10.5152/turkjnephrol.2019.3677

Turkish Journal of Nephrology

New Bio-Markers: Cell-Free DNAs and MICRO-RNAs

Emel Ekşioğlu-Demiralp[1], Mehmet Onur Elbaşı[1], Aydın Türkmen[2]

[1]Tissue Typing and Immunology Laboratory, İstanbul Memorial Hospital, İstanbul, Turkey
[2]Division of Nephrology, Department of Internal Medicine, İstanbul University, İstanbul School of Medicine, İstanbul, Turkey

Abstract

The only reason of being in the same review of cell free DNAs (cfDNA) and micro–RNAs (miRNA) , whose only common points were being tiny, showing up in the circulation and their visibility that can be increased by amplifications, is that; both of them quickly entered the research and clinical world as candidates of bio-markers for diagnosis and follow-up for many diseases with considerable number of studies published since last two decades, cfDNAs can be found in the circulation as a steady signature of a given diseased condition while miRNAs are found both as a steady marker of a disease, and a transmitter of a malign behavior into the intact cells. In our review, we tried to briefly explain the biogenesis of both and their relationship with diseases and their clinical uses. The idea that cfDNAs and miRAs can be used as a diagnostic and follow-up criterion in transplantation is a new approach. Transplant practice has long been in search of new laboratory methods that can allow for the early detection of rejection without any clinical symptoms. The early studies on the use of cfDNA and miRNAs in post- transplant monitorization indicate that both are promising candidates as bio-markers.

Keywords: Cell free DNA, micro-RNA, transplantation, cancer, rejection

Corresponding Author: Emel Ekşioğlu-Demiralp ✉ emeldemiralp@yahoo.com

Received: 26.11.2018 **Accepted:** 27.01.2019

Presented in: This study was partly presented at the 12. International Congress of Transplantation from TONKKD, October 2018, Trabzon, Turkey.

Cite this article as: Ekşioğlu-Demiralp E, Elbaşı MO, Türkmen A. New Bio-Markers: Cell-Free DNAs and MICRO-RNAs. Turk J Nephrol 2019; 28(4): 310-20.

CELL-FREE DNA (cfDNA)

Before the discovery of the double stranded DNA molecule by Francis H. C. Crick, James D. Watson, and Maurice Wilkins in 1953 that was crowned by the Nobel Prize in 1962(1), the two French scientists Mandel and Métais showed the presence of the extracellular nucleic acids in plasma in 1948 (2). However, following this publication, a limited number of studies on cell-free DNA (cfDNA) have been published, and for many years, it has not been a topic of interest. With the exception of a few studies on some autoimmune diseases, such as systemic lupus erythematosus, the issue of cfDNA began to emerge again with cancers in the late 1980s (3, 4). It was thought that these free nucleic acids found in the plasma of patients with cancer may be characteristic of tumor cells and may be related to metastases, oncogenes, and mutations (5). Technological advances have increased the interest in and the studies on cfDNA. Thus many investigations have been published on different types of cancer (6). As the number of data on cfDNA that were discovered before the identification of double-stranded DNA has increased, its implementations on research and routine use expanded from prenatal diagnosis to solid organ transplantation (7).

Resources of cfDNAs

cell-free DNAs may be introduced into the bloodstream by apoptosis, necrosis, autophagy, and mitotic or mitochondrial destruction mechanisms; by a release of newly synthesized nucleic acids into the bloodstream; or by active cellular secretions such as vesicles and exosomes (Figure 1).

Different mechanisms produce cfDNAs of different sizes. They can be as long as 80,000 base pairs (bp) (ultra-long

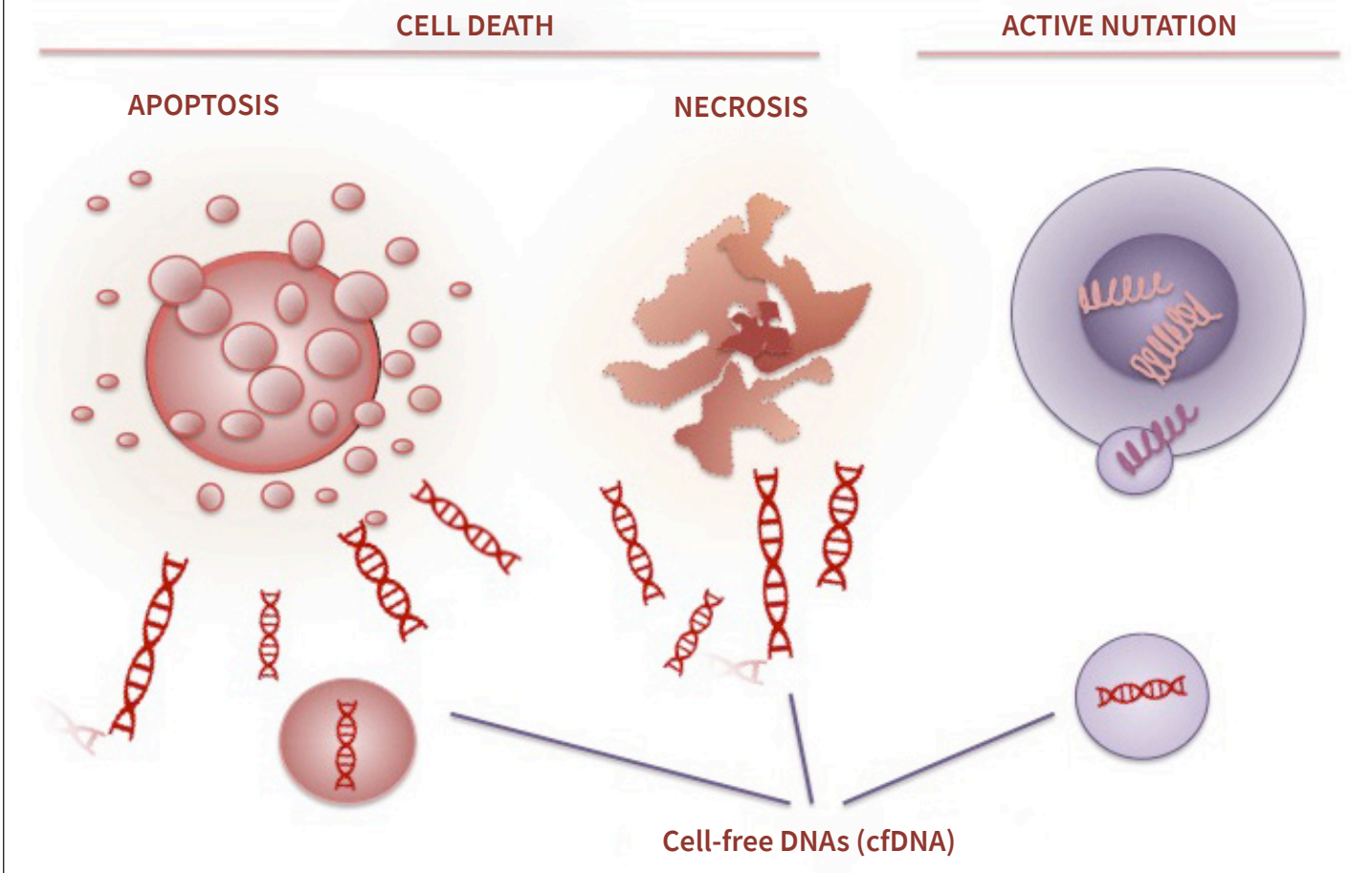

Figure 1. Sources of cell-free DNA in circulation. During apoptotic cell death, DNA surrounded by vesicles and free DNA gets into the circulation. DNA fragmented during the necrotic cell death causes longer fragments to enter into the circulation. Cell-free DNA is rapidly released from cells with rapid replication, such as tumor cells.

cfDNA) (8) or as short as 40 base pairs (ultra-short cfDNA) (9). There are several cfDNAs with similar properties, such as cell-free mitochondrial DNA (mtDNA), cell-free tumor-derived DNA (ctDNA), and fetal-derived cfDNA (7).

