

The Function and Potential of MicroRNAs

Since the discovery of the principle of inheritance and the existence of genes, there has been an effort to determine how and what controls the expression of the thousands of genes in the cell. Unraveling the mysteries surrounding the regulation of gene expression has uncovered regulatory proteins controlling translations and transcription of DNA and also structural modifications in various levels of genome organization. More recently, it was discovered that in addition to proteins, RNA also plays an important role in the regulation of gene expression. One type of regulatory RNA is microRNA (miRNA), discovered in 1993. MicroRNAs are single-stranded RNAs about 22 nucleotides long, and they are partially homologous to the genes and messenger RNAs (mRNAs) they suppress in regulation. One miRNA may control multiple genes, and one gene may be controlled by multiple miRNAs. Currently, over 500 miRNAs have been identified in human cells; it is estimated that as many as 50,000 miRNAs exist in the human genome, each regulating many different targets (Glaser, 2008).

Like proteins, miRNAs are transcribed from DNA into a primary RNA transcript. The primary transcript is much longer than the final miRNA product and undergoes processing by the nuclease Drosha to form a stem-loop structure called pre-miRNA (Zeng, 2004). This pre-miRNA is transported from the nucleus into the cytoplasm by exportin and then processed by the RNase Dicer. Dicer cleaves the pre-miRNA stem-loop into two complementary pieces, producing mature miRNAs (see Figure 1). The miRNAs are then bound by specific Argonaute proteins, ALG-1 and ALG-2 (Jannot, 2008). Argonaute proteins are active RNase enzymes in the RNA-induced silencing complex (RISC), a multicomponent nuclease. After integration into the RISC

complex, miRNAs base pair with their partially complementary target mRNA and block its translation into proteins, thereby reducing specific gene expression (Bernstein, 2001).

To perform its function of gene regulation in the cell, miRNA is complementary to a part of one or more mRNAs. In animals, the miRNA usually binds to the 3' un-translated region (3'UTR) of the mRNA. This region follows the coding region of the messenger RNA, in between the coding sequence and the 3' polyA tail. Recently, it was demonstrated that in addition to acting through the 3' UTR of target mRNA, miRNA may also target sites in the amino acid-coding regions and the 5' un-translated region between the 5' cap and the coding region (Glaser, 2008). It is believed that miRNA binding to mRNA only blocks protein translation without causing the mRNA to be degraded, though other studies suggest miRNA also causes mRNA degradation. Degradation of the mRNA can also occur through the RNA interference process, which uses small interfering RNA (siRNA) – short, double stranded RNA molecules very similar to miRNA. siRNA, however, is completely complementary to its mRNA target while miRNA is only partially complementary (Elbashir, 2001).

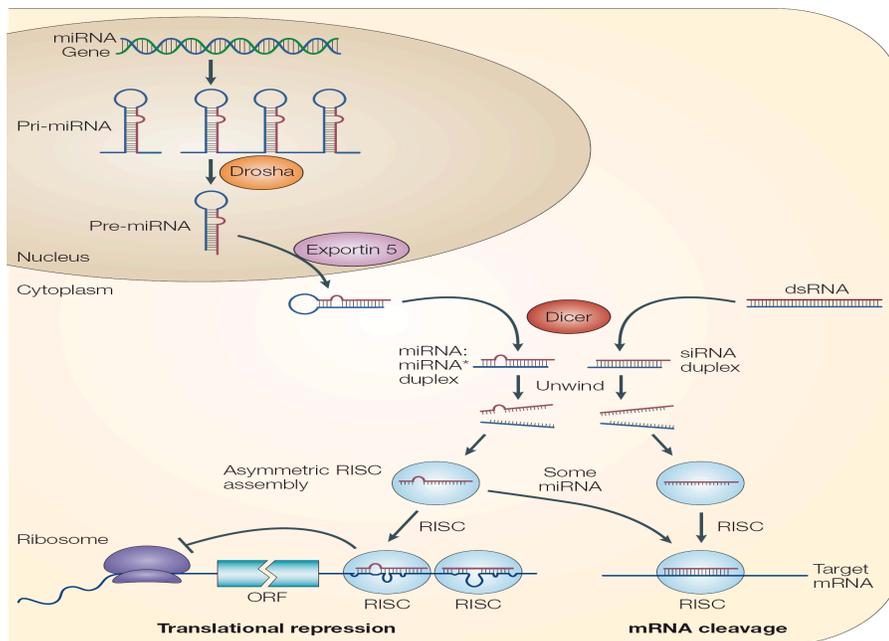


Figure 1: miRNA processing and function (Mraz, 2007)

Recent research has shown that miRNA has functions other than suppressing gene expression and blocking protein translation. It is possible that in certain cases, miRNA can bind to the promoter region of certain genes and induce gene expression (Place, 2008). Activation of gene expression is termed small RNA-induced gene activation, or RNAa. As more research is done on miRNAs, it was found that miRNA expression is extremely indicative of cell type. Out of the over 500 miRNAs so far characterized in humans, about 80-150 miRNAs are typically expressed in a particular cell type (Glaser, 2008).

