

Review

microRNAs and Alu elements in the p53–Mdm2–Mdm4 regulatory network

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p53 is a transcription factor that governs numerous stress response pathways within the cell. Maintaining the right levels of p53 is crucial for cell survival and proper cellular homeostasis. The tight regulation of p53 involves many cellular components, most notably its major negative regulators Mdm2 and Mdm4, which maintain p53 protein amount and activity in tight check. microRNAs (miRNAs) are small non-coding RNAs that target specific mRNAs to translational arrest and degradation. miRNAs are also key components of the normal p53 pathway, joining forces with Mdm2 and Mdm4 to maintain proper p53 activity. Here we review the current knowledge of miRNAs targeting Mdm2 and Mdm4, and their importance in different tissues and in pathological states such as cancer. In addition, we address the role of Alu sequences—highly abundant retroelements spread throughout the human genome, and their impact on gene regulation via the miRNA machinery. Alus occupy a significant portion of genes' 3'UTR, and as such they have the potential to impact mRNA regulation. Since Alus are primate-specific, they introduce a new regulatory layer into primate genomes. Alus can influence and alter gene regulation, creating primate-specific cancer-preventive regulatory mechanisms to sustain the transition to longer life span in primates. We review the possible influence of Alu sequences on miRNA functionality in general and specifically within the p53 network.

Keywords: microRNA, p53, Mdm2, Mdm4, Alu

p53, Mdm2, and Mdm4

p53 is a pivotal transcription factor that plays a crucial role in tumor suppression. It is activated in response to a variety of cellular stress signals including DNA damage, activated oncogenes and various metabolic challenges (Levine and Oren, 2009; Wade et al., 2013). p53 modulates the expression of a diverse set of genes to suppress cancer through cell cycle arrest, senescence, apoptosis, regulation of metabolism, autophagy, and the oxidative status of the cell (Brady and Attardi, 2010). The p53 network comprises many protein-coding genes, as well as a growing number of additional regulators such as microRNAs (miRNAs) and other non-coding RNAs. Importantly, p53 is mutated in ~50% of human cancers, and functionally inactivated in many more (Sinnott et al., 1991).

Mdm2 and Mdm4 (also called Mdmx; human orthologues often referred to as HDM2 and HDM4/HDMX, respectively) are key regulators of p53. They are structurally related proteins, containing an amino terminal p53-binding domain and a C-terminal RING finger domain through which they heterodimerize (Sharp et al., 1999), with additional contribution of the extreme C-terminal residues of both proteins (Poyurovsky and Prives, 2006; Dolezelova and

Slampa, 2007; Uldrijan et al., 2007). Mdm2 and Mdm4 regulate p53 by binding to it and physically masking its transactivation domain to limit the access of its essential co-activators and the transcription machinery (Oliner, 1993; Zambetti and Levine, 1993; Thut et al., 1997). In addition, Mdm2 possesses E3 ubiquitin ligase activity and can downregulate p53 by promoting its ubiquitylation and proteasome-dependent degradation (Haupt et al., 1997; Honda et al., 1997; Kubbutat et al., 1997). Heterodimerization with Mdm4 enhances the E3 activity of Mdm2 toward p53 (Linares et al., 2003). However, under some circumstances, Mdm4 can also inhibit Mdm2's E3 ligase activity (Barboza et al., 2008). Of note, p53 can transcriptionally activate the *Mdm2* gene through two adjacent p53-response elements, thereby establishing a negative feedback loop between p53 and Mdm2 (Barak et al., 1993; Juven et al., 1993; Wu et al., 1993).