Most cfDNA fragments are 150-200 bp in length. Nucleosomes can be released into the circulation as a result of DNA degradation during apoptosis. The nucleosome-derived fragments associated with DNA damage in the cell nucleus are approximately 147 bp in size (10). In apoptosis, when DNA electrophoresis was performed, the staggered pattern of different-sized fragments captured suggested that most of the cfDNA sources were produced by the apoptosis mechanism. However, the presence of larger cfDNAs in randomly generated by necrosis products indicates that cell necrosis is also a source of cfDNA. When the frequencies of each chromosome belonging to the fetus and the father were examined in the plasma of pregnant women, cfDNAs greater than 1000 bp were detected (11). In patients with cancer, cfDNAs are found in the circulation as the destruction products of malignant cells. As an important complication in solid organ transplantations; in rejection events donor-related cfDNAs can be detected in the circulation as a result of allograft damage by antibodies. The possibility of their quantitative evaluation could be a promising tool for non-invasive and early diagnosis of rejection.

Since cfDNAs circulate through different mechanisms in health or disease, serum concentrations, structures, and degradation times are different. Data from a cfDNA sequence analysis showed that their half-life in plasma was generally 20-30 minutes. However, due to structural differences, some fragments may remain in circulation for up to 2 hours (12-14). Fetal cfDNAs degrade faster, approximately in 16 minutes, depending on the gestational week (15).

Concentrations of cfDNAs

cell-free DNAs are easily obtained by DNA isolation from the plasma fraction by the centrifugation of venous blood. Normal plasma contains cfDNA at a concentration of 10-30 ng/mL (11). Their concentration increases with age at a rate of 0.6 ng/ml every year (7). However, plasma concentrations of cfDNAs may vary depending on their source and the health status.

Their concentration is undoubtedly increased in patients with cancer. Quantification can be compared with their own values to provide a patient-specific follow-up. However, uncertainties, such as the isolation method of the cfDNAs and in which period of the clinical course of the disease when the sample was taken, prevent a standard and general approach to this issue. Today's technological advances that include amplifying the gene regions or various translocations associated with the disease by polymerase chain reaction facilitate the visibility of isolated cfDNAs. Today, it is preferred to show the presence and amounts of a disease specific, given cell free DNA rather than measuring the direct amount of a mixture of cfDNA obtained from plasma. Thus, the disease and treatment processes can be followed by standardized methods. Methodological advances allow us to access more detailed data on cfDNAs in mutation analyzes of serum, early diagnosis of fetal anomalies, demonstration of, translocation products of tumor cells of a given cancer in serum, and detection of early graft rejection in organ transplantation.

cfDNA and Cancer

Most studies with cfDNAs have been performed in cancers. In many cancers, more than 100 ng/mL of cfDNA is detected in plasma, which is much higher than in healthy controls (16). In lung, ovarian, uterine, and cervical cancers and lymphomas, plasma cfDNA levels were shown to be reduced up to 90% following radiotherapy compared to baseline (17).

In patients with colorectal cancer, differences in the concentration and fragmentation of cfDNAs in comparison to healthy controls provide important information for developing individualized therapies (18). Similarly, comparisons between healthy individuals and patients with colorectal cancer showed that even only plasma concentration differences of cfDNA can be very helpful in predicting prognosis (19). The cfDNA detection, which benefits the treatment process management and give concurrent information about drug resistance and metastasis, can also be called as blood biopsy. It is obvious that, this provides great advantages compared to tissue biopsy, since it can be easily obtained, and that sample can be used in more than one test provides great advantage (Table 1).

Cancer studies indicate that even differences in the cfDNA concentration may be a significant prognostic criterion. However, since DNA isolation methods from plasma are limited and difficult to standardize, new methods have been sought, and new methods such as amplifying disease-specific genes and gene translocations from cfDNAs and processing and evaluating with high-tech devices have been initiated. This has led to the development of more standardized approaches for cancer monitoring. The methods used for the cfDNA amplification are the amplification methods used in the analysis of point mutations in genetics (ARMS-polymerase chain reaction [PCR], ICE COLD PZR, droplet digital PCR), examination of single-nucleotide polymorphisms in DNA, or new generation sequencing analysis. Each of these analyses has different sensitivities, ranging from 0.01% to 1%, and is used in the evaluation of treatment resistance or the risks of metastasis and recurrence during the treatment decision (20). In fact, based on the DNA methylation differences in cancers, a more sensitive method was developed based on the determination of these methylation differences in cfDNAs. The development of new methods with increasing sensitivity suggests that it will be possible to diagnose and follow-up all cancers by liquid biopsies in the very near future (21).

Table 1. The Comparison of Blood Biopsy and Tissue Biopsy*

Feature	Blood Biopsy	Tissue Biopsy
interventional process requirement	No	Yes
access to sample during illness	Yes	No
ex vivo stability of the sample	Yes (following isolation)	Yes (following process)
usability in disease monitoring	Yes	No
Cost	cheap	expensive
Processing time	Short	Long
Rejection error rate	Low	High
Sample availability for multiple tests	Enough	Limited

*: Reference 20

Although Phallen et al. (22) demonstrated that cancer-related cfDNAs can be detected in the early stages of breast, colon, lung, and ovarian cancers, and claimed that liquid biopsies can also be used as early diagnostic markers, it is unlikely that very small tumors may be detectable by DNA in the serum. (23). Thus, cfDNAs are not likely to be a biomarker in tumors that have not reached a certain size. Nevertheless, it will provide the clinician with very important information to diagnose and follow-up the patient and for the determination of the risk of recurrence, and will take its place in oncology practice in a very short time.

cfDNA and Prenatal Diagnosis

In addition to cancer research, the cfDNA analysis as a biomarker also has the advantage of being a fast and non-invasive method in prenatal diagnosis. After the detection of the fetus DNA in the mother's plasma (24), tests with fetal cfDNAs represent an alternative to high-risk invasive methods. Analyses of fetal cfDNAs isolated from maternal plasma can identify sex and sex-dependent diseases, blood mismatch, and various chromosomal abnormalities (non-invasive prenatal testing [NIPT]) (25). although all are single center studies today, many independent studies with a high number of samples agree that cfDNA analysis of trisomy 18, 21, and 13 are a very good alternative with a high positive predictive value and are accepted as the gold standard in these trisomies (26-28).

cfDNA and Transplantation

Graft survival has been significantly prolonged in the recent years, with successful surgical methods as well as the use of new generation immunosuppressive drugs and meticulous and careful follow-up after transplantation. A small number of biochemical and immunological follow up markers used to adjust drug levels and manage the treatment process are often limited in capturing data that can be obtained by tissue biopsy. Although there are some promising studies with biomarkers, such as Granzyme B and perforin, the results of these studies were not reflected into routine clinical practice (29).

Protocol biopsies are also invasive and cannot be adopted by all centers, and new biomarkers are under way to predict the outcome of the graft after transplantation. Among the many biomarker candidates, cfDNAs can be considered as a promising candidate in transplantation practice.

In the early studies on cfDNAs in transplants, the detection of donor-derived cell-free DNAs from the graft ([dd-cfDNA]; graft-derived cell-free DNA [GcfDNA]) in the serum of patients with renal and heart transplantations suggested that they can be used as an acute rejection marker (30). In the diagnosis of acute cellular rejection, especially in heart transplantation, increased percentage of plasma dd-cfDNAs may be an alternative to endomyocardial biopsies (31). In bone marrow and hematopoietic stem cell transplantations, chimerism and graft versus host disease evaluations have shown significant differences in rates of donor and recipient cfDNA (32). In solid organ transplants, before organ rejection can be recognized immunologically or by biopsy, the possibility of the detection of dd-cfDNAs, circulating into blood stream via cell destruction, appears to be an extremely important test that can directly affect the graft survival by allowing the patient an early treatment opportunity (33-35).

