miRNAs in animal development and disease

Introduction

Initially, miRNAs had been only identified in multicellular organisms, suggesting that miRNAs may be essential for organisms to differentiate into multiple cell types. Although miRNAs are indeed important for development, further studies showed that even single-celled algae do encode miRNAs enabling them to transition from one stage to another (i.e., from vegetative to reproductive state) or to respond to their environment. Here, we will discuss the role of miRNAs in animal development, in particular that of vertebrates, by looking at zebrafish as a model. Furthermore, we will discuss how miRNAs can function in human diseases and how the de-regulation of miRNAs can lead to cancer.

Characteristics of animal miRNAs

miRNA mode of action

We have already discussed the mode of action of miRNAs in lesson 1, but will briefly refresh the main points: In animals, most miRNAs bind to the 3' UTR of their target gene, although there is growing evidence that target sites can also be located within the 5' UTR or the coding region of a gene. The vast majority of animal miRNAs are imprecisely complementary to their target, resulting in translational repression coupled to target mRNA decay; however, there are exceptions where animal miRNAs bind with perfect complementarity, allowing Argonaute-catalyzed endonucleolytic cleavage (slicing) of the complementary mRNA strand (see figure 1-9). Many animal miRNA-target transcripts carry multiple miRNA target sites for several distinct miRNAs in their 3' UTR. This allows an incredibly sophisticated mode of gene-expression control at the post-transcriptional level.

miRNA locations within genomes

Over 3'000 different *miRNA* genes have been identified in animals to date, of which more than half are located within introns of genes. Many of these miRNAs are co-expressed with their host genes. Interestingly, the location of some of these miRNAs is evolutionary conserved, and they are similarly co-expressed with their host genes in different animals. This suggests that these miRNAs have important, evolutionarily conserved roles. Many *miRNA* genes are located at genomic regions that are involved in different cancer types.

miRNA genes in the animal kingdom

miRNA genes can already be found very early in the evolution of animals. Remarkably, there is a clear correlation between the number of miRNAs and the morphological complexity in the animal kingdom, suggesting that the innovation of miRNAs may have been a key player in the development of complex organisms.

But how can (novel) *miRNA* genes arise in a genome? Actually, the characteristics of miRNAs make it more straightforward to "create" a (new) miRNA than a protein-coding gene: the only requirement for a gene encoding a functional miRNA is that it produces a transcript that can form the correct secondary structure that can be recognized by Drosha and Dicer to form mature a miRNA. Because RNAs easily form non-perfect folded, hairpin-like structures, it seems more likely that a *miRNA* gene might emerge more easily than a novel protein-coding gene. Another favorable characteristic of miRNA function is that they recognize short sequences within their target gene, for interaction; thus, miRNA target sites can easily be acquired and/or lost in the transcriptome of the cell. Here, remember that the core site of animal miRNA-target recognition is only six nucleotides long (the seed sequence); thus, a few base-pair changes in the miRNA-gene sequence are very likely to alter the repertoire of possible target genes.