Upon DNA damage, when p53 activation is needed, the Mdm2 ubiquitin ligase activity is redirected preferentially toward Mdm4 (Pereg et al., 2005; Lenos and Jochemsen, 2011). In parallel, Mdm2 degradation is also enhanced (Stommel and Wahl, 2004). Together, this leads to an increase in the amount and activity of p53.

miRNAs

miRNAs are small non-coding RNAs, which are about 23 bases long and have an important role in animals as well as plants (Bartel, 2009). miRNAs bind to specific mRNAs via targets

located mostly, although not exclusively, in their 3'UTR, which are complementary to the miRNAs' seed sequence (bases 2–8 at the 5' end) (Bartel, 2009). The main role of miRNAs is post-transcriptional regulation; they are loaded on the Argonalet protein of the RISC silencing complex, and their binding to specific mRNAs through the target sequence causes translation arrest and/or degradation of the targeted mRNA, often through promoting its deadenylation (Bartel, 2009; Subtelny et al., 2014). Since the target of a miRNA is typically only seven bases long, many putative miRNA targets are located within 3'UTRs of protein-coding genes, although most of them are in reality non-responsive to the miRNA machinery and thus non-functional. Along the years, many prediction tools have been created to predict functional targets, using different features of the target that might contribute to its functionality, such as the secondary structure of the target within the mRNA, its location along the 3'UTR, number of appearances within a single mRNA molecule and more (Reyes-Herrera and Ficarra, 2012).

Alu sequences

Alu repeats are transposable elements (TEs), typically about 300 bases long, with more than one million copies in the human genome (Lander et al., 2001). They use the retrotransposition molecular machinery of the LINE TE-L1 elements to integrate into the host genome (Dewannieux et al., 2003). Alus comprise a substantial fraction (~10%) of the human genome, and are located in intergenic regions as well as within introns and 3'UTRs of annotated genes (Lander et al., 2001). In fact, 5% of the 3'UTR length, on average, consists of Alus (An et al., 2004). Alus were at first considered 'junk DNA', but over the years, it has become apparent that they may harbor functional elements. For example, they may become exons (Lev-Maor et al., 2003) and may encompass functional binding sites for transcription factors (Polak and Domany, 2006) and miRNAs (Smalheiser and Torvik, 2006).

miRNAs and the p53 regulators

The delicate balance between p53, Mdm2, and Mdm4 is crucial for cell and organismal survival. While upon cellular stress high levels of p53 protein are needed, most of the time its levels should be kept low. The transition between these two alternative states, and presumably many more intermediate states, should be quick and precise. Different factors are responsible for maintaining this balance, including also miRNAs. miRNAs play an important role in the p53 network: they target p53 itself as well as its regulators, or are targeted by p53 to regulate other components in the pathway. Thus, miRNAs offer an additional layer of post-transcriptional regulation to the network (Hunten et al., 2013; Krell et al., 2013). Here, we focus on the effect of miRNAs on the two major negative regulators of p53, namely Mdm2 and Mdm4 (Figure 1).

Mdm2 and Mdm4 mRNAs possess long 3'UTRs

The *Mdm2* and *Mdm4* mRNAs have exceptionally long 3'UTRs. *Mdm2*'s 3'UTR is ~5 kb, and *Mdm4*'s is even longer, extending to ~8.5 kb, in comparison with a genome-wide length average of 0.95 kb (Sood et al., 2006). Therefore, not surprisingly, both