Following the demonstration of very small cfDNAs in the circulation of lung transplant recipients were donor-specific mitochondrial cfDNAs, the idea that clinical approach and follow-up criteria can be established according to the structure, size and sources of cfDNAs arose. The determination of mitochondrial, nuclear and genomic cfDNA fragment patterns of both recipient and donor as well as microorganisms, the creation of single- or double-stranded DNA libraries, and cfDNA-based monitoring of microorganism sequences showed that cfDNAs are not only a marker of graft rejection, but can also be used for the follow-up of infection after transplantation (9).

A meta-analysis of cfDNAs biopsy including a total of 2,302 post-transplant patients (762 kidney; 246 liver; 785 heart; 245 lung; 34 kidney+heart+liver; 114 kidney+heart; 6 liver+kidney; 110 heart+lung) showed that cfDNAs were correlated with detected acute rejection and that they decreased after an acute rejection treatment, but that there was no association between the mild rejection and acute tubular necrosis. cfDNAs were also shown to be positively correlated with graft damage caused by factors other than rejection, BK virus nephropathy, pyelonephritis and other infections, lung infections, hepatitis B and C infections in liver recipients (not with cholestasis), and in a few studies, hospital stay and 1-year creatinine levels. Few studies in the analysis group have shown that cfDNAs are also associated with chronic rejection (36). In the light of the data evaluated in the meta-analysis, it can be considered that the negative predictive value of cfDNA is higher than the positive predictive value. Especially in the presence of the donor-specific antibody (DSA) positivity, which is a very important diagnostic criterion for antibody-mediated rejections, the positive predictive values of cfDNAs increase significantly. Therefore, the evaluation of cfDNAs together with immunological tests may provide a great support to the clinician (37).

Among the problems that need to be resolved so that cfDNAs with a very short half-live would become accepted as a definite biomarker are the standardization and facilitation of tests that could be used as DNA isolation methods and follow-up criteria, knowing the dynamics and kinetics of cfDNAs, distinguishing the structure and sources, and setting the perfect timing for different clinical conditions. However, despite all the difficulties, graft follow-up with cfDNAs will make a major contribution to organ transplants in the near future.

MICRO-RNAs (miRNA)

miRNAs were first discovered in the early 1990s, when it was recognized that the Lin-4 gene of nematode Caenorhabditis elegans was not translated into given protein and it was shown that it controlls another gene by acting as a small RNA encoder that play roles in its translation rate into protein (38). miRNAs are often encoded in introns and between genes. A very small part is also found in exons (39, 40). The discovery of miRNAs, the fact that they are encoded in the introns, which we call “genomic garbage” since we do not know their functions, has changed our perspective on biology and genetics. Despite their recent discovery, they evolutionarily constitute the oldest gene control mechanism. They are single-stranded RNAs of 20-30 nucleotides (often 22) in length. They prevent protein translation by binding to the ribosome-binding sites of the messenger RNA (41) (Figure 2).

There are more than 1900 miRNA genes in the human genome, and 60% of our genes can be silenced with these miRNAs (42, 43).

miRNAs are involved in numerous gene regulation mechanisms, from nematodes to humans. They play numerous roles in stem cells, blood cells, cell development, differentiation, proliferation, fat metabolism, endocrine mechanisms, apoptosis and cancer, and the regulation of immune system functions. miRNAs, which can also be detected in plasma and urine in addition to intracellular presence, have been associated with a variety of diseases, including cancer and organ rejection.

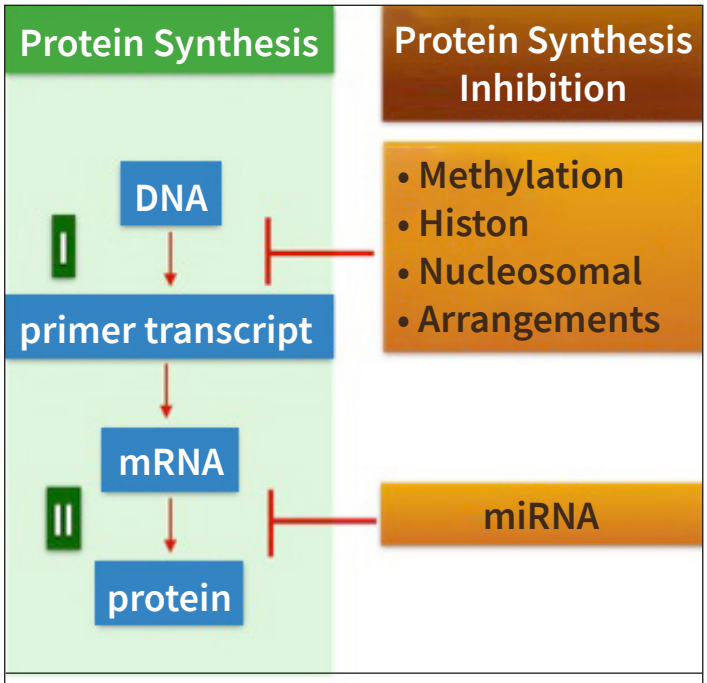

Figure 2. Central Dogma: Protein synthesis first begins with the conversion of the protein's gene into the messenger RNA (I=transcription). Protein messenger RNA (mRNA) is formed by primary transcripts and arrangements, respectively. mRNA binds to ribosomes, and protein is synthesized (II=Translation). The inhibition of protein synthesis is inhibited by closure of the gene of interest. Closure of the gene occurs through different mechanisms, such as methylation, histone modifications, and nucleosomal positioning.

miRNA Nomenclature and Classification

The nomenclature of over 1900 miRNAs uses consecutive numbers added to the miR prefix (eg miR-23, miR-155) (44). Since they are evolutionarily conserved sequences, there is no distinction between species in naming. Pre-miRNAs are expressed in italics (mir). The presence of a or b next to miRNAs designated by the same number indicates that there are one or two nucleotide differences (miR-22a; 22b). For the same miRNAs located in more than one place in the genome, a new consecutive number is added to the miR number with a dash (such as miR-155-1; miR-155-2).

Not only the multiplicity of their numbers but also, the presence of a large number of small RNAs including the siRNAs (small interfering RNAs)in the cell, make it difficult to their nomenclature and classification. Today, although there are many proposals, the full consensus on nomenclature has not been achieved.

Terminologically, nomenclature can be accomplished by referring to the mechanism by which it functions. For example, miRNAs that have different functions on apoptosis as pro- or anti-apoptotic are called apoptomiR (45), and miRNAs associated with oncogenes are called oncomiR (46). However, it is inevitable that miRNAs, which play a role in both, are regulatory in another cellular function, and such a classification does not seem to be accurate and possible.

With the idea that the expression of each may be different in different organs, it was attempted to establish a classification regarding organs. However, since miRNAs have many functions, they can also exist for different functions in different organs. A single miRNA can stop hundreds of different mRNAs (messenger RNAs), and thus different cellular functions. An mRNA can also be silenced by hundreds of different miRNAs. These functional overlapping conflicts make it impossible to classify. For example, more than 40 miRNAs have been identified for the liver, and more than 50 miRNAs for the lung, but many miRNA overlaps have been identified for both organs (47). Although a very small number of miRNAs have been identified for organ specificity, such as miR-122 being liver specific, the preferred pathway for classifications today is creating different scenarios using sophisticated bio-informatics approaches and taking into account the extent of the expression of a large number of miRNAs, thus creating patterns specific to organs and tissues, and identifying deviations from these patterns and finding relationships with disease states (48).

