Oxidative Stress-Related MicroRNAs as Diagnostic Markers: A Newer Insight in Diagnostics

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6.1 MicroRNA Biogenesis and Mechanism of Action

MicroRNAs (miRNAs) are evolutionarily conserved small noncoding RNAs (18–25 nucleotides). miRNAs are transcripts of individual genes having their own promoter or produced from protein coding genes by transcribing spliced portions intragenically. RNA polymerase II produces pri-miRNA, a primary transcript (Czech and Hannon 2011). It has basic structural features of mRNA transcripts such as 7-methylguanosine cap and poly-(A) tail. Introns are also present sometimes. Drosha ribonuclease along with DGCR8 (double-stranded RNA-binding protein) recognizes pri-miRNAs for further processing. In humans, the DGCR8 microprocessor complex subunit is encoded by *DGCR8* gene. It is localized in cell nucleus and binds to Drosha to form the microprocessor complex which cleaves the characteristic stem-loop structure, i.e., pri-miRNA, which is processed further to miRNA fragments by enzyme Dicer (Bertoli et al. 2015). pri-miRNAs produces precursor miRNAs (approx. 70 nucleotides). It is also known as pre-miRNAs. pre-miRNAs is also produced from some intronic miRNAs (also known as mirtrons) by using splicing machinery because they bypass Drosha processing (Czech and Hannon 2011). The pre-miRNAs are then transported to cytoplasm from the nucleus by exportin 5 (XPO5). In cytoplasm, RNase III enzyme Dicer 1 along with AGO2 (DICER complex) and transactivation-responsive RNA-binding protein 2 (TARBP2) cleaves pre-miRNAs resulting in a double-stranded miRNA-miRNA* duplex formation (Bertoli et al. 2015). Two strands then separate. The mature miRNA, also known as guide strand, gets integrated into the RNA-induced silencing complex (RISC). Two routes

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are followed by the passage miRNA* strand, i.e., either it is loaded in RISC or it is degraded. To repress the expression of target, AGO protein of the RISC is guided by mature miRNA to the complementary mRNA sequence (Czech and Hannon 2011).

miRNA has a 6–8-nucleotide seed sequence at the 5′ end which determines specificity for its binding to target mRNA (Czech and Hannon 2011). The complementarity between seed sequence and the loaded miRNA causes a measurable decline in the expression of target mRNA. Complementarity matching may take place in any segment of mRNA. However, it is likely to occur in 3′ untranslated region of a mRNA (3′ UTR) (Bertoli et al. 2015). Degree of homology between 3′ UTR of mRNA and miRNA determines whether translation will be repressed or target mRNAs will be degraded. Since each miRNA has got capability to regulate the expression of many genes, it might be inferred that each miRNA is capable of controlling many cellular signaling pathways simultaneously.

There are reports which advocate an entirely different opinion about the mechanism of miRNA action. Some findings suggested that miRNAs could enhance target mRNA translation by inducing AU-rich region through recruitment of protein complexes at this site. Else, they could in some way raise the level of target mRNA by altering repressor proteins which inhibit the translation process. miRNAs could also be implicated in increased ribosome biogenesis, thus affecting protein biosynthesis, or bypassing cell cycle arrest, which ultimately activates repression of target gene (Bertoli et al. 2015). The role of miRNAs as biomarkers has been found to be a tempting area in health and diseases. They will revolutionize the diagnostic and patient care processes including screening and diagnosis of diseases, evaluation of disease progression, and identification of accurate treatment. Since they control the target-specific gene expression, their dysregulation is concerned with modulation of biochemical processes at molecular level in cells and tissues ultimately progressing toward diseases. Diagnostic miRNAs can be found at both extracellular (whole blood, sera, plasma, and urine) and intracellular levels. Studies also revealed that miRNAs found in seminal fluid or cerebrospinal fluid may also be used to study the expression profile of miRNAs as prognostic marker for particular disease (Bertoli et al. 2016). Isolation and characterization of miRNAs are mostly done on samples derived from body fluids and tissues. Their extraordinary stability in blood and urine makes them attractive target for noninvasive tests.