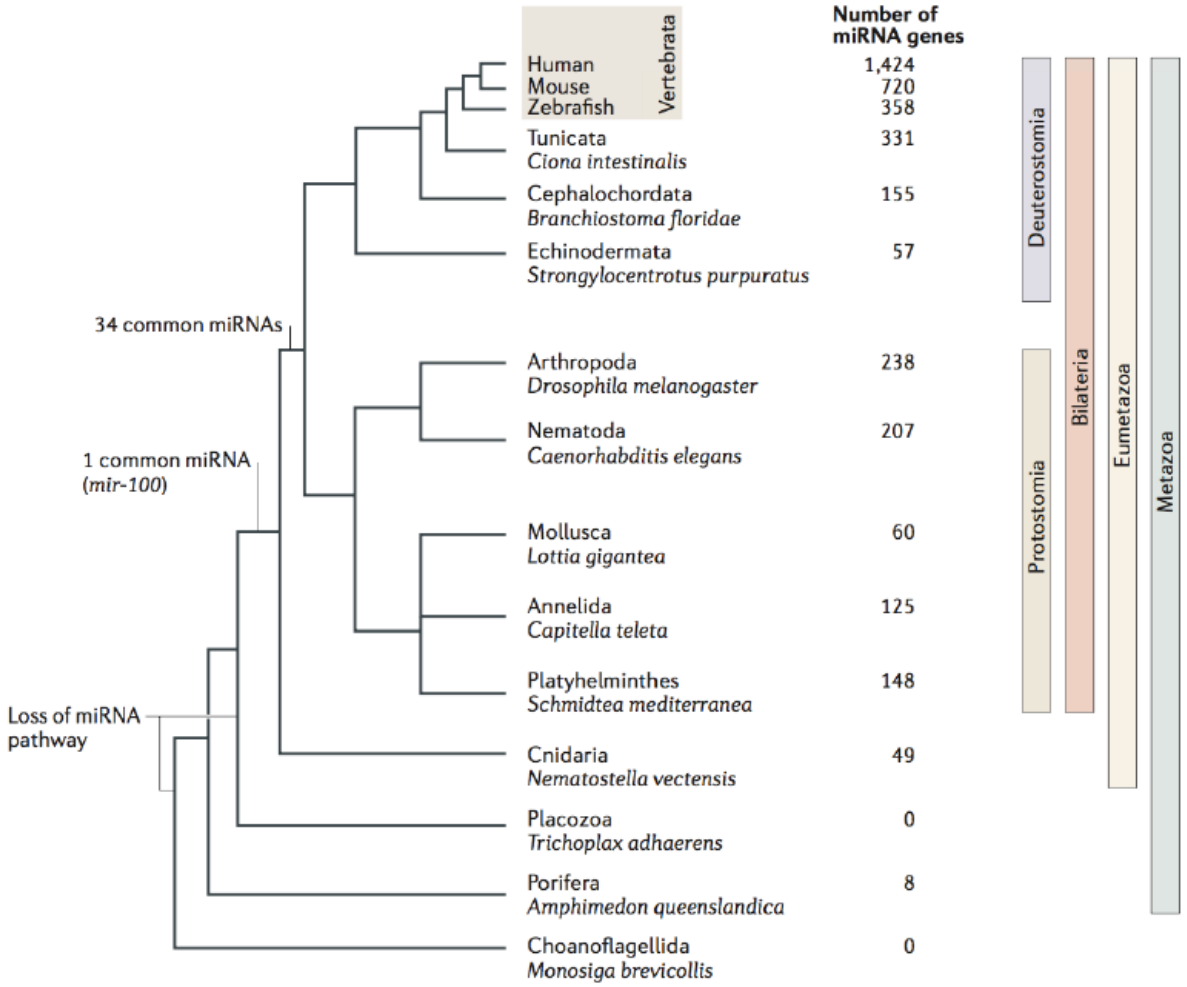

Figure 3-1 Distribution of miRNA genes in the animal kingdom. miRNAs are present already at the base of animal evolution. miRNAs abundance increases within the vertebrate lineage and further increases in the lineage leading to placental mammals (note the doubling of miRNA numbers from zebrafish to mice). Interestingly, after a miRNA gene has emerged in a particular lineage, it is rarely lost in the descendant lineages. (Adapted from E. Berezikov, *Nat. Rev. Genet.*, 2011)

The role of miRNAs in vertebrate development: lessons learned from zebrafish

One problem in studying the function of miRNAs during animal development is the lack of miRNA loss-of-function mutations. Many miRNAs have been missed in classical forward genetic screens, because *miRNA* genes are relatively small (roughly 50 times smaller than the average protein-coding gene), and it is statistically less likely that a mutation will be induced into a small gene during mutagenesis. In fact, only three miRNAs were identified in classical screens in *C. elegans*.

How would one go about studying the very early role of miRNAs in development if loss-of-function mutants for most *miRNA* genes are not available? Here, organisms with defective miRNA biogenesis have been extremely useful, where the enzyme Dicer was knocked out or knocked down. In animals lacking Dicer function, pri-miRNAs are transcribed, but the precursors cannot be processed into mature miRNAs. Dicer full or hypomorphic mutants have been generated in many different animals, including *C. elegans*, *Drosophila* and vertebrates, and all of these have shown that miRNAs are required for normal development. However, these studies have also shown that miRNAs exert their function

beyond the developmental stages and are required in adults to maintain tissue integrity. Here, we will discuss the results obtained in vertebrate models with a focus on research done in zebrafish.

In zebrafish, loss-of-function mutations in Dicer lead to a developmental arrest at a rather late developmental stage (larval stage, day 8 after fertilization), indicating that the maternally provided Dicer is sufficient to reach this point. At this relatively late stage, all the major organs have already been formed, and the fish has no obvious abnormalities. Thus, researchers generated mutants lacking maternally provided Dicer in addition to the Dicer knockout. These animals are deficient in both maternally derived as well as zygotic Dicer and are therefore called MZ (for maternal-zygotic) mutants. Although MZ animals do not process pre-miRNAs into mature miRNAs, they only have mild defects during early development. They show an intact axis formation and differentiate into different cell types, indicating that embryonic patterning is not affected (see figure 3-2B, showing a fish at 48 h after fertilization). However, at later stages, they show some morphogenesis defects, mostly during brain formation, neural differentiation, somite formation, and heart development.

In the MZ-Dicer-mutant embryos, neurulation is severely affected. Here, we will discuss two of the abnormalities that occur in Dicer-mutant animals: The formation of the brain ventricles and the formation of the midbrain-hindbrain boundary. The ventricles are cavities within the brain that produce and transport the cerebrospinal fluid, which bathes the central nervous system. The developing brain is subdivided by several constrictions into distinct regions, and the boundary between midbrain and hindbrain is very prominent (see figure 3-2A,E). When looking at the MZ-Dicer mutants, it was found that the brain ventricles are not formed correctly. Furthermore, the constrictions that divide the brain into distinct regions did not form in MZ-Dicer mutants, and the midbrain-hindbrain boundary was not developed (see figure 3-2B,F).