Sources of circulating miRNAs

miRNAs can be found in various body fluids, such as plasma, urine, and saliva. The ability of miRNAs to circulate suggests that they play a role in cell-to-cell communication, and studies have proven that exosomal miRNAs in particular show both paracrine and endocrine effects as a new way of intercellular communication. In addition, the presence of miRNAs in plasma and urine has led to new horizons in the diagnostic field. Hundreds of studies are under way to identify markers for diagnosis and follow-up of many different conditions, from cancer to atherosclerosis and transplantations. In addition, miRNAs are also found in breast milk, and these are mostly involved in the regulation of the immune system, affecting the development of the immune system of the newborn (49).

miRNAs are double-stranded hairpin-shaped RNAs transcribed with RNA polymerases II and III. They need to be processed to stop translation. They are processed with ribonuclease enzymes called Drosha in the nucleus and Dicer in the cytoplasm, which produce short double-stranded RNAs. As a single chain, it participates in the RNA-induced silencing complex (RISC) structure, including Argonaute (Ago) proteins, and inhibits mRNA translation (Figure 3) (50).

Newly synthesized miRNAs carried by exosomes or vesicles are actively released into the bloodstream. It is also circulated freely or in apoptotic bodies as passive end products of necrotic and/or apoptotic cells (51, 52). Circulating miRNAs are much more stable than cfDNAs. They remain stable in response to pH and temperature changes and RNase enzyme activity. Since miRNAs are found in circulation as in a complex with lipid vesicles, lipoproteins, or RNA-binding Ago proteins, they are protected from the degradation of ribonucleases found in body fluids (53). However, long-term storage by freezing still leads to a reduction in their amount (54). Some of the circulating miRNAs are trans-

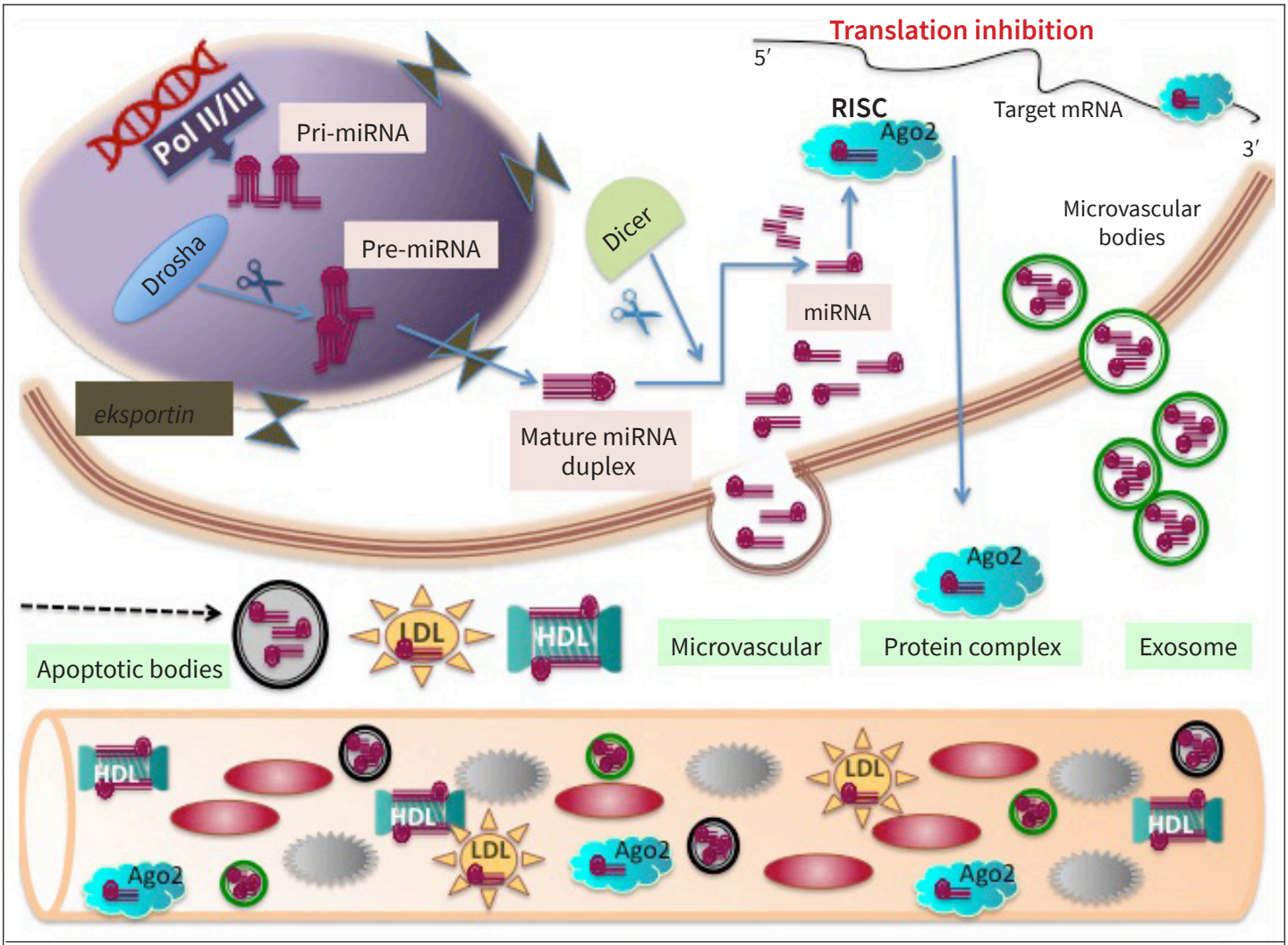

Figure 3. Sources of miRNAs in circulation: Primary miRNAs (pri miRNAs) synthesized with Polymerase II and III were converted to hairpin, precursor miRNAs (pre-miRNA) by a ribonuclease called Drosha, and they were passed to the cytoplasm via exportin gates on the nucleus membrane. In the cytoplasm, the nucleotide is cut into nucleotide duplexes via Dicer. A chain of mature miRNA duplexes binds with Ago proteins (Ago2) to form RISC (ribonuclease-mediated silencing complex RNA mediated Silencing Complex) structures. RISC binds to mRNA and suppresses translation into the protein of interest. The miRNAs actively secreted from the cell are either bound by Ago proteins or in exosomes, or within the apoptotic cell if the source is an apoptotic cell. A portion of the free-released miRNA is also transported in plasma by HDL and LDL (redrawn using references [47, 50] and [90]).

ported by high-density lipoproteins (HDLs) and low-density lipoproteins (LDLs). HDLs mostly carry miRNAs that are related to inflammation. This transport may be considered as the endocrine effect of miRNAs. Namely, these miRNAs carried by lipoproteins and Ago proteins can silence genes in neighboring or distant cells (55). The transport of miRNAs with lipoproteins suggests that they have other tasks beyond the task of promoting gene silencing. MiRNAs playing roles in lipid metabolism and lipid homeostasis have been discovered (56).

Obtaining circulating miRNAs

Circulating miRNAs are separated from plasma or serum from 10 milliliters of venous blood via plasma, serum RNA, or exosomal RNA isolation methods (57). The simplest method of analysis is the amplification of miRNAs to be examined by quantitative, real-time polymerase chain reaction (Q-rtPCR) after transcription of the obtained RNAs to complementary DNA (cDNA), and evaluation of their copy numbers. For further evaluations, high throughput sequence analysis is performed after amplification, and examinations and groupings are performed using bioinformatic approaches by taking into account existing miRNA libraries.

miRNA and Cardiovascular Diseases

miRNAs have been shown to play an important role in the development of the cardiovascular system. In mouse studies performed by silencing DGCR8, Dicer, or Ago2 genes, which are involved in the miRNA formation and complexing with Drosha, it was shown that the embryo died in the early stages of gestation with multiple vascular and cardiac development defects. However, studies that examined the miRNA blocking showed no lethal effect at this level. MiRNAs confirmed thgeir role in cardiac development were miR-1, miR-133, miR-15, miR-208, and miR-17-92. miR-126 is responsible for the endothelial cell development (58, 59).