6.2 Oxidative Stress- and Disease-Related miRNAs in Cardiovascular Diseases

Oxidative stress-linked modulation of various miRNAs is implicated in endothelial and vascular dysfunction. It has been observed that characteristic features are shown by miR-200 family in oxidative stress-induced vascular cell response. miR-141 and miR-200c are members of miR-200 family. They have been found as the most upregulated miRNAs in response to oxidative stress in endothelial cells (ECs) (Magenta et al. 2011). Magenta et al. (2013) reported that glutathione reductase inhibitor 1,3-bis(2 chloroethyl)-1-nitrosourea (BCNU), which inhibits the

formation of reduced glutathione from oxidized glutathione, has ability to increase expression of miR-200c. They described the significance of miR-200 family upregulation, and especially of miR-200c in ECs reaction to oxidative stress, indicating the important role of ZEB1 (zinc finger E-box-binding homeobox 1) downmodulation in ROS-induced apoptosis and senescence. Several studies have established that oxidative stress is involved in upregulation of miR-200 family. Experiments on oxidative stress induction in a cell model with tert-butyl hydroperoxide (t-BHP) proved upregulation of miR-200c and miR-141. miRNA profiling studies on H_2O_2-induced oxidative stress in primary hippocampal neurons of mouse further supported upregulation of miR-200c. Nitric oxide (NO), a free radical known to play important role in endothelium metabolism, also stimulates overexpression of miR-200 family. These events and ZEB2 knockdown bring forth the expression of CXCR4 and Flk1, which are early cardiovascular and mesendoderm precursor markers in mouse embryonic stem cells (Magenta et al. 2011). A relationship between the expression of miR-200 family and oxidative stress has also been portrayed by workers in other systems (Mateescu et al. 2011). The same seed sequences are present in miR-141 and miR-200a. These sequences are used to target mitogen-activated protein kinase (MAPK) p38α, a signal molecule involved in regulation of cell proliferation, stress management, and cell survival. This illustration highlights the potential mechanism of action used in many cell types by miR family (Magenta et al. 2011).

Upregulation of an NAD^+-dependent class III histone deacetylase, sirtuin 1 or SIRT1, is reported in many organisms during caloric restriction, aging, and extension of the life span processes. SIRT1 plays a major role in metabolic regulation at cell, tissue, and organ levels by actively deacetylating countless enzymes, stress-responsive transcription factors, and co-regulators. It also has intense anti-inflammatory and antioxidant activity, suggesting a beneficial role for SIRT1 upregulation in endothelial cell biology (Magenta et al. 2011). However, decreased expression of SIRT1 is found during aging and overproduction of ROS (reactive oxygen species) which is related to EC dysfunction. Activation of SIRT1 in ECs mitigates oxidative stress, increases bioavailability of NO through eNOS induction, promotes biogenesis of mitochondria, and averts endothelial senescence. Interaction between oxidative stress-miRNA-SIRT1 pathways plays crucial role in vascular disease development processes. Its role has been highlighted in abdominal aortic aneurysm and atherosclerosis. miR-217 increases with age progression and affects endothelial senescence. miR-217 negatively modulates expression of SIRT1, resulting in loss of functional interaction with eNOS and Forkhead box protein O1 (FoxO1) which are main endothelial targets of SIRT1 (Menghini et al. 2001). Inverse relationships between FoxO1 acetylation and miR-217 level as well as between expression of SIRT1 in atherosclerotic plaques and miR-217 level are explicit. HIF1A destabilization is directly related to SIRT1 expression which is downregulated by miR-199a. Low SIRT1 consecutively causes inactivation of prolyl hydroxylase domain-containing protein 2 (PHD2) which is essential for HIF1A destabilization. Similarly miR-34a also targets SIRT1.