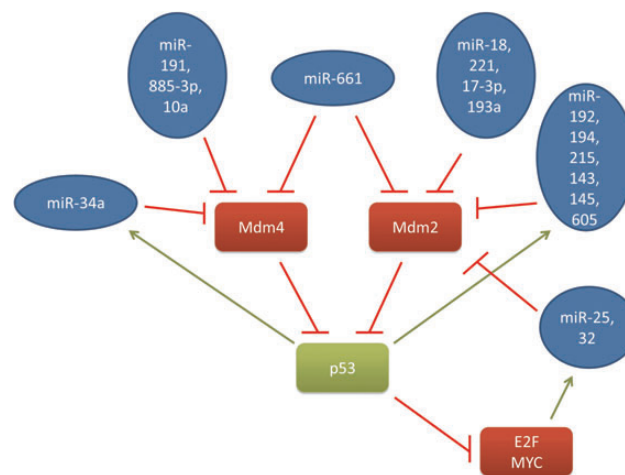


Figure 1 miRNAs targeting Mdm2 and Mdm4. Depicted are miRNAs that indirectly affect the p53 network, through downregulation of two major negative regulators of p53—Mdm2 and Mdm4. Some of those miRNAs are also positively or negatively regulated by p53. The lines in the graph represent different levels of regulation—transcriptional, translational, and functional. Green represents positive regulation, while red stands for negative regulation.

Mdm2 and *Mdm4* harbor a multitude of potential miRNA targets within their 3'UTRs: according to TargetScan, *Mdm2* has 760 potential miRNA targets and *Mdm4* has 1276 (Lewis et al., 2005). However, it is highly likely that the vast majority of those putative targets are non-functional, and only a relatively small subset of them plays actual roles in the regulation of Mdm2 and Mdm4 protein levels. Of note, both *Mdm2* and *Mdm4* contain several Alu sequences within their 3'UTR.

miRNAs regulating Mdm2

miR-192, miR-194, and miR-215 belong to two related clusters, the miR-194-2-192 cluster at 11q13.1 and the miR-215-194-1 cluster at 1q41.1, and all share the same seed. It was first reported that these three miRNAs are upregulated by p53 (Braun et al., 2008; Georges et al., 2008), and while they are found in high levels in colon tissues, they are almost depleted from colon cancer samples (Braun et al., 2008). miR-192 and miR-215 enhance CDKN1A/p21 levels, colony growth suppression, cell cycle arrest, and cell detachment from a solid support (Braun et al., 2008; Georges et al., 2008). Two years later, Pichiorri et al. (2010) identified *Mdm2* as a direct target of these three miRNAs, and it was therefore concluded that their anti-cancerous effect may at least in part be mediated through downregulation of Mdm2, causing an elevation of p53 functionality. They further showed that miR-192, miR-194, and miR-215 are transcriptionally upregulated by p53 in multiple myeloma (MM) cells, and thus inhibit cell growth and enhance apoptosis (Pichiorri et al., 2010). Moreover, when combining transfection of these miRNAs with a pharmacological inhibitor of Mdm2 (MI-219), they showed in a mouse model, as a proof of concept, that this combination might be a lead to successful treatment of MM (Pichiorri et al., 2010). Recently, Khella et al. (2013) found that these three miRNAs are significantly downregulated in metastatic tumors, and identified

Mdm2 as their target in renal cell carcinoma (RCC). Knockdown of these miRNAs led to enhanced migration and invasion of RCC cells, and miR-215 expression correlated positively with survival of RCC patients, with low levels of miR-215 predicting reduced disease-free survival. In agreement with identification of Mdm2 as a relevant target, an inverse correlation was observed between the levels of *Mdm2* mRNA and the three miRNAs in specimens from clear cell RCC patients (Khella et al., 2013).

Another pair of Mdm2-regulating miRNAs is miR-143 and miR-145. These two miRNAs, which belong to the same cluster, were found to be under-expressed in head and neck squamous cell carcinoma relative to normal tissues and cells, while Mdm2 is highly expressed in these tumors (Zhang et al., 2013). Moreover, Mdm2 was identified as a direct target of miR-143 and miR-145, and both miRNAs are post-transcriptionally upregulated by WT but not mutant p53 (Zhang et al., 2013). miR-143 and miR-145 induce apoptosis and suppress proliferation of epithelial cancer cells in a p53-dependent manner, both *in vitro* and *in vivo* (Zhang et al., 2013). Furthermore, under conditions where DNA damage elicits oscillations in p53 and Mdm2 levels, expression of the two miRNAs correlates with the increase in p53, and blocking of these miRNAs during the oscillations reduces the p53 pulses (Zhang et al., 2013). Hence, miR-143 and miR-145 are important for the equilibrium that maintains these pulses.