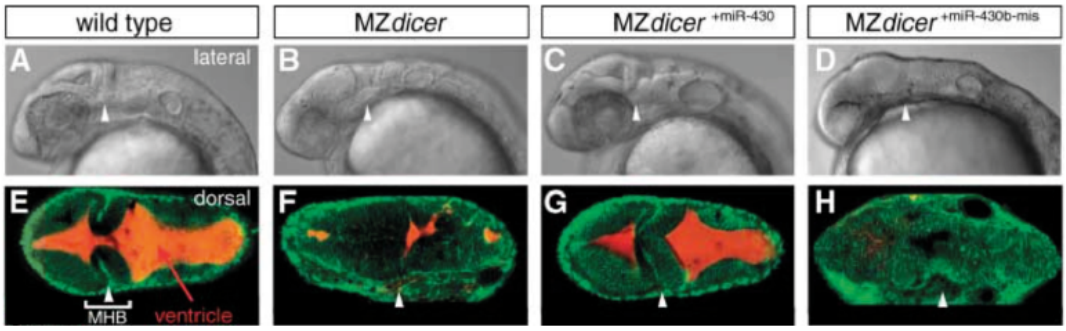

Figure 3-2 Fish deprived of Dicer activity (and therefore, deprived of all miRNA function) do not develop a functional brain. The mutant fish are depleted of both maternally provided as well as zygotic Dicer, and are therefore called MZ*dicer* mutants. The upper panels show lateral light-microscopy images **(A-D)**. In the lower panels, confocal dorsal views of the embryos are shown where cell membranes are labeled in green and brain ventricles labelled with red **(E-H)**. **(A, E)** Wild-type fish display the characteristic fold of the midbrain-hindbrain boundary (MHB, arrowhead) and have brain ventricles (orange). **(B, F)** MZ*dicer* embryos lack the midbrain-hindbrain boundary and ventricles. **(C, G)** The ventricles as well as the midbrain-hindbrain boundary formed in MZ*dicer* mutants injected with miRNA (miR-430), similar as in wild-type embryos. **(D, H)** Injection of a miRNA with point mutations that result in sequence mismatches with its target (miR-430b-mis) fails to rescue brain development in MZ mutants. (Adapted from A.J. Giraldez *et al.*, *Science*, 2005)

Interestingly, the researchers had found that one specific family of miRNAs, miR-430, were highly expressed between fertilization and the first 48 h of development. They therefore tested whether injection of a pre-processed, mature miR-430 (a duplex miRNA that resembles the Dicer-processed form of a miRNA) could rescue the brain morphogenesis defects in the Dicer mutants. Indeed, local injection of the processed miR-430 into Dicer mutants resulted in the formation of normal brain ventricles and brain constrictions (see figure 3-2C,G). Amazingly, a single miRNA is sufficient to

restore near-wild-type brain development, indicating that miR-430 alone is responsible for a major switch in brain development. This also indicates that miR-430 most likely controls many different target-mRNA transcripts that are involved in brain morphogenesis.

These findings in zebrafish indicate that miRNAs are not essential for cell-fate determination and early patterning during the first 48 h of development, but that they are essential for later steps of embryogenesis. Indeed, when looking at the expression patterns of a large number of miRNAs in zebrafish, most miRNAs were not expressed during early development. The researchers analyzed the expression of over 100 miRNAs and found that until segmentation, most miRNAs could not be detected, and most miRNAs became only visible 1 to 2 days after fertilization and showed strong expression when organogenesis is virtually completed at 96 h after fertilization (see figure 3-3A). When analyzing where the miRNAs were expressed in the different zebrafish tissues they found that, interestingly, the expression of miRNAs is highly tissue-specific in fully developed tissues (see figure 3-3B). Even within an organ, different miRNAs are specifically expressed within distinct regions. For example, in the zebrafish pancreas, miR-217 can be seen to be expressed in the exocrine pancreas, and miR-7 in the endocrine pancreas (Langerhans islets, see figure 3-3C). These results suggest that the role of miRNA during development is not only in tissue-fate establishment, but also (and in this example, mainly) in differentiation or maintenance of tissue identity and integrity.

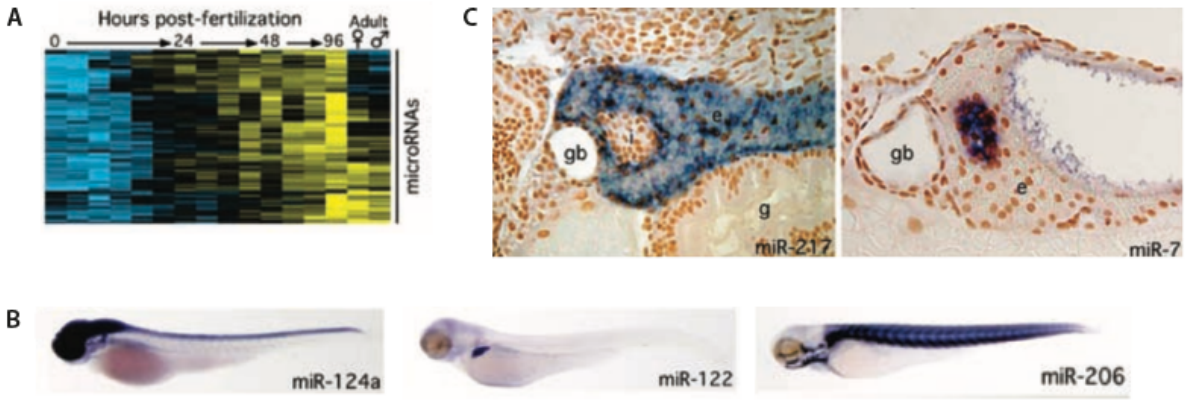

Figure 3-3 miRNA expression during zebrafish embryonic development. (A) Expression levels of 90 miRNAs during embryonic development. Colors indicate relative expression for each miRNA: blue, low; black, average; yellow, high. **(B)** Expression of miRNA in different organ systems of 72h-old embryos. miR-124a: nervous system; miR-122: liver; miR-206: muscles. **(C)** Histological analysis of miRNA expression in the pancreas 5 days after fertilization shows that different miRNAs are differentially expressed even within the same organ. ***Abbreviations***: e, exocrine pancreas; i, pancreatic islet; gb, gall bladder; g, gut. (Adapted from E.Wienholz *et al.*, *Science*, 2005)