In cultured bone marrow cells (BMCs), overexpression of miR-34a stimulates cell death and SIRT1 downregulation, while its inhibition stops H_2O_2-induced cell death. It has been observed that BMCs, with blocked miR-34a expression ex vivo, when injected in mice after acute myocardial infarction boost cardiac function (Magenta et al. 2011). Targeting of SIRT1 by oxidative stress-induced miR-200a further confirmed the major role played by miR-200 family in EC dysfunction resulting from oxidative stress. It has been reported that stress-induced increased expression of miR-21 safeguards ECs by promoting eNOS and NO levels and lowering apoptosis. Overexpression of miR-21 in atherosclerotic plaques is also associated with decline in mitochondrial antioxidant protein SOD-2 and SPRY-2. This, further, causes activation of ERK/MAP kinase with consequential increase in ROS production and migratory defects in angiogenic progenitor cell (APC). During heart failure, mitochondrial structural modification coupled with size reduction and increased numbers has been observed (Magenta et al. 2011). In addition, mitochondrial damage is related to several manifestations of ventricular dysfunction in congestive heart failure including end-diastolic pressure, ejection volume, and the extent of orthosympathetic stimulation. One study has revealed that mitochondrial integrity is affected by specific miRNAs in cardiomyocytes which include miRNAs of miR-15 family (miR-15b, miR-16, miR-195) and miR-424 having same seed nucleotide sequences. These modulate ATP levels and decrease the expression of ADP-ribosylation factor-like 2 (Arl2) mRNA which is their common protein target. The evidences explained above suggest that miRNAs play key roles in pathophysiological processes, and hence they are good diagnostic biomarker for oxidative stress-mediated vascular ailments (Magenta et al. 2011).

6.3 Oxidative Stress-Induced miRNAs in Liver Injury

Several studies have revealed the role of oxidative stress in the pathophysiology of liver injury. In adult liver, miR-122 constitutes approximately 70% of the total miRNA. These are involved in functional aspects of the liver in health and diseases comprising lipid metabolism, progression of cell cycle, fibrosis, and hepatocellular carcinogenesis. Moreover, miR-122 is a prospective biological marker for diagnosing liver toxicity resulting from alcohol, acetaminophen, and drug-induced liver injury (Zhang et al. 2011). In addition, miR-122 has also been proposed as a biomarker for the early detection and diagnosis of antitubercular drug-induced liver injury (ADLI). Correlation between oxidative stress and miR-122 in ADLI has been proven by measuring alterations in oxidative stress indicators and levels of miR-122 in liver tissue of mice during hepatic damage.

Isoniazid (INH) is used as a first-line drug to treat tuberculosis. However, ADLI is the most frequent offshoot of INH treatment and progresses to liver cirrhosis. ADLI occurs because of excessive oxidative stress and mitochondrial dysfunction attributable to the formation of reactive metabolites during metabolic breakdown of drug. Studies indicate that mitochondrial ribosomal protein S11 (MRPS11) is targeted and regulated by miR-122. It is well known that oxidative stress causes ADLI. It has been

hypothesized that certain types of miR-122, acting as a potential biomarkers for ADLI, regulate the synthesis of mitochondria, thus participating in oxidative stress (Zhang et al. 2011). miR-122, as a dominant liver-specific miRNA, may adjust the targets to affect liver cells. It has been reported that miR-122 expression is more closely correlated with liver damage as compared to levels of GPT and GOT, the serum enzymes indicating that miR-122 is an important biomarker having diagnostic potential. It is concluded that tissue miR-122 is closely involved in INH-induced ADLI and might be implicated in oxidative stress by altering its target levels.