In human studies, individuals with cardio-metabolic disease, which is the precursor of cardiovascular and metabolic diseases, in genomewide analysis studies performed to identify risk, were found to present with multiple single nucleotide polymorphisms associated with disease, and interestingly, these polymorphisms were found mostly in noncoding regions (60). Some of the regions where polymorphisms were found are associated with miRNAs. There are many studies showing that miRNAs carried in HDLs in individuals with coronary artery diseases who have unstable angina or myocardial infection have a different profile than healthy individuals (61, 62).

Although the data do not yet allow the development of a hypothesis for the mechanism, it at least shows that miRNAs are rapidly evolving into being a biomarker for monitoring coronary artery disease.

miRNA and Cancer

The initial association of miRNAs with cancer has been described in chronic lymphocytic leukemia (CLL). miR15 and 16 in this region were also shown to disappear in CLLs with the 13q14 deletion (63). The deletion of miR15 and 16 increases the expression of bcl-2, an anti-apoptotic protein, which explains the long survival of CLL B lymphocytes. Following this initial study of the miRNA-cancer relationship, a study of the hypothesis that miRNAs in the region of the cell cycle regulatory p53 gene with tumor suppression potential may have changed in cancers demonstrated the relationship between the miR-34 family members and the p53 protein. Among the members of the miR-34 family, miR-34-a is most commonly transported in the brain, miR-34b is most commonly carried in the lungs, and miR34c is usually transported in equal amounts in the cells and tissues in which miR-34b is present. Expressions of miR-34bc have been shown to be significantly reduced in non-small cell lung cancers with P53 mutations. Subsequently, using anti-sense oligonucleotides, the p53 protein produced by mutations in the p53 gene, which is known to be the most affected in cancers, was the first to show that as predicted, it was a transcription inhibitor protein (bound to DNA) and suppressed transcription of miRNAs (64). The Mir-34 family is a direct regulator of bcl-2, which is an anti-apoptotic (64). In other words, p53 gene mutations are predictors of the survival of tumor cells via miRNAs. Hundreds of studies have been conducted with different miRNAs in different types of cancers after these early studies that show not only differences in expression rates but also causality between miRNA and cancer and bring a new perspective to cancer formation. After the identification of the relationship of mi-RNAs with members of the Bcl-2 family, their roles in the immune system and their effects on the immune system and tumor cell apoptosis have been described in detail (65). Tumor suppressor miRNAs, such as miR-7, miR-124, miR-137, miR-146b, miR-15b, miR-128, and miR-326 have been shown to act in different organs, systems, and diseases (66).

Today, miRNA profiles that can be used in the diagnosis and follow-up of esophageal, gastric, pancreatic, colorectal, and hepatocellular cancers have been defined and started to be used in clinical practice (67).

Similar studies have also been performed in breast cancer, and the very high density of miR-21 and miR-1246 in exosomes compared to healthy controls proved that miRNAs can be used in the diagnosis of breast cancer (68).

Especially in the central nervous system malignancies, where biopsy is almost impossible to perform, studies on the follow-up of cancer with liquid biopsy will be the most useful area for miRNAs in terms of providing a better follow-up of these cancers and producing new treatment approaches (69).

mi-RNAs also affect angiogenesis, which is the most important indicator of cancer. Numerous miRs have been identified that increase or inhibit angiogenesis. Identification of the miRNA profiles that determine tumor angiogenesis in the tumor specific area brings the hope that miRNAs can be used for inhibition of angiogenesis, and treatment methods can be developed to stop cancer metastases (70).

miRNA and Infections

In viral infections, it has been shown that viruses encode their own miRNAs (v-miRNA) and direct them to targets in the host to escape antiviral mechanisms and to regulate these mechanisms (69). Identification of these v-miRNAs and their targets appears to be important in regulating the antiviral immune response. The association of v-miRNAs with replication has been shown in viral infections, such as EBV and HIV (71, 72). The association of many miRNAs with hepatitis B virus (HBV) and liver damage has also been reported (73). In addition, these viral miRNAs, which can be transported in the circulation by exosomes, are of diagnostic importance.

miRNA and Transplantation

miRNAs have been shown to be associated with many immunological factors that play a role in acute or chronic rejection mechanisms (50). Among these factors, the transforming growth factor-beta (TGF-β) is particularly important as a negative regulator of many inflammatory processes. However, it also causes fibrosis. In this sense, the behavior of TGF-β in organ rejection can be defined as “the determinant of the process.” MiRNAs that negatively regulate the TGF-β gene transcription are miRNA-548d, miRNA-203, and miRNA-146a. The absence or possibly suppression of the TGF-β release in inflammation and rejection processes with mi-RNAs may cause the balance to shift in the direction of the positive regulators of inflammation and lead to rapid rejection response and graft loss. However, the blockade of mi-RNAs that inhibit the TGF-β increase also leads to an uncontrolled release of TGF-β and increased fibrosis with FGF and complete loss of function. Indeed, TGF-β was shown to be associated with certain miRNAs in kidney and liver transplants (74, 75). In animal experiments, it was suggested that miRNAs can be biomarkers for organ rejection, and the

roles of different miRNAs in different organ transplants were determined (50, 76).

Let-7c, which plays a role in the development of TH1 cells and serves as the regulator of stem cells with the TGF-βeta signal, was reported as a common miRNA precursor in three different types of organ transplants (kidney, liver, and lung) (50).

Another mi-RNA involved in organ transplantation is MiR-182-5p. Activated by IL-2 and STAT5, it inhibits FOXO1, which plays a role in many basic cellular functions such as cell survival, apoptosis, and proliferation. FOXO1 is a critical factor that acts upon T cells, B cells, and neutrophils and the immune homeostasis (77). In its absence, regulatory T cells cannot develop. The inhibition of FOXO1 by miRNA leads to a change in the balance toward inflammation and thus to the rejection side in organ transplantations as it eliminates the suppression of regulatory cells. Since the inhibition of FOXO1 by miRNA will eliminate the suppression created by regulatory cells, it leads to a change in the balance toward inflammation and thus to the rejection side in organ transplantations.

In many human and animal studies, miRNA-122 has been defined as a liver-specific miRNA. More than 70% of mi-RNAs in the liver are miR-122. miR-122 is associated with acute rejection following liver damage and liver transplantation in correlation with miR-148a and miR-194 (76). It has also been found that serum levels of these miRNAs show a significant increase that correlates with aminotransferases in rejection-induced liver damage (78). In recent years, many independent studies have shown that miR-122 is a noninvasive biomarker for a wide range of liver diseases, such as liver viral diseases, hepatocellular cancer, liver damage, and cirrhosis. Studies indicate that miR-22b, miR-15b, miR-99a, and miR-192, in addition to miR-122, are also biomarkers for the liver (47).