An additional Mdm2-regulatory miRNA is miR-605. Xiao et al. (2011) found that miR-605 targets directly Mdm2, thereby indirectly upregulating p53. In addition, miR-605 is a transcriptional target of p53, which interacts with the miR-605 gene promoter to upregulate its transcription (Xiao et al., 2011). This is therefore an additional example of a miRNA-based positive feedback loop that helps to rapidly accumulate p53 in response to stress.

In contrast to the above examples of miRNAs whose expression is positively regulated by p53, Suh et al. (2012) identified two miRNAs that are downregulated by p53 in glioblastoma—miR-25 and miR-32. The regulation of these two miRNAs, which share the same seed sequence, is via the transcription factors E2F1 and MYC, which regulate positively the expression of the two miRNAs and are repressed by p53 (Suh et al., 2012). Furthermore, *Mdm2* is a direct target of miR-25 and miR-32, and downregulation of Mdm2 by these miRNAs leads to p53 accumulation with subsequent cell cycle arrest and inhibition of cell proliferation (Suh et al., 2012). miR-25 and miR-32 also significantly inhibited tumor growth in a mouse model. Importantly, an inverse correlation was observed between expression of the two miRNAs and *Mdm2* mRNA in glioblastoma patient tissues, a tumor type in which *Mdm2* mRNA is often increased relative to normal brain tissues (Suh et al., 2012). Unlike the positive feedback loops discussed above, this is an example of negative feedback loop where p53 indirectly downregulates miR-25 and miR-32, which in their turn downregulate Mdm2.

miR-18b was recently identified as a tumor suppressor in melanoma. miR-18b is silenced by hypermethylation in melanoma and its low levels in primary cutaneous melanoma specimens are associated with reduced survival (Dar et al., 2013). Importantly, miR-18b directly targets *Mdm2* mRNA, affecting the p53 pathway, and its overexpression causes cell cycle arrest and apoptosis. Accordingly, stable overexpression of miR-18b suppressed tumor

growth in nude mice (Dar et al., 2013).

The miRNAs discussed above downregulate Mdm2 and therefore affect p53 indirectly. Some miRNAs also target Mdm2 with no reported effect on p53. Kim et al. (2010) screened for miRNAs that regulate the chondrogenic differentiation of chick limb mesenchymal cells, and found miR-221 to be upregulated upon inhibition of differentiation. They identified Mdm2 as a key target of the miRNA in this process; the reduction in Mdm2 protein increases the level of the Slug protein, which negatively regulates the proliferation of chondroprogenitors (Kim et al., 2010).

Li and Yang (2012) investigated miR-17 in glioblastoma, and found it to have two roles: under normal conditions it suppresses tumor cell growth, while under unfavorable conditions it promotes cell survival. They showed that miR-17-3p directly targets Mdm2, which leads to decreased cell proliferation and attenuates drug resistance. On the other hand miR-17-5p, the mature miRNA derived from the complementary strand of miR-17-3p, targets the tumor suppressor PTEN. The authors therefore proposed miR-17 as a biomarker of response to chemotherapy and anti-angiogenic treatment in glioblastoma patients. Intriguingly, while overexpression of miR-17 led to reduction of Mdm2, it did not upregulate p53, leading to the proposal that the effect of Mdm2 in these cells is not via the p53 pathway. Interestingly, Mdm2 has been suggested to regulate miR-17-5p (together with miR-20), by a yet unknown mechanism (Wang et al., 2013).