6.4 Oxidative Stress-Induced miRNAs in Neurodegenerative Disease

miRNAs are possibly related to several pathophysiological processes, viz., antioxidant response, cholesterol trafficking, pathological proteins clearance, neuroinflammation, and cell cycle anomalies. All these processes aid in progression of neurodegenerative diseases (NDs). Postmortem of ND brains in humans and animal models (rodent mouse) has reported oxidative stress responses (Coppedè and Migliore 2010). In ND patients, oxidative stress affects calcium homeostasis, peroxidation of membrane lipids, proper protein folding, aggregation, DNA repair, and clearance of damaged proteins. ROS is mainly produced during mitochondrial respiration and inflammatory processes. This indicates a strong linkage among oxidative stress-associated redox processes vis-a-vis mitochondrial dysfunction and neuroinflammatory response. Current scientific literature has highlighted the role of miRNA in pathological and physiological stress-induced responses (Coppedè and Migliore 2010; Prendecki and Dorszewska 2014).

In Parkinson's disease (PD), Alzheimer's disease (AD), and other NDs, undue neuronal apoptosis takes place in different parts of the human brain which adversely affects the central nervous system (CNS). PD and AD are nonhomogeneous group of disorders. The nature of the abnormalities in behavioral and cognitive functions as well as motor responses of the brain are determined by part of brain and types of neurons affected, which are disease specific. However, perfect diagnosis of NDs is possible simply after postmortem and histopathological evaluation of brain tissue because of the considerable heterogeneity of clinical signs in NDs. Today, NDs constitute one of the most important healthcare issues. About 24 million people are affected by AD worldwide. Scientific community across the globe is looking for specific markers essential for the pathogenesis and diagnosis of ND. It appears that miRNA, whose biosynthesis is clearly known, could be one of the potential biomarkers. At present, about 2600 miRNAs are known in humans who are engaged in many pathophysiological processes (Prendecki and Dorszewska 2014). Out of these, specific miRNAs related to pathogenesis and diagnosis of NDs have been identified. Majority of miRNAs are of common occurrence in various NDs. Only a small number of them are exclusive to specific NDs such as miR-132 and miR-212 for frontotemporal dementia; miR-19b, miR-34b/miR-34c, and miR-133b for PD; and let-7f, miR-125b, and miR-193b for AD. Thus ND-specific miRNAs may pave

the way for early and definite diagnosis, and accordingly, clinicians may introduce suitable treatment regimen for particular disease. Association between NDs and oxidative stress-dependent miR-153 has been displayed by workers (Narasimhan et al. 2014). The study reported the effect of paraquat, an environmental pollutant, on PD resulting from increased risk of dopaminergic neurons (DNs) damage. Real-time quantitative PCR analysis demonstrated that paraquat considerably upregulated the expression of brain-enriched miR-153 and downregulated nuclear factor Nrf2 which binds and activates antioxidant response elements (ARE, the transcription initiator) involved in mitigation of oxidative stress. Thus, paraquat induces neurotoxicity by damaging DNs, suggesting a decisive role of ROS (resulting from oxidative stress) interaction in miR-153-Nrf2/ARE pathway (Narasimhan et al. 2014). Intensive efforts are required for the development of miRNA-based methods merging data obtained from the expression studies of various miRNAs of CSF or blood origin. The effort will go ahead in making available, specific, and sensitive assays for early diagnosis of neurodegenerative disorders.

