The Guardian’s Little Helper: MicroRNAs in the p53 Tumor Suppressor Network

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Abstract

Several microRNAs (miRNAs) have been implicated in tumor development based on both changes in their expression patterns and gene structural alterations in human tumors. However, we are only now beginning to see how miRNAs interact with classic oncogene and tumor suppressor mechanisms. Several recent studies have implicated the miR-34 family of miRNAs in the p53 tumor suppressor network. The expression of miR-34a, miR-34b, and miR-34c is robustly induced by DNA damage and oncogenic stress in a p53-dependent manner. When overexpressed, miR-34 leads to apoptosis or cellular senescence, whereas reduction of miR-34 function attenuates p53-mediated cell death. These findings, together with the fact that miR-34 is down-regulated in several types of human cancer, show that miRNAs can affect tumorigenesis by working within the confines of well-known tumor suppressor pathways. [Cancer Res 2007;67(23):11099–101]

Background

p53 responds to DNA damage or deregulation of mitogenic onco-genes through the induction of cell cycle checkpoints, apoptosis, or cellular senescence (1, 2). Mutations in p53 are often associated with aggressive tumor behavior and poor patient prognosis (3). The p53 tumor suppressor network has been intensively studied; however, genetic analyses long hinted at the existence of components that remained elusive (1, 2). For example, although p53 is clearly a transcriptional activator, numerous reports indicated that p53 also represses the expression of specific genes either directly or indirectly (4). The manner in which this occurred was obscure, with both transcriptional and posttranscriptional suppression as possible mechanisms. In the latter case, the discovery of extensive networks of microRNAs (miRNAs), which act through the RNA interference pathway (RNAi), offered the possibility that p53-mediated control of miRNA expression could allow it to act indirectly to repress target gene expression at the posttranscriptional level.

miRNAs are small, 18- to 24-nucleotide noncoding RNAs that regulate gene expression largely through effects on productive translation and miRNA stability (5–7). Nascent miRNA transcripts (pri-miRNAs) are often RNA polymerase II products, with promoters that closely resemble those of protein-coding genes. Rather than being exported to the cytoplasm for translation, pri-miRNAs are processed by the microprocessor complex in the nucleus, and the hairpin products (pre-miRNAs) are then cleaved by Dicer in the cytoplasm to generate mature miRNA duplexes (5–7). The mature miRNA joins RNA-induced silencing complex, which it guides to target mRNAs by imperfect base pairing that depends mainly on the “seed,” sequences comprising bases 2 to 7 of the mature miRNA (6).

Increasing evidence has suggested that miRNAs are components of oncogene and tumor suppressor pathways. Inappropriate expression and structural alterations of miRNA genes have been found in a variety of tumor types (8, 9), and several functional studies have shown the oncogenic or tumor-suppressive potential of specific miRNA families (10–13). Among these are five recent reports that uncover miRNA components in the p53 tumor suppressor network (14–18).

Key Findings

miR-34s form an evolutionarily conserved miRNA family, with three members in vertebrate genomes (miR-34a, miR-34b, and miR-34c) and single orthologues in invertebrate species. Initial links to tumorigenesis emerged from Welch et al. (19) who showed that the miR-34a gene, residing at 1p36, is often lost in human neuroblastoma. 1p36 deletions frequently encompass very large numbers of genes; however, primary neuroblastomas and cell lines often showed low miR-34a expression (19). Artificial elevation of miR-34a in these cells inhibited proliferation and activated cell death pathways (19).

Shortly afterward, links to tumor biology solidified with the finding that miR-34 levels correlated with p53 status. Four groups approached the general relationship between p53 and miRNAs using a variety of different profiling approaches and cellular contexts. Chang et al. (15) compared miRNA profiles of p53+/+ and p53−/− HCT116 cells in response to DNA damage, whereas Ravera-Shapira et al. (17) and Tarasov et al. (18) probed miRNA levels in lung carcinoma cells (H1299) harboring regulated p53 alleles. He et al. (16) compared miRNA expression in a series of p53+/+ and p53−/− mouse embryonic fibroblasts (MEF) bearing several different oncogenic lesions. In addition, Bommer et al. (14) identified miR-34 family from a previously published genome-wide p53 chromatin immunoprecipitation analysis (20). Several potentially p53-responsive miRNAs emerged from these studies, but all five groups zeroed in on the miR-34 family for further study.