Lastly, miR-193a also directly targets Mdm2 (Li et al., 2013). miR-193a expression is epigenetically silenced by the fusion protein AML1/ETO, product of a gene translocation that occurs in acute myeloid leukemia (AML) and is considered the leukemia-initiating event (Li et al., 2013). miR-193a induces G1 arrest and apoptosis and restores leukemic cell differentiation; however, it is presently unknown whether this occurs via activation of p53.

miRNAs regulating Mdm4

The miR-34 family (miR-34a, b, c) has an important role in the p53 network: it is a direct transcriptional target of p53; it can induce apoptosis, cell cycle arrest, and senescence; and its loss can lead to resistance to p53-mediated apoptosis (Hermeking, 2010). miR-34b and miR-34c are encoded by the same genetic locus, whereas miR-34a is encoded by a different locus. The corresponding promoters undergo CpG hypermethylation in many tumors, causing their inactivation (Hermeking, 2010). Recently, miR-34a was shown to also target Mdm4; specifically, miR-34a targets a conserved region of the *Mdm4* mRNA ORF, rather than its 3'UTR (Mandke et al., 2012). The target sequence is within the region encoding the C-terminal RING domain of Mdm4, which is also present in Mdm2; interestingly, in *Mdm2* mRNA, there is a different codon in the homologous region, eliminating the putative miR-34a target (Mandke et al., 2012).

Wynendaele et al. (2010) identified a single nucleotide polymorphism (SNP) in the 3'UTR of the human *Mdm4* gene, creating a functional target site for miR-191, which is highly expressed in ovarian cells and carcinomas. In the *Mdm4-A* allele the target has one base substitution, and ovarian cancer cells harboring this allele display increased Mdm4. In contrast, in the *Mdm4-C* allele the miR-191 target is intact, and in cells harboring this allele Mdm4 levels are reduced. Downregulation of Mdm4 by miR-191

delays ovarian carcinoma progression and death (Wynendaele et al., 2010). Accordingly, absence of the target in both alleles (A/A genotype) is more frequent in high grade than low grade carcinoma, and correlates with increased risk of recurrence and death. In a related study, McEvoy et al. (2012) found Mdm4 to be highly expressed in retinoblastoma in comparison with the developing human retina, and proposed that these high levels are due to down-regulation of miR-191 and somatic mutations that eliminate the target of the miRNA in *Mdm4* RNA in a subset of the tumors.

Huang et al. (2011b) found that miR-885-3p directly binds a target in the 5'UTR of *Mdm4* mRNA; in these cases, the consequence is an increase, rather than a decrease, in Mdm4 protein. The effect of the miRNA is enhanced by cisplatin treatment of squamous cell carcinomas (SCCs) that overexpress WT Δ Np63 α (the dominant-negative isoform of p63, which is particularly abundant in many SCCs), as miR-885-3p is upregulated by cisplatin-induced p- Δ Np63 α (Huang et al., 2011a). After DNA damage, Mdm4 translocates from the cytoplasm to the mitochondria where it facilitates apoptosis (Mancini et al., 2009). Notably, the upregulation of Mdm4 by miR-885-3p together with cisplatin facilitates the export of Mdm4 to the mitochondria (Huang et al., 2011a).

Another miRNA that targets Mdm4 directly is miR-10a. Interestingly, there is an inverse correlation between miR-10a and Mdm4 in AML patients, according to their nucleophosmin (NPM1) mutation status; specifically, miR-10a is upregulated in NPM1-mutant AML (Ovcharenko et al., 2011). Of note, NPM1 stabilizes p53 in the nucleus upon DNA damage.