6.5 Oxidative Stress-Induced miRNAs in Sepsis

Infection causing a systemic inflammatory response is known as sepsis which could be responsible for high mortality because of multiple organ dysfunction syndromes (MODS). Studies related to sepsis prevention and treatments during the last 10 years have achieved some success. Nevertheless, sepsis is still the most important cause of ICU mortality (Yao et al. 2015). For diagnosis and appraisal of sepsis conditions before time, biomarkers play significant role. C-reactive protein (CRP), procalcitonin (PCT), N-terminal pro-atrial natriuretic peptide, and interleukins are the early sepsis markers having some limitations. They need to have more precision with respect to their role as a biomarkers. For instance, CRP is not appropriate alone as diagnostic marker because of its high sensitivity and low specificity. However, specificity of PCT is higher than that of CRP. It has been reported that PCT alone is unable to differentiate between bacterial sepsis and nonbacterial systemic inflammatory response syndrome (SIRS). Hence, development of new biomarkers is much needed. Altered level of miRNA expression has been observed in some sepsis patients. The studies on miRNAs in rat model of septic shock revealed that 17 out of 351 miRNAs had higher expression, while only 9 among them notably regulated specific genes (Yao et al. 2015). Sepsis causes upregulation of miR-27a in mice, and its inhibition is correlated with reduction of the inflammatory responses by lowering IL-6 and TNF-α levels (Wang et al. 2014). Hence, miR-27a acts as biomarker for sepsis detection. In addition, it is also a good target for drug action. In PBMCs of sepsis patients, dysregulated miRNAs are present. Downregulation of miR-146a is associated with raised level of IL-6 and monocyte proliferation in sepsis (Zhou et al. 2015). Oxidative stress is involved in sepsis-induced MODS, a major cause of patient death. During sepsis, ROS generation resulting from activation of enzymes (NADPH oxidase and xanthine oxidase) by ischemia reperfusion injury and/or endotoxins produces redox imbalance which eventually causes tissue damage (Von-Dessauer et al. 2011).

miR-25 and oxidative stress levels are inversely related in sepsis patients. Increased stress indicates lower levels of miR-25 whose target is NOX4 (NADPH oxidase 4) gene. Lower levels of miR-25 enhance NOX4 expression which induces ROS production and ultimately triggering the oxidative damage (Yao et al. 2015). Earlier studies have also revealed that linkage of miR-25 with oxidative stress may be the main cause for sepsis-induced MODS. Hence, miR-25 could be used as oxidative stress-mediated prognostic biomarker of sepsis (Varga et al. 2013).

6.6 Oxidative Stress-Induced miRNAs in Diabetes

Diabetes is known to be an important and established threat factor for diverse ailments. Hyperglycemia is a common feature of both type 1 and type 2 diabetes. It enhances ROS production, changes the oxidant status of cells, and quickly modifies membrane function, followed by other malfunctioning in different organs of the body. Endogenous antioxidant defense system gets compromised during oxidative stress which is associated with diabetes-induced decline in different body functions (Kumar and Pandey 2015). Several studies have probed the causes underlying role of oxidative stress in diabetes. Emerging facts specify that miRNAs functioning as translational repressors are key regulators of important biological processes and might be related to the pathophysiology of diabetes. Tissue-specific miRNAs have been recognized in complications associated with diabetes. Dysregulation and differential expression of cardiac-enriched miRNA levels in heart of diabetic animals have been shown to play vital roles in the progression of diabetic cardiomyopathy (Yildirim et al. 2013). In diabetic heart, miR-133 expression level has been shown to change (Feng et al. 2010). Glucose-stimulated apoptosis of cardiomyocytes is mediated by miR-1. However, lowered expression of miR-1 brings about enhancement in the level of a cytoskeleton regulatory protein which induces cardiac hypertrophy (Yu et al. 2008). Hyperglycemia-induced oxidative stress-dependent reduction of four miRNAs (miR-1, miR-133a, miR-133b, and miR-499) has been experimentally shown in diabetic rats. In addition, redox imbalance further influences many intracellular targets of these miRNAs. Therapeutic efficacy of miRNA therapeutic intervention in diabetic complications has been proven, and miR-21 has been identified as a disease target (Yildirim et al. 2013).