When looking at figure 3-3A, you realize another striking phenomenon: many miRNAs that are expressed during development stay highly expressed in the adult. This again suggests that many miRNAs have specific roles other than developmental patterning and that they act as guardians of established cell fates. The failure to maintain an acquired cell fate is one of the causes of cancer, as we will see later in this lesson.

miRNA in human disease

In humans, approximately 2'200 *miRNA* genes have been reported, and one third of the human genes are estimated to be regulated by miRNAs. miRNAs play a major role in a wide range of developmental processes including cell proliferation, cell cycle, cell differentiation, metabolism, apoptosis, developmental timing, neuronal cell fate, neuronal gene expression, brain morphogenesis, muscle differentiation, and stem-cell division. Remember that animal miRNAs can repress the gene translation of hundreds of their targets. Thus, it is easy to imagine that changing the miRNA composition or action results in a cell nightmare, which might be leading to disease. What are the mechanisms by which miRNA function can be altered?

Mechanisms that change miRNA action

There are different ways how miRNA action can be changed. First, let's consider changes that affect a small number of miRNAs or their targets. Here, a miRNA may acquire a mutation resulting in loss of its function. Alternatively, there may be a gain-of-function mutation in a miRNA, and overexpression by amplification of the miRNA locus may work like overexpression of an oncogene, resulting in cancer. On the other hand, the binding sites of miRNAs within their target mRNAs might be mutated such that the miRNA cannot interact with its target any longer. Or, a gene may acquire a new and undesired miRNA target sequence that results in its aberrant silencing. Here, remember that the seed sequence is only six nucleotides long, and that many miRNAs are expressed simultaneously in the same tissue. The likelihood that a mutation will lead to a sequence that can be targeted by any of these miRNAs is quite high. If a sequence is only one mutation away from becoming a target for one of these miRNAs, a single mutation will result in an undesired reduction of this gene's activity, which may be the cause of disease. In the second scenario, global changes in miRNA and/or target accumulation can occur. For example, mutations could render the miRNA biogenesis pathway non-functional or lead to an increase in miRNA biogenesis. Thus, miRNA action may be altered both by mutations or by gene amplification.

We will now look, in more detail, into the mechanisms how single point mutations can affect miRNA-mRNA interactions and lead to a different miRNA response eventually causing disease. For that, we will briefly refresh the different steps of miRNA biogenesis and processing necessary for their sequence-specific gene silencing. *miRNA* genes are transcribed by RNA polymerase II to produce a 500-3'000-nucleotide transcript, called the pri-miRNA, which is then cropped to form a pre-miRNA hairpin of 60-100 nucleotides by a multi-protein complex including Drosha. This double-stranded hairpin structure is exported from the nucleus to the cytoplasm by Exportin 5. Finally, the pre-miRNA is cleaved by Dicer to produce a double-stranded miRNA of approximately 20 nucleotides in length. The guide strand is incorporated into the RNA-inducing silencing complex (RISC), while the passenger strand is discarded. The single-stranded miRNA that was incorporated into RISC guides it to the 3'-UTR mRNA sequence of the target to facilitate translational repression coupled to mRNA decay, or, in rare cases, mRNA endonucleolytic cleavage (see figure 1-7).

Mutations affecting miRNA biogenesis

Point mutations or single-nucleotide exchanges are referred to as single-nucleotide polymorphisms (SNPs) when they occur with a certain frequency within a population. When you think about the biogenesis of miRNAs, there are several possibilities how SNPs can affect miRNA functions: on the level of transcription of the primary transcript, on the level of miRNA biogenesis, or by affecting miRNA-mRNA interactions. Let's start by looking at how SNPs in some *miRNA* genes were shown to affect miRNA biogenesis or processing. The first evidence that mutations in *miRNA* genes can have a functional effect came from studies on chronic lymphocytic leukemia, where a mutation in

mir-16-1 led to low levels of miR-16-1 expression. The mutation led to a change in the sequence of the pri-miR-16-1 (see figure 3-4A). Apparently, this SNP changes the processing and the levels of mature miRNA. Several other studies have shown that SNPs in the pri regions (sequences present only in the pri-miRNAs) lead to decreased mature miRNA levels, and in most cases, this decrease was associated with a higher risk to develop cancer. SNPs have also been found in the sequences of pre-miRNAs, and these SNPs were also associated with various types of cancer. In figure 3-4B, the case for miR-196a-2 is depicted, in which a SNP has been found to affect the 3' sequence of the passenger strand. This SNP is suspected to affect miRNA maturation, since the presence of this SNP reduces the levels of mature miR-196a-2. Importantly, although the two examples shown in figure 3-4 depict the down-regulation of mature miRNA levels, SNPs can also lead to an up-regulation of miRNA processing and therefore an increase in mature miRNAs.

