IMPDH2, but not other metabolic enzymes in this pathway, and the reduction was executed through miR-34. Thus, this study suggests that p53 can indirectly regulate GTP biosynthesis by inducing the expression of miR-34, which in turn suppresses the expression of IMPDH1 and IMPDH2 (Kim et al., 2012). Also, p53 was shown to repress glycolysis and enhance mitochondrial respiration by targeting a number of glycolytic enzymes, such as hexokinase1, hexokinase 2, and glucose-6-phosphate isomerase, via miR-34 (Kim et al., 2013). These studies suggest that p53-regulated miRNAs are involved in p53 regulation of cellular metabolism (Figure 3).

Roles of miRNAs in p53 regulation of stem cell renewal

It has been shown that p53 lowers the efficiency of generating human induced pluripotent stem (iPS) cells and functions as a barrier in iPS cell regeneration (Hong et al., 2009). Recently, this function was linked to miR-199a-3p, which can mediate the inhibitory effect of p53 on iPSCs generation. It was found that ectopic p53 can induce the transcription of miR-199a-3p, which in turn suppresses the reprogramming of MEF cells. Amazingly, miR-199a-3p could almost completely reverse the p53 knockdown-initiated reprogramming of MEF cells (Wang et al., 2012). This study demonstrates that miR-199a-3p is the main player that executes p53-dependent suppression of stem cell self-renewal, although its targets specifically for the stem cell regulation still remain to be identified.

Roles of miRNAs in linking p53 with others diseases

Emerging evidence suggests that miRNA might play a role in mediating p53 regulation of the development of rheumatoid arthritis (RA), which is a systemic autoimmune disease with chronic joint inflammation. p53 mutations were identified in RA synovium more than a decade ago (Sun and Cheung, 2002). Remarkably, some of the RA-pertinent molecular or cellular phenotype changes, such as increased IL-6 expression and invasion of synovial cells, could be observed when p53 activity was inhibited (Migita et al., 2001; Yamanishi et al., 2002). These early studies suggest that p53 might be crucial for RA development. However, the underlying mechanism(s) remained largely unknown until a recent study revealed the inverse correlation between miR-22 and Cyr61, an RA-associated protein, in RA synovial tissues (Lin et al., 2014). Interestingly, Cyr61 was shown to be a direct target of miR-22. Because p53 can promote miR-22 expression, this finding uncovers miR-22 as a crucial player that implements the inhibition of Cyr61 expression by p53. Cyr61 has been shown to promote proliferation of fibroblast-like synoviocytes and contribute to the pathogenesis of RA (Zhang et al., 2009a). Thus, p53 might retard the development of RA (Lin et al., 2014), which is consistent with the fact that p53 is mutated in some RA synovial tissues.

Similarly, our lab recently identified miR-1246 as a p53 transcriptional target that mediates p53-dependent suppression of a Down syndrome-associated protein kinase called DYRK1A (Gwack et al., 2006). In our initial attempt to search miRNAs that could be induced specifically by the p53 family members, we found that the expression of miR-1246 can be transcriptionally activated by all of the three p53 family members p53, p63, and p73. Our further study revealed that DYRK1A is one of the top targets for miR-1246 in response to p53 activation (Zhang et al., 2011). DYRK1A is one of the vital proteins that are highly expressed in Down syndrome patients (Guimera et al., 1996; Hattori et al., 2000). Overexpression of DYRK1A can also cause Down syndrome-like phenotypes in mouse (Altafaj et al., 2001). Our studies hence imply that the p53 family members might play a role in the development of Down syndrome via miR-1246 (Zhang et al., 2011; Liao et al., 2012), which would be an interesting topic for future study.