Association of particular miRNAs, viz., miR-15a, miR-107, miR-103, and miR-143, with glucose metabolism (insulin induced) and progression of the diabetes have been discussed by several workers. Studies have revealed the linkage between some of the diabetic problems and the modulated expression of miRNA consequently leading to increase in transcription factors, matrix components, and growth factors. Lowered level of miRNA expression causes failure of inhibition at certain targets in diabetic complications. This is the first trend observed in diabetes. Expression of fibronectin (FN) is negatively regulated by miRNA-146a related to retinopathy. Downregulation of miR-146a in diabetic animals finds direct correlation with overexpressed FN (Feng et al. 2013). Further, miR-200b and VEGF expression are inversely related. Therefore, loss of miR-200b in retinopathy results in endothelial

proliferation and permeability due to overexpression of VEGF. Association of miRNA-133a with insulin growth factor 1 receptor also results in cardiomyopathy (Feng et al. 2013). Finally, hyperglycemia-induced oxidative stress led downregulation of miRNA results in augmentation in the level of growth regulators and inflammatory factors. However, some miRNAs are overexpressed in diabetic nephropathy, e.g., miR-377 which is directly related to SOD1/SOD2 levels. The increment in miR-377 level increases the instability of *SOD2* transcripts which ultimately lead to reduced Mn-SOD activity (Wang et al. 2008). This shows that hyperglycemia-induced overexpression of a miRNA compromises with the antioxidant defense. Similarly, hyperglycemia causes overexpression of miR-192 in renal mesangial cells which directly targets zinc finger E-box-binding homeobox 1 (*ZEB1/ZEB2*) resulting in decreased expression of *ZEB1/ZEB2* in the experimental subjects (Kato et al. 2007). Under normal conditions, *ZEB1/ZEB2* is responsible for repressing TGF-β. So decreased *ZEB1/ZEB2* levels in diabetes ultimately causes increase in TGF-β expression and leading to decline in renal function. Above examples demonstrate that inhibition mediated by overexpression of miRNA in the cell can produce varied results on protective signaling in diabetic individuals.

6.7 Oxidative Stress-Induced miRNAs in Cancer

Since hundreds of mRNAs are targeted by a single miRNA, anomalous expression of miRNA may have an effect on a large number of transcripts and strongly affect signaling pathways related to cancer. miRNA was primarily portrayed in 1993, its physiological and pathological significance became known after their characterization in several species in 2001. Microarray expression data obtained from variety of cancers have proved that unusual expression of miRNA is the essential feature rather than the exception. Notably, miRNA overexpression or ablation in mouse models have confirmed the fundamental association between miRNAs and cancer growth, and therefore, miRNAs are making their entry in biomedical fields as potential biomarkers and accepted as targets for therapy. Studies on several mammalian differentiation pathways have revealed that individual miRNAs may serve as switches, for example, smooth muscle cell differentiation is regulated by miR-143 and miR-145, while skin differentiation is regulated by miR-203 (Jansson and Lund 2012). A single miRNA has capability to affect identity of the cell. Early studies on overexpression substantiated that HeLa cells transfected with single miR-124 showed changed pattern of expression exemplifying characteristics of brain expression profile. Brain tissues show higher expression of miR-124 (Lim et al. 2005). miRNAs play critical roles in many vital processes including noise-dampening effect, differentiation, and cellular identity. Hence, functional loss of miRNA may lead to enhanced dedifferentiation, cellular plasticity, and a greater tendency toward oncogenic alterations. miRNAs also play remarkable roles in differentiation of stem cells and pluripotency induction. Recently, human and mouse fibroblasts have shown ability to produce miR-302-induced iPSC (induced pluripotent stem cells) by a single miRNA cluster. At present, about 1400 human miRNAs are known.

Many of them show strong conserved sequences among distantly related animal taxa (Jansson and Lund 2012). Most of the miRNAs put forth their complete functional effects by targeting many mRNAs; some of them might be the part of same cellular pathway. A specific miRNA may be augmented in some cancer types suggesting its oncogenic behavior, while in other cancers it may be downregulated signifying its tumor suppressor function. Therefore, it is imperative to be careful while drawing conclusions regarding usage of miRNA.