Unlike the liver, miR208, the heart-specific miRNA, is not the most common miRNA in the heart. Despite of that, its involvement in circulation in cardiac injury has led to its recognition as a biomarker in cardiac damage (79)

MiRNAs also play an important role in renal physiological processes. Blood filtration takes place in the Bowman capsule. When the ultrafiltrate is excreted as urine from the glomerulus to the bladder, different reabsorption and secretions occur in each flow path. Fine control of these processes and healthy operation of the nephron unit are achieved by mi-RNAs in the ultrafiltrate (80). Major mi-RNAs in the kidney are miR 215, miR 146a, and miR 886. miR 192, miR 194, miR 21, miR 200a, miR 204, and let-7a -g are also found in the kidneys. Most mi-RNAs are activated under hypertonic conditions and control osmolarity balance over the Na^+ and K^+ levels. miR 192 controls the gene of Na^+/K^+-ATPase β1 subunit in renal epithelial cells (81). MiRNA expression patterns are different in fetal and adult kidneys (82). In addition to miRNAs found in the kidney, the aberrant miRNA expression may also be present in different pathologies of the kidney, such as kidney cancers, acute kidney damage, end-stage kidney disease, diabetic nephropathy, or polycystic kidney (83-85). It has been shown that the level of miR-146 increases in serum and urine in acute renal injury following renal transplantation. This increase is correlated with the severity of ischemia-reperfusion injury. miR146a is considered to be a rejection risk factor (86). Mutations in miR-146a double the risk of rejection. MiR-10b is another kidney-specific miRNA (85). It regulates the Bcl2L11 expression. A decrease or absence of miR-10 b causes a decrease in Bcl-2 and plays a role in renal rejection by causing the pro-apototic/anti-apoptotic balance to shift to the direction of apoptosis (87).

Additionally, the amounts of miR-16, miR200-c, miR-21, and miR-423 are increased in urine in acute kidney injury.

miRNAs and New Therapeutic Approaches

As miRNAs are rapidly becoming biomarkers for disease diagnosis and follow-up, they have been found to have paracrine and endocrine effects by being transported physiologically in exosomes. The use of this mechanism as a therapeutic approach has emerged as a brilliant idea. Cellular therapies may be administered by introducing mi-RNAs into exosomes, for example, which inhibit proliferation. Therefore, cancer progression and growth can be stopped. miR21 and miR34 are the first validated miRNAs in RNA-based therapies and have been included in phase studies to be used in the treatment of breast cancer (88). Phase 2 studies for HCV are also carried out with mir-122. Preliminary results show that it is quite successful, especially in cases that do not respond to other antivirals (89).

CONCLUSION

When compared to invasive methods such as biopsy, it seems very likely that obtaining reliable data on the health of organs transplanted, with cfDNAs or miRNAs, which can be easily obtained from body fluids such as serum, plasma, or urine, will be highly possible. The establishment of multiple gene expression panels in patients who underwent transplantation, detection of mRNAs that are the target of miRNAs, instant monitoring, and understanding the mechanisms of rejection are extremely important for developing a treatment strategy. Determining the mechanisms, adjusting the doses of immunosuppressive drugs after transplantation, and being able to determine the risks of cancer and/or infection with a single panel will increase the chance of early intervention, prolong graft survival, and make a significant contribution to transplantation success in the near future.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - E.E.D.; Design - E.E.D., A.T., M.Ö.E.; Supervision - E.E.D., A.T., M.O.E.; Resource - E.E.D., M.O.E., A.T.; Materials - E.E.D., M.O.E., A.T.; Data Collection and/or Processing - E.E.D., M.O.E., A.T.; Analysis and/or Interpretation - E.E.D., A.T., M.O.E.; Literature Search - E.E.D., M.O.E., A.T.; Writing - E.E.D.; Critical Reviews - E.E.D., A.T.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

1. Watson JD, Crick FH. Molecular structure of nucleic acids; a structure for deoxyribose nucleic acid. Nature 1953; 171: 737-8. [CrossRef]
2. Mandel P, Metais P. Les acides nucléiques du plasma sanguin chez l'Homme. C R Seances Soc Biol Fil 1948; 142: 241-3.
3. Tan EM, Schur PH, Carr RI, Kunkel HG. Deoxybonucleic acid (DNA) and antibodies to DNA in the serum of patients with systemic lupus erythematosus. J Clin Invest 1966; 45: 1732-40. [CrossRef]
4. Stroun M, Anker P, Maurice P, Lyautey J, Lederrey C, Beljanski M. Neoplastic characteristics of the DNA found in the plasma of cancer patients. Oncology 1989; 46: 318-22. [CrossRef]
5. Vasioukhin V, Anker P, Maurice P, Lyautey J, Lederrey C, Stroun M. Point mutations of the N-ras gene in the blood plasma DNA of patients with myelodysplastic syndrome or acute myelogenous leukaemia. Br J Haematol 1994; 86: 774-9. [CrossRef]
6. Anker P, Mulcahy H, Chen XQ, Stroun M. Detection of circulating tumour DNA in the blood (plasma/serum) of cancer patients. Cancer Metastasis Rev 1999; 18: 65-73. [CrossRef]
7. Sherwood K, Weimer ET. Characteristics, properties, and potential applications of circulating cell-free dna in clinical diagnostics: a focus on transplantation. J Immunol Methods 2018; 463: 27-38. [CrossRef]
8. Dennin RH. DNA of free and complexed origin in human plasma: concentration and length distribution. Klin Wochenschr 1979; 57: 451-6. [CrossRef]
9. Burnham P, Kim MS, Agbor-Enoh S, Luikart H, Valantine HA, Khush KK, et al. Single-stranded DNA library preparation uncovers the origin and diversity of ultrashort cell-free DNA in plasma. Sci Rep 2016; 6: 27859. [CrossRef]
10. Stroun M, Lyautey J, Lederrey C, Olson-Sand A, Anker P. About the possible origin and mechanism of circulating DNA apoptosis and active DNA release. Clin Chim Acta 2001; 313: 139-42. [CrossRef]
11. Cheng SH, Jiang P, Sun K, Cheng YK, Chan KC, Leung TY, et al. Noninvasive prenatal testing by nanopore sequencing of maternal plasma DNA: feasibility assessment. Clin Chem 2015; 61: 1305-6. [CrossRef]
12. Chandrananda D, Thorne NP, Bahlo M. High-resolution characterization of sequence signatures due to non-random cleavage of cell-free DNA. BMC Med Genomics 2015; 8: 29. [CrossRef]
13. Fatouros IG, Jamurtas AZ, Nikolaidis MG, Destouni A, Michailidis Y, Vrettou C, et al. Time of sampling is crucial for measurement of cell-free plasma DNA following acute aseptic inflammation induced by exercise. Clinical Biochem 2010; 43: 1368-70. [CrossRef]
14. Beiter T, Fragasso A, Hudemann J, Niess AM, Simon P. Short-term treadmill running as a model for studying cell-free DNA kinetics in vivo. Clinical Chem 2011; 57: 633-6. [CrossRef]
15. Lo YM, Leung TN, Tein MS, Sargent IL, Zhang J, Lau TK, et al. Quantitative abnormalities of fetal DNA in maternal serum in preeclampsia. Clin Chem 1999; 45: 184-8.
16. Gormally E, Hainaut P, Caboux E, Airoldi L, Autrup H, Malaveille C, et al. Amount of DNA in plasma and cancer risk: a prospective study. Int J Cancer 2004; 111: 746-9. [CrossRef]
17. Anker P, Stroun M. Circulating DNA in plasma or serum. Medicina 2000; 60: 699-702.
18. Mouliere F, El Messaoudi S, Pang D, Dritschilo A, Thierry AR. Multi-marker analysis of circulating cell-free DNA toward personalized medicine for colorectal cancer. Mol Oncol 2014; 8: 927-41. [CrossRef]
19. Spindler KL, Appelt AL, Pallisgaard N, Andersen RF, Brandslund I, Jakobsen A. Cell-free DNA in healthy individuals, noncancerous disease and strong prognostic value in colorectal cancer. Int J Cancer 2014; 135: 2984-91. [CrossRef]
20. McLarty J, Yeh C. Circulating cell-free DNA: The blood biopsy in cancer management. MOJ Cell Sci Rep 2015; 2: 0021. [CrossRef]
21. Shen SY, Singhania R, Fehringer G, Chakravarthy A, Roehrl MHA, Chadwick D, et al. Sensitive tumour detection and classification using plasma cell-free DNA methylomes. Nature 2018; 563: 579-83. [CrossRef]
22. Phallen J, Sausen M, Adleff V, Leal A, Hruban C, White J, et al. Direct detection of early-stage cancers using circulating tumor DNA. Sci Transl Med 2017; 9(403). pii: eaan2415. [CrossRef]
23. Diamandis EP, Fiala C. Can circulating tumor DNA be used for direct and early stage cancer detection? F1000Res 2017; 6: 2129. [CrossRef]
24. Lo YM, Corbetta N, Chamberlain PF, Rai V, Sargent IL, Redman CW, et al. Presence of fetal DNA in maternal plasma and serum. Lancet 1997; 350: 485-7. [CrossRef]
25. Lewis C, Hill M, Chitty LS. Offering non-invasive prenatal testing as part of routine clinical service. Can high levels of informed choice be maintained? Prenat Diagn 2017; 37: 1130-7. [CrossRef]
26. Galeva S, Konstantinidou L, Gil MM, Akolekar R, Nicolaides KH. Routine first-trimester screening for fetal trisomies in twin pregnancies: cell-free DNA test contingent on results from the combined test. Ultrasound Obstet Gynecol 2019; 53: 208-13. [CrossRef]
27. Liang D, Lin Y, Qiao F, Li H, Wang Y, Zhang J, et al. Perinatal outcomes following cell-free DNA screening in >32 000 women: Clinical follow-up data from a single tertiary center. Prenat Diagn 2018; 38: 755-64. [CrossRef]
28. Bianchi DW, Parker RL, Wentworth J, Madankumar R, Saffer C, Das AF, et al. DNA Sequencing versus Standard Prenatal Aneuploidy Screening. N Engl J Med 2014; 370: 799-808. [CrossRef]
29. Heng B, Li Y, Shi L, Du X, Lai C, Cheng L, et al. A Meta-analysis of the Significance of Granzyme B and Perforin in Noninvasive Diagnosis of Acute Rejection After Kidney Transplantation. Transplantation 2015; 99: 1477-86. [CrossRef]
30. Garcia Moreira V, Prieto Garcia B, Baltar Martin JM, Ortega Suarez F, Alvarez FV. Cell-free DNA as a noninvasive acute rejection marker in renal transplantation. Clin Chem 2009; 55: 1958-66. [CrossRef]
31. Snyder TM, Khush KK, Valantine HA, Quake SR. Universal noninvasive detection of solid organ transplant rejection. Proc Natl Acad Sci U S A 2011; 108: 6229-34. [CrossRef]
32. Duque-Afonso J, Waterhouse M, Pfeifer D, Follo M, Duyster J, Bertz H, et al. Cell-free DNA characteristics and chimerism analysis in patients after allogeneic cell transplantation. Clin Biochem 2018; 52: 137-41. [CrossRef]
33. Beck J, Bierau S, Balzer S, Andag R, Kanzow P, Schmitz J, et al. Digital droplet PCR for rapid quantification of donor DNA in the circulation of transplant recipients as a potential universal biomarker of graft injury. Clin Chem 2013; 59: 1732-41. [CrossRef]
34. Beck J, Oellerich M, Schulz U, Schauerte V, Reinhard L, Fuchs U, et al. Donor-Derived Cell-Free DNA Is a Novel Universal Biomarker for Allograft Rejection in Solid Organ Transplantation. Transplant Proc 2015; 47: 2400-3. [CrossRef]