miRNA regulating both Mdm2 and Mdm4

As Mdm2 and Mdm4 greatly overlap in their function and mission, it is only reasonable to speculate that there may be miRNAs that would target both of them simultaneously. Indeed, we recently identified such miRNA—miR-661 (Hoffman et al., 2014). miR-661 targets *Mdm2* and *Mdm4* mRNA in a cell-specific manner: while in MCF7 breast cancer cells it targets both, in other cell lines it targets only *Mdm2*. This leads to increased p53 activity, cell cycle arrest, and a reduction in long-term colony-formation capacity. In agreement with the p53-activating role of miR-661, high miR-661 expression in ER⁺ breast cancer patients correlates with better prognosis; notably, ER⁺ breast cancers usually retain WT p53, and therefore miR-661 presumably acts as a tumor suppressor in these cells by augmenting WT p53 activity. On the other hand, the miR-661 locus is frequently amplified in several types of human tumors that harbor frequent *TP53* mutations. Indeed, in tumor-derived cell lines with mutant p53, miR-661 promotes migration, a well-documented feature of mutant p53 gain-of-function. Therefore, we propose that miR-661 can be either a tumor suppressor or an oncogenic miRNA (oncomiR), depending on the mutational status of p53 (Hoffman et al., 2014).

Another interesting feature of miR-661 as a regulator of both Mdm2 and Mdm4 is the location of its targets within the corresponding mRNA molecules. *Mdm4* mRNA contains nine targets for miR-661 within its 3'UTR, all but one residing within Alus. *Mdm2* mRNA contains three putative miR-661 targets, all within Alus. We suggest that *Mdm2* and *Mdm4* are most probably targeted by miR-661 via sequences residing within Alus. Like Alus, miR-661 is a primate-specific miRNA, and its targets within *Mdm2* and *Mdm4*

exist only in primates. This suggests that additional layers of regulation were added to the highly conserved p53 pathway during primate evolution, through emergence of a new miRNA and integration of Alus that inserted targets for this miRNA into the 3'UTRs of p53 network genes.

Role of Alu sequences in miRNA regulation and in the p53 network

As discussed above, miR-661 likely regulates *Mdm2* and *Mdm4* mRNA through their Alu sequences. Alus are very abundant in the human genome, and therefore it is only reasonable to assume that they affect the microRNA machinery at multiple levels. For example, there is growing evidence that some miRNAs are derived from TEs and specifically from Alu sequences (Smalheiser and Torvik, 2005; Piriyaopongsa et al., 2007; Yuan et al., 2010; Dahary et al., 2011). Of particular interest is the contribution of Alus to the 3'UTR of genes, since the insertion of these repetitive elements into thousands of primate genes has introduced a vast number of new potential miRNA targets into these 3'UTRs (Hoffman et al., 2013). Smalheiser and Torvik (2006) found that the seed region of a few dozen human miRNAs possesses complementarity to a highly conserved sequence within the Alu family, which seems open enough to serve as a functional binding site for miRNAs. Another feature of these potential targets within Alus is their multiple appearances within a single mRNA, considered an important feature of a functional target (Grimson et al., 2007). This multiplicity is not surprising, as in some genes, Alus appear several times within the same 3'UTR (Smalheiser and Torvik, 2006). Lehnert et al. (2009) identified a small number of miRNAs with over 1000 targets per megabase in Alu sequences; the majority of these miRNAs are derived from the C19MC cluster on human chromosome 19. The C19MC cluster harbors many miRNAs surrounded by Alu sequences, embedded in repetitive cassettes (Bailey et al., 2003). It has been hypothesized that miRNAs that target Alus serve to protect against Alu transposition (Bailey et al., 2003). Likewise, miRNAs may repress TEs to maintain genomic stability; in cancer cells, which often display compromised miRNA-mediated regulation, such deregulation may enable derepression of the TEs, eventually promoting genomic instability (Shalgi et al., 2010).