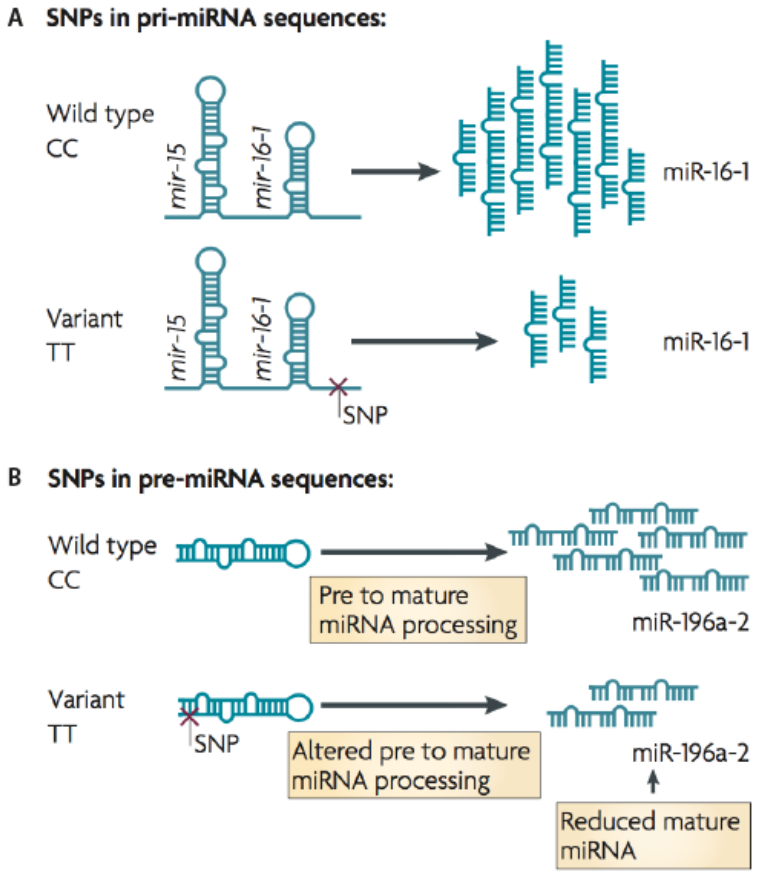

Figure 3-4 SNPs affecting miRNA processing lead to changes in mature miRNA levels. SNPs can occur at different positions in pri-miRNA **(A)** and pre-miRNA sequences **(B)**. Such SNPs can lead to either an increase or decrease in processing (here, examples for a decrease are depicted). (Adapted from B.M. Ryan *et al., Nat. Rev. Cancer,* 2010)

Mutations affecting miRNA-target interactions

We have already mentioned above that mutations can also affect the binding of miRNAs to their targets. This can either be due to mutations in the seed sequence of the miRNA or due to mutations in the target genes. For the miRNA miR-146a, a SNP was identified that affects the seed sequence in the passenger strand. Interestingly, individuals that are heterozygous for this mutation showed a greater risk of developing cancer than homozygous individuals. Why would a heterozygous situation pose a greater risk to develop the cancer? This can be explained by the effect of this mutation for the action of miR-146a: the SNP falls within the seed region of the passenger strand, thus creating a new target binding site. In heterozygous individuals, there are now three different mature miRNAs: one from the guide strand and two different ones from the passenger strand, the latter two differing only in the SNP in the seed sequence. Thus, each of these three mature miRNAs can target different mRNAs. In homozygous individuals, however, only two species of mature miR-146a are produced (the original 5p strand and the 3p strand containing the SNP); thus, the repertoire of putative target mRNAs is smaller than in heterozygous individuals. In patients with miR-146a-associated tumors, researchers have found that somatic mutations exist that change miR-146a-homozygous cells into heterozygous ones.

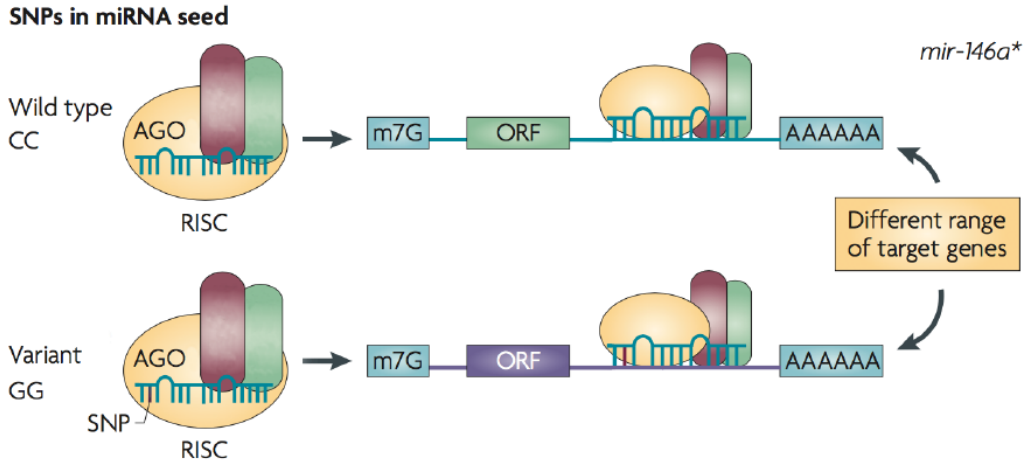

Figure 3-5 Single nucleotide polymorphisms (SNPs) in mature microRNAs (miRNAs) within the seed sequence can strengthen or reduce binding between the miRNA and its mRNA target. Moreover, such SNPs can create or destroy target-binding sites, as is the case for *mir-146a** (* denotes the passenger strand). AGO-associated proteins are shown in red and green. ***Abbreviations***: AA, poly(A) tail; m7G, 7-methylguanosine cap. (Adapted from B.M Ryan *et al., Nat. Rev. Cancer*, 2010)

Finally, SNPs can also occur in the miRNA binding sites within target genes. Analogous to mutations in the seed sequence of miRNAs, SNPs in the 3' UTR of a gene may create or destroy a miRNA binding site. The disruption of miRNA-dependent regulation of target genes by SNPs in the target mRNA is found in many cancer types. For example, *let-7* binds to the 3' UTR of *KRAS*, a proto-oncogene that is mutated in many cancers. Over-activation of *KRAS* leads to several malignancies. Interestingly, a SNP was found in one of the *let-7* complementarity sites within the 3' UTR of *KRAS*, and this SNP was associated with a higher risk for developing lung cancer. Thus, by destroying the target site for *let-7*, *KRAS* expression was up-regulated (because it was no longer repressed by *let-7*), leading to increased cell growth and proliferation, two hallmarks of cancer cells (see figure 3-6, right). On the other hand, SNPs can also create a target site for a miRNA, a scenario that was shown for the 3' UTR of *CD86*, a receptor involved in the immune response. The SNP increased the binding affinity of some miRNAs, thus leading to inhibition of *CD86* translation (see figure 3-6, left).