Gene regulation by miRNAs can affect a wide variety of cell functions, such as regulation of cell differentiation, proliferation, and apoptosis. Just as miRNAs are important in the normal functioning of cells, a dysfunction of the miRNA regulation system would result in disruption of normal cell function and cause disease. It was first noted in 2002 that miRNA may be connected to cancer. Calin *et al.* made this connection by showing that *miRNA-15* and *miRNA-16* genes are located in a deleted region present in more than half of B-cell chronic lymphocytic leukemia (CLL) cases, and both genes are deleted or mutated in more than 68% of CLL cases (Calin, 2002). In 2005, the *miR-17-92* cluster of miRNAs on chromosome 13 was found to be associated with B-cell lymphoma and may be potential oncogenic miRNAs. Comparing cancer cells to normal cell types, it was found that cancer cells had substantially increased expression of these miRNAs, which acted with c-MYC oncogene expression to accelerate tumor development in B-cell lymphoma (He, 2005). miRNAs act as oncogenes in some types of cancers, but it can suppress tumor development in other types of cancers. The normal form of c-MYC is a proto-oncogene that encodes a transcription factor important in regulation of cell proliferation and growth; a mutation in the c-MYC gene or gene expression is one of the most common abnormalities in human cancers. It was shown that c-MYC activates

expression of a cluster of miRNA on human chromosome 13, and expression of two miRNAs in the cluster represses the expression of E2F1, a transcription factor that promotes cell cycle progression (O'Donnell, 2005). This discovery suggests a mechanism by which miRNAs play a role in the regulation of the normal cell cycle and prevent cancer cell proliferation.

As more and more research is done on miRNAs, it is found that miRNAs are implicated as either oncogenes or tumor suppressor genes in a wide range of human cancers and play a significant role in tumorigenesis. Hayashita *et al.* found that *miR-17-92* was markedly over-expressed in lung cancer, especially with small-cell lung cancer. Their findings suggested that over-expression of the *miR-17-92* cluster with occasional gene amplification might play a role in the development of lung cancer (Hayashita, 2005). Other studies showed that specific miRNAs were repressed or activated in human breast cancer compared with normal breast tissue. The miRNA expression correlated with specific breast cancer features, such as estrogen and progesterone receptor expression, tumor stage, vascular invasion or proliferation index (Iorio, 2005). Chang *et al.* showed that *miR-34a* is frequently absent in pancreatic cancer cells. They demonstrated that this miRNA was directly trans-activated by *p53* gene, and it was likely that an important function of *miR-34a* is the modulation and fine-tuning of the gene expression program initiated by *p53* (Chang, 2007). Papillary thyroid carcinoma, glioblastoma, colorectal cancer, neuroblastoma and many other cancer types have all been connected to abnormal expressions of miRNA (Cho, 2007).

In addition to cancer, miRNAs are implicated in other diseases. It was reported that a cardiac-specific knockout of the Dicer gene leads to rapidly progressive dilated cardiomyopathy (DCM), heart failure, and postnatal lethality. Dicer mutant mice also had significantly reduced heart rates. Also, Dicer expression decreased in end-stage human DCM and heart failure. These

findings suggest that Dicer function and miRNAs play critical roles in normal cardiac function and in heart diseases (Chen, 2008). Another study by Zhao *et al.* showed miRNA production in the mouse heart is essential for cardiogenesis. Furthermore, targeted deletion of the muscle-specific miRNA, *miR-1-2*, revealed numerous functions in the heart, including regulation of cardiac morphogenesis, electrical conduction, and cell-cycle control (Zhao, 2007). In neurodevelopment, overabundance of miRNAs has been associated with fragile X mental retardation, and miRNA pathways are important for neuronal plasticity and remodeling in general (Qurashi, 2007).

miRNAs are also involved in stem cell division and development. Stem cells have the ability to escape cell cycle stop signals and divide for long periods of time in conditions where most cells enter the G₀ phase. On the basis of cell cycle markers and genetic interactions, Hatfield *et al.* reported that Dicer mutant stem cells, which are unable to produce miRNAs, were delayed in the G₁ to S phase transition. This suggested that miRNAs are required for stem cells to bypass the normal G₁/S checkpoint and continue on the cell cycle. The miRNA pathway might be part of a mechanism that makes stem cells insensitive to environmental signals that normally stop the cell cycle at the G₁/S transition and allows stem cells to escape cell division stop signals (Hatfield, 2005).

Discovery of the role of miRNAs in various pathological processes has opened up possible applications of miRNA in molecular diagnostics and prognostics, particularly for cancer. miRNA expression profiles could be used to discriminate lung cancers from non-cancerous lung tissues, and over-expression of certain miRNAs were found to correlate with poor survival of lung adenocarcinomas (Yanaihara, 2006). These results indicate that miRNA expression profiles can serve as diagnostic markers as well as prognostic markers for lung

cancer. In pancreatic tumors, over-expression of *miR-21* was strongly indicative of presence of liver metastasis and related to progression of cancer malignancy (Roldo, 2006).

With increased knowledge of miRNAs and their roles in the origin and progression of disease, novel clinical therapies and treatments are being developed for the treatment of these diseases. Production of miRNA microarrays, referred to as MMChips, have enabled the easy detection and profiling of miRNAs, important for diagnosis and prognosis of cancers. Some miRNA expressions are controlled by epigenetic alterations in cancer cells, including DNA methylation and histone modification (Kozaki, 2008). Using chromatin-modifying drugs to demethylate DNA and activate tumor suppressor miRNAs can regulate target oncogenes and may lead to novel cancer therapies in the future. More recently, attention has been focused on exploring miRNA-replacement therapies, in which miRNA is reintroduced in a diseased cell to reinitiate pathways that have been turned off by the down-regulation of the miRNA (Glaser, 2008). Stem cells and the role miRNA plays in stem cell division are also of great interest in the cancer field. Stem cells possess features required for sustained tumor propagation, and there is increasing evidence that cancers develop from a small subset of cells called cancer stem cells. Understanding how these stem cells continue to divide and escape detection by the immune system and the role miRNAs play in the process would be an important step in finding a cure for cancer (Cho, 2007).

Though miRNAs are a relatively recent discovery, they are becoming increasingly important in the field of biology and medicine. Realizing the function and role of miRNAs can open the way for deeper understanding of the molecular mechanisms in normal cell function as well as in diseased cells and suggest new treatment methods with the potential to advance medical cures and save lives.

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