35. Grskovic M, Hiller DJ, Eubank LA, Sninsky JJ, Christopherson C, Collins JP, et al. Validation of a Clinical-Grade Assay to Measure Donor-Derived Cell-Free DNA in Solid Organ Transplant Recipients. J Mol Diagn 2016; 18: 890-902. [CrossRef]
36. Knight SR, Thorne A, Faro MLL. Donor-specific Cell-Free DNA as a Biomarker in Solid Organ Transplantation. A Systematic Review. Transplantation 2019; 103: 273-83. [CrossRef]
37. Bloom RD, Bromberg JS, Poggio ED, Bunnapradist S, Langone AJ, Sood P, et al. Cell-Free DNA and Active Rejection in Kidney Allografts. J Am Soc Nephrol 2017; 28: 2221-32. [CrossRef]
38. Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 1993; 75: 843-54. [CrossRef]
39. Rodriguez A, Griffiths-Jones S, Ashurst JL, Bradley A. Identification of mammalian microRNA host genes and transcription units. Genome Res 2004; 14: 1902-10. [CrossRef]
40. Macfarlane LA, Murphy PR. MicroRNA: Biogenesis, Function and Role in Cancer. Curr Genomics 2010; 11: 537-61. [CrossRef]
41. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-97. [CrossRef]
42. Manchester TUo. miRBase: the microRNA database 2018, November 23 [Available from: http://www.mirbase.org/index.shtml.
43. Jan CH, Friedman RC, Ruby JG, Bartel DP. Formation, regulation and evolution of Caenorhabditis elegans 3'UTRs. Nature 2011; 469: 97-101. [CrossRef]
44. Ambros V, Bartel B, Bartel DP, Burge CB, Carrington JC, Chen X, et al. A uniform system for microRNA annotation. RNA 2003; 9: 277-9. [CrossRef]
45. Brennecke J, Hipfner DR, Stark A, Russell RB, Cohen SM. bantam encodes a developmentally regulated microRNA that controls cell proliferation and regulates the proapoptotic gene hid in Drosophila. Cell 2003; 113: 25-36. [CrossRef]
46. Bhaskaran M, Mohan M. MicroRNAs: history, biogenesis, and their evolving role in animal development and disease. Vet Pathol 2014; 51: 759-74. [CrossRef]
47. Zhou M, Hara H, Dai Y, Mou L, Cooper DK, Wu C, et al. Circulating Organ-Specific MicroRNAs Serve as Biomarkers in Organ-Specific Diseases: Implications for Organ Allo- and Xeno-Transplantation. Int J Mol Sci 2016; 17(8). pii: E1232. [CrossRef]
48. Stepien EL, Costa MC, Enguita FJ. miRNAtools: Advanced Training Using the miRNA Web of Knowledge. Noncoding RNA 2018; 4(1). pii: E5. [CrossRef]
49. Zhou Q, Li M, Wang X, Li Q, Wang T, Zhu Q, et al. Immune-related microRNAs are abundant in breast milk exosomes. Int J Biol Sci 2011; 8: 118-23. [CrossRef]
50. Hamdorf M, Kawakita S, Everly M. The Potential of MicroRNAs as Novel Biomarkers for Transplant Rejection. J Immunol Res 2017; 2017: 4072364. [CrossRef]
51. Etheridge A, Lee I, Hood L, Galas D, Wang K. Extracellular microRNA: a new source of biomarkers. Mutat Res 2011; 717: 85-90. [CrossRef]
52. Kosaka N, Iguchi H, Yoshioka Y, Takeshita F, Matsuki Y, Ochiya T. Secretory mechanisms and intercellular transfer of microRNAs in living cells. J Biol Chem 2010; 285: 17442-52. [CrossRef]
53. Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, Lotvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol 2007; 9: 654-9. [CrossRef]
54. Grasedieck S, Scholer N, Bommer M, Niess JH, Tumani H, Rouhi A, et al. Impact of serum storage conditions on microRNA stability. Leukemia 2012; 26: 2414-6. [CrossRef]
55. Vickers KC, Palmisano BT, Shoucri BM, Shamburek RD, Remaley AT. MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. Nat Cell Biol 2011; 13: 423-33. [CrossRef]
56. Vickers KC, Landstreet SR, Levin MG, Shoucri BM, Toth CL, Taylor RC, et al. MicroRNA-223 coordinates cholesterol homeostasis. Proc Natl Acad Sci U S A 2014; 111: 14518-23. [CrossRef]
57. Lasser C, Eldh M, Lotvall J. Isolation and characterization of RNA-containing exosomes. J Vis Exp 2012: e3037. [CrossRef]
58. Wojciechowska A, Braniewska A, Kozar-Kaminska K. MicroRNA in cardiovascular biology and disease. Adv Clin Exp Med 2017; 26: 865-74. [CrossRef]
59. Hata A. Functions of microRNAs in cardiovascular biology and disease. Annu Rev Physiol 2013; 75: 69-93. [CrossRef]
60. Dechamethakun S, Muramatsu M. Long noncoding RNA variations in cardiometabolic diseases. J Hum Genet 2017; 62: 97-104. [CrossRef]
61. Zhou Y, Liu M, Li J, Wu B, Tian W, Shi L, et al. The inverted pattern of circulating miR-221-3p and miR-222-3p associated with isolated low HDL-C phenotype. Lipids Health Dis 2018; 17: 188. [CrossRef]
62. Wang SS, Wu LJ, Li JJ, Xiao HB, He Y, Yan YX. A meta-analysis of dysregulated miRNAs in coronary heart disease. Life Sci 2018; 215: 170-81. [CrossRef]
63. Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, et al. Frequent deletions and down-regulation of micro- RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proc Natl Acad Sci U S A 2002; 99: 15524-9. [CrossRef]
64. Bommer GT, Gerin I, Feng Y, Kaczorowski AJ, Kuick R, Love RE, et al. p53-mediated activation of miRNA34 candidate tumor-suppressor genes. Curr Biol 2007; 17: 1298-307. [CrossRef]
65. Schickel R, Boyerinas B, Park SM, Peter ME. MicroRNAs: key players in the immune system, differentiation, tumorigenesis and cell death. Oncogene 2008; 27: 5959-74. [CrossRef]
66. Sayed D, Abdellatif M. MicroRNAs in development and disease. Physiol Rev 2011; 91: 827-87. [CrossRef]
67. Kawaguchi T, Komatsu S, Ichikawa D, Tsujiura M, Takeshita H, Hirajima S, et al. Circulating MicroRNAs: A Next-Generation Clinical Biomarker for Digestive System Cancers. Int J Mol Sci 2016; 17: E1459. [CrossRef]
68. Hannafon BN, Trigoso YD, Calloway CL, Zhao YD, Lum DH, Welm AL, et al. Plasma exosome microRNAs are indicative of breast cancer. Breast Cancer Res 2016; 18: 90. [CrossRef]
69. Zorofchian S, Iqbal F, Rao M, Aung PP, Esquenazi Y, Ballester LY. Circulating tumour DNA, microRNA and metabolites in cerebrospinal fluid as biomarkers for central nervous system malignancies. J Clin Pathol 2019; 72: 271-80. [CrossRef]
70. Goradel NH, Mohammadi N, Haghi-Aminjan H, Farhood B, Negahdari B, Sahebkar A. Regulation of tumor angiogenesis by microRNAs: State of the art. J Cell Physiol 2019; 234: 1099-110. [CrossRef]
71. Triboulet R, Mari B, Lin YL, Chable-Bessia C, Bennasser Y, Lebrigand K, et al. Suppression of microRNA-silencing pathway by HIV-1 during virus replication. Science 2007; 315: 1579-82. [CrossRef]
72. Cameron JE, Fewell C, Yin Q, McBride J, Wang X, Lin Z, et al. Epstein-Barr virus growth/latency III program alters cellular microRNA expression. Virology 2008; 382: 257-66. [CrossRef]
73. Chen Y, Li L, Zhou Z, Wang N, Zhang CY, Zen K. A pilot study of serum microRNA signatures as a novel biomarker for occult hepatitis B virus infection. Med Microbiol Immunol 2012; 201: 389-95. [CrossRef]
74. Wilflingseder J, Reindl-Schwaighofer R, Sunzenauer J, Kainz A, Heinzel A, Mayer B, et al. MicroRNAs in kidney transplantation. Nephrol Dial Transplant 2015; 30: 910-7. [CrossRef]