Recently, we showed that the majority of miRNA targets within Alus are non-responsive to manipulations of miRNA levels (Hoffman et al., 2013). By scanning a large dataset of mRNA expression arrays and Ago2 photoactivatable-ribonucleoside-enhanced cross-linking and immunoprecipitation (PAR-CLIP) data, we found that targets within Alus typically do not affect target mRNA expression, probably because there is no binding between the Ago-miRNA complex and the Alu-derived targets within the mRNA. The dysfunctionality of such targets presumably reflects a combined effect of their relatively closed secondary structure and their depletion from the ends of the 3'UTR, which are known to be preferentially miRNA-responsive (Grimson et al., 2007), and possibly also due to post-transcriptional RNA editing that occurs mainly within Alus (Hoffman et al., 2013). In addition, there might be other features and mechanisms that prevent the majority of Alu-derived miRNA targets from being functional, as suggested by Spengler et al. (2014), who performed further analysis of microarray data and luciferase assays and confirmed that indeed miRNA targets within Alus lack functionality or are weakly responsive to

microRNA-mediated regulation. However, on the background of this general dysfunctionality, a subset of target sites within Alus can be effectively recognized by specific miRNAs, as illustrated above for miR-661 and *Mdm2/Mdm4* mRNA.

Alus represent a unique opportunity for primates to gain new regulatory mechanisms and expand existing pathways. Alus are primate-specific, and therefore any functional target within an Alu in an mRNA's 3'UTR will create a primate-specific regulatory edge. This is extremely interesting in the case of a highly conserved pathway such as p53. It may present a novel opportunity for primates to create slight but significant changes in the regulation of highly important pathways. Another example of the importance of TEs in general and Alus in particular for creating new functional miRNA targets is provided by miR-24 and the gene sideroflexin2 (*SFXN2*). In both human and mouse, miR-24 targets are present in *SFXN2* mRNA within repetitive elements (Alu in humans and B1 element in mouse), but the chimpanzee genome contains one base substitution at this target. Indeed, in both human and mouse, but not chimpanzee, a 3'UTR luciferase construct carrying the corresponding region of the *SFXN2* 3'UTR was responsive to miR-24 overexpression (Spengler et al., 2014). This is an example of convergent evolution enabled by TEs, which highlights the importance of the mechanisms that endow Alu-resident miRNA targets with functionality in particular genes.

In summary, by introducing simultaneously many potential miRNA targets into numerous mRNAs, repetitive TEs pose a serious threat to proper genome function. Cells appear to employ multiple means of avoiding such TE-based miRNA targets, effectively excluding them from the miRNA regulatory network. Notwithstanding, while restraining this harmful potential by bulk exclusion of such targets, individual targets are gradually admitted into the network, expanding its diversity, and providing new regulatory opportunities. Such opportunities represented by TEs, in the p53 network and in other important pathways, may contribute toward addressing the need for more effective cancer-preventive mechanisms in primates. The lifespan and age of reproduction is appreciably higher in most primates compared with rodents. This is a major evolutionary shift that must have required the evolution of more effective mechanisms to prevent cancer, so that primates would sustain longer cancer-free life. It is therefore conceivable that the primate cancer-preventive networks would have acquired capabilities extending beyond those of their rodent counterparts. It is tempting to speculate that the observed Alu-mediated miRNA regulation of the p53 network might represent such additional capacity, further to other improved tumor suppressive mechanisms.

Conclusion

miRNAs are important regulators of cellular processes, which play a key role in the p53 network. In this review we focused on miRNAs targeting *Mdm2* and *Mdm4*, two major negative regulators of p53. Multiple miRNAs regulate either *Mdm2* or *Mdm4*, while miR-661 targets both. p53 regulates directly many of these miRNAs, mostly in a positive manner, thereby establishing a positive feedback loop. All of these miRNAs contribute to maintaining correct levels of p53 activity through *Mdm2* and *Mdm4*. Alu elements also play a role in miRNA-mediated regulation of the p53 network. Alus are primate-specific and are distributed throughout the human genome.

Intriguingly, Alus introduce many potential miRNA targets within the 3'UTRs of human genes. Although most of these potential miRNA targets are non-functional, there exist some exceptions. Thus, the downregulation of *Mdm2* and *Mdm4* by miR-661 probably involves functional targets within Alus. Alus therefore introduce a new layer of primate-specific regulation in the p53 network, involving the activity of miRNAs.

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