There are two mir-34 loci in vertebrate genomes, one encoding miR-34a and the other yielding both miR-34b and miR-34c from a single primary transcript. Both genes show little conservation even among closely related species, except in the miRNA-encoding sequences and in short promoter proximal regions that each contains a consensus p53-binding site (14–18). The miR-34a and miR-34b/miR-34c loci are regulated directly by interaction of p53 with these consensus sites, as can be shown by chromatin immunoprecipitation (14, 16–18) and by the ability of miR-34 promoters to drive expression of luciferase reporters in a p53-dependent manner (14–17). The consequences of miR-34 activation may vary depending on the cell type. In some cases, ectopic expression or delivery of synthetic mir-34 mimetics resulted in cell cycle arrest or senescence.

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In other cases, the output was apoptosis, with the response being reduced by inhibition or depletion of miR-34. Achieving these different outcomes may depend on the spectrum of miR-34 regulatory targets that are expressed in a given cell type. A combination of bioinformatic predictions and experimental analysis led to the notion that miR-34s control broad programs of targets involved in cell cycle control, apoptosis, and DNA repair, among which cyclin-dependent kinase (cdk) 4, cyclin E2, cMet, cdk6, and bcl-2 were shown as possible candidates.

**Implications**

Activation of p53 leads to diverse cellular responses, including apoptosis, cell cycle arrest, blockade of angiogenesis, and activation of DNA repair. The output of the p53 pathway is determined by the coordinated transcriptional activation of p53 target genes in a context-dependent manner (Fig. 1). For example, p53-induced apoptosis is dependent on the induction of not only bax but also puma and noxa. The identification of the miR-34 family as p53 targets expands the repertoire of p53-regulated genes to include small RNAs. An important lesson to be drawn from this finding is the potential for small RNAs to fill roles in signaling network, which have persisted as long-standing mysteries. p53 induces growth arrest through its activation of the cdk inhibitor p21. However, studies of p21-deficient MEFs indicated the existence of another pathway that worked in parallel with p21 to enforce p53-dependent G1 arrest. Searches for protein-coding mediators of the cell cycle effects of p53 yielded several candidates, but these mainly promote G2 arrest. Notably, miR-34 can induce G1 arrest independently of p21 in specific cell types, raising the possibility that this small RNA may normally act in parallel with p21, filling the genetically predicted gap in this arm of the p53 pathway.

With accumulating evidence revealing the importance of miRNAs in cancer, it is now accepted that miRNAs can have tumor suppressor or oncogenic activity. For example, 13q14, a chromosomal locus deleted in >50% of B-cell chronic lymphocytic leukemias, contains two miRNAs, miR-15 and miR-16, which suppress the expression of bcl-2 and likely act as tumor suppressors. The miR-17-92 gene, which is amplified in B-cell lymphomas and shows altered expression in numerous tumor types, displays oncogenic activity in a variety of models. Now, we see that miR-34s may at least participate in tumor suppression as part of the p53 network. Whether or not miR-34s are bona fide tumor suppressors in their own right awaits further study. The mir-34a gene maps to 1p36, a region of common loss in many human tumor types. In addition, reduced miR-34a expression is a frequent feature of both pancreatic tumors and neuroblastomas and reduced miR-34b and miR-34c expression has been observed in a subset of non-small cell lung cancers. In these cases, the lack of miR-34...
may not simply reflect the loss of p53 as p53 is often wild-type in these tumors. Overall, accumulating evidence is forcing us to remodel our notions of oncogenes and tumor suppressors to include noncoding RNAs, and as a class, these may afford new opportunities for diagnosis and treatment of human cancer.

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