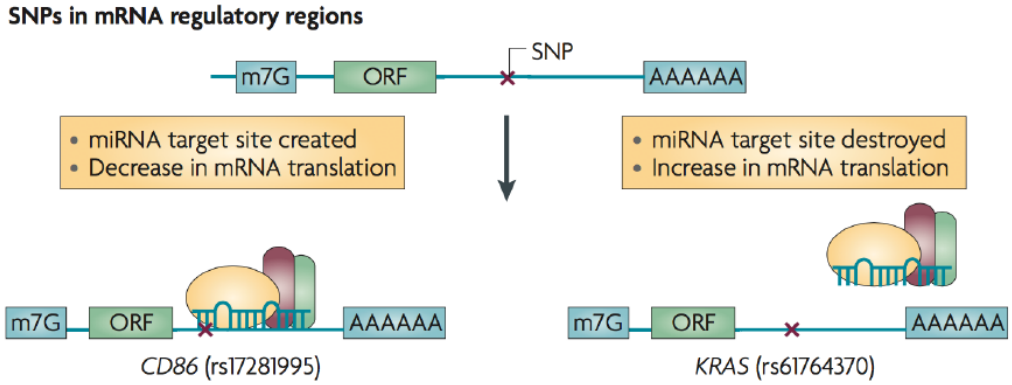

Figure 3-6 SNPs located within the 3'-UTR-miRNA binding sites function analogously to seed-region SNPs and modulate the miRNA–mRNA interaction. They can create (as in the case for *CD86*) or destroy (as in the case for *KRAS*) miRNA binding sites and affect subsequent mRNA translation. ***Abbreviations***: AA, poly(A) tail; m7G, 7-methylguanosine cap. (Adapted from B.M Ryan *et al.*, *Nat. Rev. Cancer*, 2010)

As we have seen, mutations of a single nucleotide can affect miRNA action. We will now discuss how SNPs have been shown to affect miRNA function in three different areas: Animal breeding, inheritable diseases and the development of cancer.

Mutations in the miRNA pathway affect animal breeding

Just like mutations affecting miRNA action can function during natural selection in evolution, these mutations also function in artificial selection in animal breeding. Here, we will present an example of how a miRNA-target-specific mutation resulted in an advantageous outcome in sheep breeding by a SNP that occurs in the target gene. A point mutation that creates an illegitimate *mir-1* target site in the 3' UTR of the *myostatin* gene was found to inhibit *myostatin* expression, which contributes to increased muscle mass of sheep of the Texel breed. Texel is a breed originally from the island of Texel in the Netherlands, and is now a popular lean-meat sheep all over the world, because it is heavily muscled. Myostatin acts to inhibit muscle-cell growth and differentiation (myogenesis). Thus, inhibition of *myostatin* expression by the *mir-1* miRNA through a mutation as it occurred in the Texel breed increases muscle mass.

Figure 3-7 Normal vs. Texel breed.

Mutations in the miRNA pathway are involved in cancer development

Taking into account that 30% of human genes are regulated by miRNAs, it is not surprising that miRNAs have been shown to be implicated in many different human diseases. We have already discussed many examples of how miRNAs are implicated in different cancer types. miRNAs can act as oncogenes and tumor suppressors and are involved in a variety of pathways deregulated in cancer. Above, we already presented the case of *KRAS*, where a mutation in its 3'-UTR target site for *let-7* leads to an up-regulation of *KRAS*, resulting in increased cell growth and proliferation, hallmarks of cancer cells. Genes encoding proteins that regulate cell proliferation, differentiation, or cell death are often mutated in cancer cells. Genes that act as negative regulators of growth and differentiation are referred to as tumor-suppressor genes, while those acting to enhance growth and differentiation are called oncogenes. Mutations in these protein-coding genes are selected for in cancer cells, and there are key proteins (e.g., KRAS or PTEN) that are mis-regulated in various cancer types.

Recently, miRNAs were also shown to act as tumor suppressors or oncogenes, and some miRNAs are even able to exert both roles, depending on the cellular context. Cancer-associated miRNAs are also called "oncomirs", and they can be used in diagnosis and treatment of cancer.

50% of annotated human *miRNA* genes are located in areas of the genome that are known as fragile sites, sites within a chromosome that are susceptible for chromosome breakage, amplification and fusion with other chromosomes. These fragile sites are commonly associated with the development of cancer. This shows that miRNAs might have a crucial function in cancer progression.

Figure 3-8 summarizes the current view on how miRNAs can act as tumor suppressors or oncogenes in cancer progression. Keep in mind the different ways how miRNA action can be affected by mutations: through alterations in miRNA biogenesis, through loss or gain of a seed sequence, or through loss or gain of a target sequence.