75. Xu Z, Nayak D, Yang W, Baskaran G, Ramachandran S, Sarma N, et al. Dysregulated MicroRNA Expression and Chronic Lung Allograft Rejection in Recipients With Antibodies to Donor HLA. Am J Transplant 2015; 15: 1933-47. [CrossRef]
76. Wei L, Wang M, Qu X, Mah A, Xiong X, Harris AGC, et al. Differential Expression of MicroRNAs During Allograft Rejection. Am J Transplant 2012; 12: 1113-23. [CrossRef]
77. Hanniford D, Hernando E. Characterization of MicroRNAs Regulating FOXO Expression. Methods Mol Biol 2019; 1890: 13-28. [CrossRef]
78. Farid WR, Pan Q, van der Meer AJ, de Ruiter PE, Ramakrishnaiah V, de Jonge J, et al. Hepatocyte-derived microRNAs as serum biomarkers of hepatic injury and rejection after liver transplantation. Liver Transpl 2012; 18: 290-7. [CrossRef]
79. Huang Y, Li J. MicroRNA208 family in cardiovascular diseases: therapeutic implication and potential biomarker. J Physiol Biochem 2015; 71: 479-86. [CrossRef]
80. Thomas MJ, Fraser DJ, Bowen T. Biogenesis, Stabilization, and Transport of microRNAs in Kidney Health and Disease. Noncoding RNA 2018;4(4). pii: E30. [CrossRef]
81. Mladinov D, Liu Y, Mattson DL, Liang M. MicroRNAs contribute to the maintenance of cell-type-specific physiological characteristics: miR-192 targets Na+/K+-ATPase beta1. Nucleic Acids Res 2013; 41: 1273-83. [CrossRef]
82. Akkina S, Becker BN. MicroRNAs in kidney function and disease. Transl Res 2011; 157: 236-40. [CrossRef]
83. Trionfini P, Benigni A, Remuzzi G. MicroRNAs in kidney physiology and disease. Nat Rev Nephrol 2015; 11: 23-33. [CrossRef]
84. Simpson K, Wonnacott A, Fraser DJ, Bowen T. MicroRNAs in Diabetic Nephropathy: From Biomarkers to Therapy. Curr Diab Rep 2016; 16: 35. [CrossRef]
85. Wonnacott A, Bowen T, Fraser DJ. MicroRNAs as biomarkers in chronic kidney disease. Curr Opin Nephrol Hypertens 2017; 26: 460-6. [CrossRef]
86. Amrouche L, Desbuissons G, Rabant M, Sauvaget V, Nguyen C, Benon A, et al. MicroRNA-146a in Human and Experimental Ischemic AKI: CXCL8-Dependent Mechanism of Action. J Am Soc Nephrol 2017; 28: 479-93. [CrossRef]
87. Liu X, Dong C, Jiang Z, Wu WK, Chan MT, Zhang J, et al. MicroRNA-10b downregulation mediates acute rejection of renal allografts by derepressing BCL2L11. Exp Cell Res 2015; 333: 155-63. [CrossRef]
88. Kaboli PJ, Rahmat A, Ismail P, Ling KH. MicroRNA-based therapy and breast cancer: A comprehensive review of novel therapeutic strategies from diagnosis to treatment. Pharmacol Res 2015; 97: 104-21. [CrossRef]
89. Sarnow P, Sagan SM. Unraveling the Mysterious Interactions Between Hepatitis C Virus RNA and Liver-Specific MicroRNA-122. Annu Rev Virol 2016; 3: 309-32. [CrossRef]
90. Michell DL, Vickers KC. Lipoprotein carriers of microRNAs. Biochim Biophys Acta 2016; 1861: 2069-74. [CrossRef]