Besides, the p53–miR-34 axis has been shown to act as a potential barrier for the infection of human herpes viruses. Viral DNAs and proteins have been found in human tumors to inhibit apoptosis and promote oncogenic activity (Michaelis et al., 2009; Ablashi et al., 2010; Jensen et al., 2010; Michelow et al., 2012). Consistent with this statement, viral infection has been proved to play important roles in the development of a number of cancers, including cervical, liver, breast, colon, and prostate cancers and so on (Michaelis et al., 2009). In fighting virus-initiated or associated tumorigenesis, p53 also utilizes its miRNA troupe. One vital fighter in this battle is its prime miRNA target, miR-34, as discussed above (He et al., 2007). Interestingly, it was found that a number of viral entry receptors, such as PDGFRA, CD46, CCR2, HVEM family, and PEA15 (Kofman et al., 2013), possess miR-34-binding sites. Predictably, the expression of these cellular genes could be inhibited by miR-34 in response to p53 activation, though more studies are necessary to confirm this. Since most viruses rely on the host cellular machineries for their infection, survival, and propagation, targeting these viral entry receptors by the p53–miR-34 pathway

provides a new approach to eliminate virus-initiated or associated cancer cells.

In summary, p53-regulated miRNAs have emerged as important downstream players in facilitating or implementing p53 functions involved in the regulation of multiple cellular pathways and the development and progression of the aforementioned and yet discussed or unknown diseases, including cancers and genetic diseases. Further systematic studies on these miRNAs will unravel both new p53 functions in diseases and new molecule targets in the p53 pathway for future drug discovery.

Remaining questions

In addition to the above-mentioned p53 target miRNAs, other miRNAs have also been reported to regulate p53 activity in response to other signaling cues. For example, miR-122 (Fornari et al., 2009; Burns et al., 2011), miR-29 (Park et al., 2009), and miR-18b (Dar et al., 2013) promote p53 activity, while miR-504 (Hu et al., 2010a), miR-125a (Le et al., 2009, 2011; Zhang et al., 2009b; Ghose et al., 2011), miR-33 (Herrera-Merchan et al., 2010), miR-30 (Li et al., 2010), miR-380-5p (Swarbrick et al., 2010), miR-1285 (Tian et al., 2010), miR-150 (Ghose et al., 2011; Wang et al., 2013; Zhang et al., 2013b), miR-138 (Ye et al., 2012), miR-214 (Xu et al., 2012), and miR-374 (Liu et al., 2013) have been shown to inhibit p53 expression by directly binding to p53 mRNA. Clearly, miRNAs have become a group of important players in the p53 pathway, controlling cell growth, metabolism, apoptosis, and disease development. It is anticipated that more miRNAs will be identified to play a role in mediating p53 functions in cells. Thus, it is crucially important and biologically significant to obtain a relatively full list of p53-responsive miRNAs. This miRNA profiling or landscape would be considerably informative and useful for our better understanding of the relatively complete list of p53 functions in pathophysiology of tumorigenesis and other diseases as well as for future drug discovery.

Although a great progress has been made to link miRNAs with the p53 pathway, a number of outstanding questions or conjectures still remain to be addressed. First, even though individual target has been identified for most of the p53-responsive miRNAs, numerous cellular mRNAs can be targeted based on the nature of miRNA action. Therefore, it is also important to identify all potential target mRNAs for each of the p53-activated miRNAs. This would provide a whole picture of the entire p53–miRNA–target pathway. Also, since p53 is often activated in response to a variety of extracellular and/or intracellular stresses, it is crucial to learn whether this dynamic regulation is also reflected in the landscape or profiling of p53-responsive miRNAs. This goal could be achieved when the miRNA sequencing technology becomes available and financially feasible. It is similarly important to identify tissue- or cancer stage-specific miRNAs that are regulated by p53 in a biologically relevant setting, as this would reveal not only new miRNA targets, but also previously undiscovered functions of p53. Furthermore, as circulating miRNAs in blood have been identified for several human cancers (Heneghan et al., 2010; Yu et al., 2014), it is thus of great interest to determine whether p53-regulated miRNAs could also be used as molecular markers for any specific cancer, monitoring the progression of cancers or the therapeutic response. For example, circulating miR-221 has been found well correlated with

the protein level of p53 in colorectal cancer (Pu et al., 2010), suggesting that miRNA-221 might be used as a biomarker for this cancer. As such, the miRNA profiling of blood samples from cancers at different stages and p53 status (wild type or mutant) would help to identify more circulating miRNAs that might be useful as an indicator of the p53 status in cancer patients. Hence, addressing these big questions would better depict the whole p53–miRNA–target pathway and divulge its role in the development and prevention of human diseases, including cancers and other genetic disorders.

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