If the loss of a miRNA function leads to tumor formation, this miRNA is supposed to act as a tumor suppressor. The loss or reduction of a miRNA can be due to mutations that affect the biogenesis of miRNAs as it was described for the case of *mir-16-1* above (see figure 3-4). miR-15 and *mir-16-1* negatively regulate the expression of an oncogene, the anti-apoptotic gene *BCL2*; thus, reducing the amount of miR-15/miR-16-1 up-regulates the expression of *BCL2*, resulting in cell-death inhibition. Hence in this case, the normal function of *mir-15/mir-16-1* is to suppress tumor formation, i.e., *mir-15/mir-16-1* act as tumor suppressor genes (see figure 3-8B).

On the other hand, miRNAs can also function as oncogenes. Here, alterations that enhance the expression or processing of miRNAs lead to increased amounts of miRNA that inhibit the expression of a target tumor suppressor gene, leading to cancer progression (see figure 3-8C).

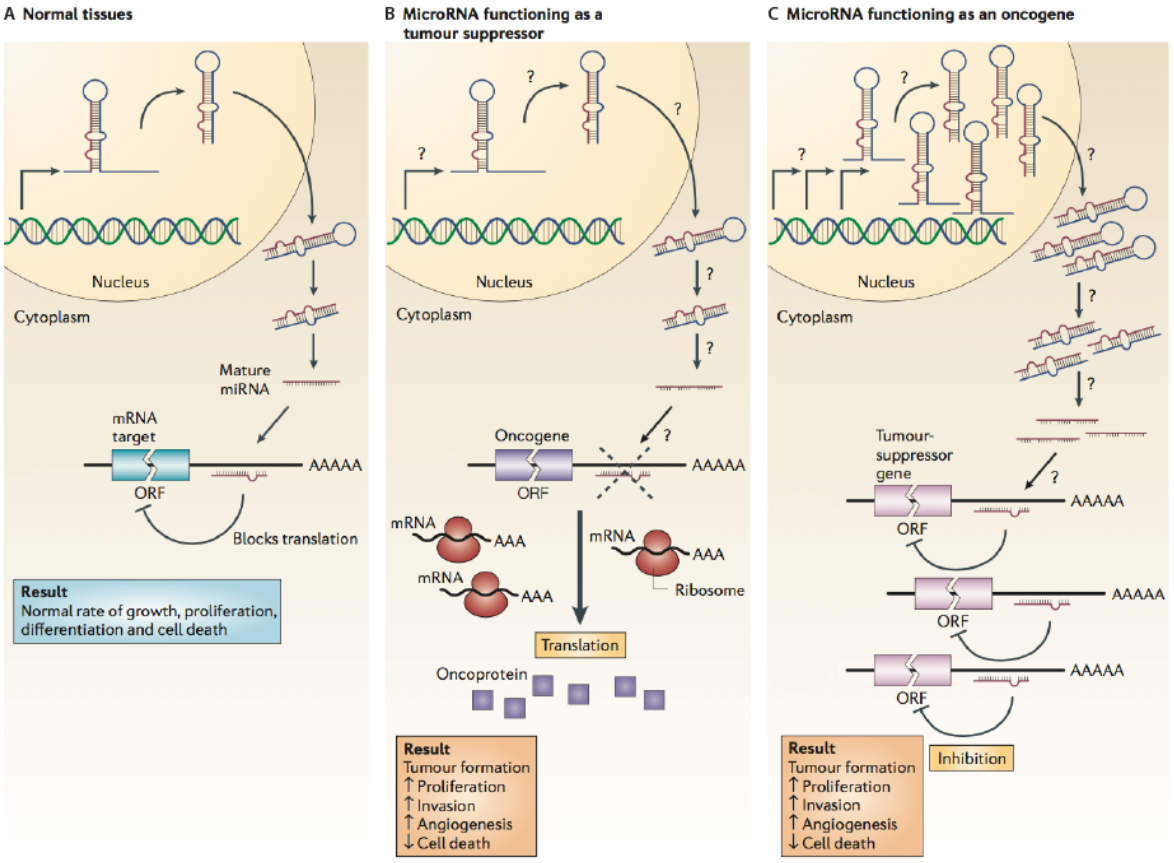

Figure 3-8 How miRNAs can function as tumor suppressors or oncogenes. (A) In normal tissues, proper microRNA (miRNA) transcription, processing, and binding to complementary sequences on the target mRNA results in the repression of target-gene expression. This ensures normal rates of cellular growth, proliferation, differentiation, and cell death. **(B)** If a miRNA functions as a tumor suppressor, the reduction or deletion of this miRNA leads to tumor formation. A reduction of mature miRNA levels can occur because of defects at any stage of miRNA biogenesis (indicated by question marks) and leads to the inappropriate expression of the miRNA-target oncoprotein (purple squares). The overall outcome might involve increased proliferation, invasiveness, or angiogenesis, decreased levels of apoptosis, or undifferentiated or de-differentiated tissue, ultimately leading to tumor formation. **(C)** If a miRNA functions as an oncogene, the amplification or overexpression of this miRNA results in tumor formation. Here, increased amounts of the miRNA eliminate the expression of a target tumor-suppressor gene (pink) and lead to cancer progression. The miRNA levels can be affected at different stages of the miRNA pathway (indicated by question marks). (Adapted from A. Esquela-Kerscher and F.J. Slack, *Nat. Rev. Cancer*, 2006)

There are several examples where miRNAs can act both as tumor suppressors as well as as oncogenes. This reflects the fact that a single miRNA can control many unrelated targets, such that the same miRNA can control opposing cellular processes. This ability seems to depend on in which cell types the miRNA is expressed and which target mRNAs are present in this cell type. Let's look at how this works in the case of miR-125b, which has opposite roles (oncogene and tumor suppressor) in different cancer types or cell lines. miR-125b targets a number of genes, including transcription factors, growth factors, and members of the *BCL2* family and plays important roles in cell differentiation, proliferation, and cell death. miR-125b functions as a tumor suppressor in ovarian, thyroid, or breast cells, it targets oncogenes that control cell proliferation or cell-cycle progression to reduce tumor formation. Accordingly, miR-125b was found to be downregulated in ovarian, breast, or thyroid cancer (see figure 3-9, right). In other cellular contexts, however, miR-125b acts as an oncogene to promote tumor formation by increasing cell proliferation and inhibiting apoptosis. Thus, in cancers of the prostate or the thyroid as wells as in glioblastoma and neuroblastoma, the expression of miR-125b is often upregulated (see figure 3-9, left).