6.7.1 Ovarian Cancer

Among gynecological malignancies, epithelial ovarian cancer (EOC) is the major cause of fatality in women worldwide. It accounts for approximately 5% of all cancer cases and about 4.2% of all cancer deaths in women globally. Despite rapid progress in diagnostics and therapeutics, only limited success in the survival rate of ovarian cancer patients ahead of 5 years has been realized after initial diagnosis. Several factors are responsible for the high mortality of ovarian cancer patients. These include the lack of early stages specific symptoms and delayed diagnosis causing problem in designing therapeutic intervention as well as the developing resistance to chemotherapy in cancer cells. This necessitates for better EOC detection and screening approach at early stages coupled with effective treatment regimen for advanced stage patients (Pal et al. 2015).

Redox regulation has been suggested to play important role in cancers. However, its role in tumor prognosis is still elusive. Several studies have shown relationships between oxidative stress and the miRNAs that affect tumorigenesis and chemosensitivity. miR-141 and miR-200a have been shown to alter the oxidative stress response by targeting p38a. Higher concentrations of miR-200a and lower concentrations of p38a are observed in human ovarian adenocarcinomas patients, which act as an oxidative stress marker (Mateescu et al. 2011). Correlation has been found between the level of stress biomarker miR-200a and the improvement in patients' survival under treatment regimen. Therefore, miR-200a could acts as a prognostic marker during stress for evaluation of clinical efficacy in EOC. Besides its tumor-promoting role, oxidative stress also enhances sensitization of cancerous tissue to drug treatment. This could provide explanation to clinical trials using antioxidants with the partial success. Thus, there is an urgent requirement to investigate the biochemical rationale of ovarian cancer for exploration of early diagnostic markers/classifiers which can consistently identify patients for interventional therapy. ROS buildup in cancer cells damages cellular machinery and modifies diverse processes at biochemical and molecular levels including cell proliferation, gene expression, and stability of genome. Among stress-linked modulation of gene expression, the mitogen-activated protein kinase p38α family carries out the redox-sensing function for monitoring oxidative stress status. The sensor function is necessary for controlling tumor progression. The miRNAs of miR-200 family have been shown to alter cellular motility and manage "stemness" and apoptosis. During redox imbalance and ovarian tumorigenesis, the miR-200 performs a new function. Mateescu et al.

(2011) have demonstrated inhibition of p38α by miR-141 and miR-200a (the members of the miR-200 family) which play important role in redox sensing. Upsurge of these miRNAs in mouse imitates deficiency of p38α and supports malignancy.

6.7.2 Breast Cancer

Various reviews have presented the diagnostic, prognostic, and the therapeutic roles of miRNAs in breast cancer (BC). miRNAs can play dual role in BC either by acting as oncogenes or tumor suppressor genes. Outcome of studies has indicated the role of miRNAs as promising biomarkers for diagnostic, prognostic, and therapeutic applications in BC. miR-9, miR-10b, and miR-17-5p are diagnostic makers for BC, while miR-148a and miR-335 are prognostic markers. Some miRNAs, viz., miR-30c, miR-187, and miR-339-5p, are emerging as potential biomarkers for testing therapeutic efficacy of drugs. All these miRs are associated with BC control functions, i.e., proliferation, invasion, metastasis, apoptosis, resting death, and genomic instability (Bertoli et al. 2015). Other new and easily available miRNAs, viz., miR-155 and miR-210, circulating in body fluids have drawn attention as markers for management of BC patients because they are affordable and noninvasive tools. Many circulating miRNA have shown better diagnosis and prognosis results in BC along with better sensitivity. New miRNA-based drugs containing miR-9, miR-21, miR-34a, miR-145, and miR-150 have emerged as potential therapy for BC. Other miRNAs such as miR-21, miR-34a, miR-195, miR-200c, and miR-203 in combination with chemotherapy have also shown a basic response in modulation of other non-miRNA treatments (Bertoli et al. 2015). miRNAs having oncogenic activities are designated as oncomirs. These are constitutively overexpressed and responsible for promoting tumor growth by inhibiting tumor suppressor genes or regulatory genes that affect cell cycle progression and differentiation or apoptosis. miR-21 is an excellent example of oncomirs. Its target tumor suppressor gene is PTEN (phosphatase and tensin homolog), and several studies have shown that miR-21 overexpression, correlating to PTEN downregulation, leads to proliferation and metastasis (Corsini et al. 2012). miRNAs having invasive skills are called metastamiRs. These miRNAs regulate positively or negatively epithelial-to-mesenchymal transition (EMT), loss of cellular adhesion, can play a pro- and anti-metastatic role. Examples include miR-192/miR-215, which targets ZEB1 and ZEB2 (E-cadherin repressors), miR-30, and miR-200 family that regulate the TGF-beta pathway (Corsini et al. 2012).