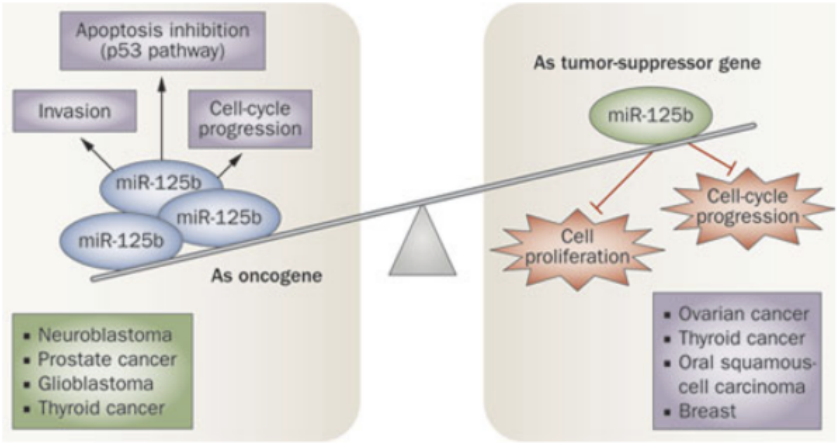

Figure 3-9 A miRNA can function both as oncogene and tumor-suppressor gene depending on the cancer type and cellular context. As a tumor suppressor, miR-125b acts on genes to inhibit cell proliferation and cell-cycle progression. In ovarian, thyroid, breast, and oral squamous-cell carcinomas, miR-126b is downregulated, which promotes tumor progression. On the other hand, miR-125b functions as oncogene in cancers such as prostate, thyroid, glioblastoma, and neuroblastoma. In these cancer cells, miR-125b expression is upregulated to inhibit tumor-suppressor genes. (Adapted from M.A. Cortez *et al.*, *Nat. Rev. Clin. Onc.*, 2011)

Predicting tumor origins by miRNA profiling

You have seen that, one the one hand, miRNAs are expressed with high tissue specificity, and, on the other hand, they are important regulators of cell proliferation and their malfunction is involved in tumor formation. Consequently, it is possible to use the differential expression profiles of miRNAs in cells to aid in diagnosing and classifying cancers. This is useful, because it is often difficult to trace from which tissue a tumor arises, either because it metastasized or because the tumor cells are undifferentiated.

A group of researchers found that the expression profiles of approximately 200 *miRNA* genes are sufficient to accurately classify human cancer types. They studied and compared normal and tumorous tissues and clustered the tissues according to their miRNA expression profiles. They noticed that within the clusters, the tumors were grouped according to their embryonic lineage, e.g., tumors of endothelial origin such as colon or liver were clustered together. The classification quality they achieved by using miRNA expression profiles was in many cases better than using profiles of protein-coding mRNAs. Additionally, that miRNA signatures within tumors reflect their developmental history is also compatible with the evidence that miRNAs direct tissue-specific developmental functions, as we have discussed for zebrafish. Using miRNA expression profiles, the group was even able to classify very poorly differentiated tumors into the tissue-specific lineage, which had not been possible until then.

miRNA circulating in plasma or serum can be used as biomarkers

Current techniques for cancer diagnosis commonly involve a biopsy of the cancer tissue, i.e., an extraction of sample cells. Because this technique is rather invasive and unpleasant for patients, efforts have been made to search for biomarkers in human fluids, such as blood plasma or urine. In medicine, biomarkers are substances that indicate the presence or severity of a disease. Interestingly, there are several reports of miRNAs that circulate in the blood plasma or serum and can be utilized as biomarkers for disease, because their level is significantly different in patients compared to healthy people. These miRNAs appear to be very stable, because they are not diffusing freely in the blood, but are incorporated in small vesicles.

There are several hypotheses about the origin of miRNAs in the blood serum. One theory is that they are present in apoptotic bodies, cell fragments produced during cell death that are taken up by phagocytes. The contents of these small vesicles still contain a part of the ingredients of the dead cell, e.g., miRNAs present before apoptosis. There is, however, ongoing research on the question where miRNAs in the circulation could come from.

Summary

In this lesson, we have seen that miRNAs are regulators of cell or tissue differentiation. Undifferentiated (or poorly differentiated) cells do not require miRNAs to survive, as we have seen from the example of early zebrafish development. miRNAs show a highly tissue-specific expression at later stages when most of the cell types have been formed, indicating that miRNAs function to maintain an already established cell fate. A de-regulation of miRNAs often results in a failure to maintain cell fates, and this failure often results in cancer. In line with this, many types of human cancer cells have reduced miRNA expression compared to their fully differentiated tissue of origin. Due to their tissue-specific expression, miRNA profiling can predict the cancer tissue origin, an information relevant for cancer treatment.