6.7.3 Prostate Cancer

In males prostate cancer (PC) is the sixth major cause of cancer death and its prevalence increases with age. Like other cancer, oxidative stress also contributes major role in pathophysiology of prostate cancer. Intrinsic and extrinsic factors may cause higher production of ROS in the prostate and thereby affect the function of prostate.

Altered redox status in prostate tissue resulting from imbalance between oxidants and antioxidants plays an important role in the initiation of PC.

Androgens play key role in mitigating ROS imbalance in the prostate. In addition, the transcription factor Nrf2-mediated expression of major antioxidant defense enzymes through the upregulation of ARE is responsible for lowering the ROS levels in prostate cancer. Current reports indicate that in human prostate cancer, Nrf2 and its target genes are considerably downregulated. Thus, cells repeatedly face rising oxidative stress levels that ultimately result in their continuous advancement toward metastatic conditions (Pekarik et al. 2013). One of the approaches to study miRNAs in PC is to analyze the exosomal miRNA profiles of cancerous and non-cancerous prostate samples. It has been shown that miR-711 and miR-4258 have comparatively higher expression in exosomes of PC sample (Liu et al. 2011). Another study has reported that serum level of miR-141 can differentiate between healthy control and PC samples (Huang et al. 2010). It was further substantiated by upregulation of miR-141 in the plasma of PC patients that confirmed its role as diagnostic biomarker (He et al. 2012). The use of miR-141, miR-298, miR-346, and miR-375 as diagnostic markers of PC has been authenticated by using microarray and RT-PCR techniques (Felicetti et al. 2008). Compared with healthy control sera, upregulation of 15 miRNAs (miR-16, miR-92a, miR-103, miR-107, miR-197, miR-34b, miR-328, miR-485-3p, miR-486-5p, miR-92b, miR-574-3p, miR-636, miR-640, miR-766, miR-885-5p) have also been reported in serum of PC patients (Fanjul-Fernandez et al. 2010). In addition, miR-12, miR-34, miR-129-5p, miR-203, miR-302, miR-372, miR-373, and miRNA cluster miR-183-96-182 are known to be involved in oxidative stress-mediated prostate cancer pathophysiology (Bertoli et al. 2016).

6.8 Conclusion

MicroRNAs are specific targets for diagnostic and prognostic applications. They are helpful in evaluation of disease progression and in recognition of the therapeutic target for disease management in patients suffering from many degenerative diseases. Expression of particular target genes is regulated by miRNAs and their dysregulation cause altered biochemical and molecular processes in intracellular milieu. Oxidative stress is concerned in up-/downregulation of numerous miRNAs. Identification of various miRNAs as prospective targets for diagnosis and prognosis of cardiovascular, liver, and neurodegenerative diseases, diabetes, sepsis, and different types of cancers has revolutionized the field of biomarker discovery. Diagnostic miRNAs can be found at extracellular and/or intracellular levels. miRNAs are extremely stable in biological samples making them attractive molecules for noninvasive tests.

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