miRNAs Related to Immune Checkpoint Inhibitor Response

Subjects: Oncology

Contributor: José Luis García-Giménez , Wiam Saadi , Angel L. Ortega , Agustin Lahoz , Guillermo Suay , Julián Carretero , Javier Pereda , Ahlam Fatmi , Federico V. Pallardó , Salvador Mena-Molla

The advent of immune checkpoint inhibitors (ICIs) has represented a breakthrough in the treatment of many cancers, although a high number of patients fail to respond to ICIs, which is partially due to the ability of tumor cells to evade immune system surveillance. Non-coding microRNAs (miRNAs) have been shown to modulate the immune evasion of tumor cells, and there is thus growing interest in elucidating whether these miRNAs could be targetable or proposed as novel biomarkers for prognosis and treatment response to ICIs.

cancer immunity epigenetic regulation immune checkpoint inhibitor microRNAs immune checkpoint

1. Introduction

Study of the tumor microenvironment has uncovered a wide battery of mechanisms exploited by neoplastic cells to evade immune system action, which enables them to survive and fosters tumorigenesis [1]. Immune cell activation requires T cell receptor (TCR) recognition of antigens presented by major histocompatibility complex class I or II molecules (MHC-I/II) expressed on normal cells or antigen presenting cells (APCs), and a costimulatory pathway wherein the receptor CD28 binds to ligands expressed on APC membrane such as B7 family ligands B7-1/CD80 or B7-2/CD86. This interaction stabilizes the signal and triggers the complete activation, proliferation, survival, and cytokine production of T lymphocytes. This same signaling initiates a regulatory mechanism to prevent overactivation of the immune system, in which the expression of immune checkpoint (IC) molecules such as cytotoxic T lymphocyte-associated antigen-4 (CTLA-4; CD152) is upregulated on the lymphocyte cell membrane after T cell activation to compete for the same ligands as CD28, avoiding T cell overactivation and hyperactivity. Similarly, programmed cell death protein 1 (PD-1; CD279) and its ligands PD-L1 (PD-1 ligand 1; CD274; B7-H1) and PD-L2 (CD273; B7-DC); or B7-H3 (CD276) and its ligandstriggering receptor expressed on myeloid cell (TREM)-like transcript 2 (TLT-2), toll-like receptor 2 (TLR2) or interleukin-20 receptor subunit alpha (IL20RA), that inhibit T cell effector functions and induce T cell apoptotic death [2][3][4][5][6]. Another IC is the cluster of differentiation 47 (CD47), which binds to signal regulatory protein alpha (SIRPα) of membrane macrophages to inhibit tumor cell phagocytosis [7].

The use of inhibitors against these ICs (immune checkpoint inhibitors (ICIs), also known as immune checkpoint blockade (ICB)) has shown clinical benefits in the treatment of different types of cancer. The inhibitors currently

available are PD-1 inhibitors (i.e., Cemiplimab, Nivolumab and Pembrolizumab, and the recently approved Retifanlimab and Dostarlimab, approved in March and July 2023, respectively; www.fda.gov, accessed on 8 November 2023), and PD-L1 inhibitors (i.e., Atezolizumab, Avelumab and Durvalumab), and CTLA-4 inhibitors (Ipilimumab)), all approved by the U.S. Food and Drug Administration (FDA) [8]. However, treatment response depends on tumor type; good responses can be observed in immunogenic tumors, as seen in metastatic melanoma, which show a five-year overall survival (OS) rate of up to 52% when combining Nivolumab and Ipilimumab (compared to the five-year OS rate of about 35% for targeted therapy) [9]. Nonetheless, other tumors do not respond well to ICIs, and these inhibitors can even favor tumor progression, as has been observed in T cell leukemia–lymphoma after treatment with Nivolumab [10].

This differential response to ICIs highlights the need to find novel biomarkers that can guide decision making to select the most personalized treatment for each patient. The FDA has approved different biomarkers for ICIs, such as PD-L1 expression in tumor cells, microsatellite instability (MSI), and Tumor Mutational Burden (TMB), referring to the totality of somatic mutations (single nucleotide polymorphisms (SNPs) and variations of copy number (CNVs)) per million bases. Other biomarkers are being studied, such as the tumor proportion score (TPS; evaluates expression of PD-L1 on tumor cells) [11], tumor immune dysfunction and exclusion (TIDE) signature (stratifies patients into high or low cytotoxic T lymphocyte count based on gene signature) [12], and Immunoscore (based on the density of total and cytotoxic tumor infiltrating T cell). However, these biomarkers present certain limitations of use for selecting the most appropriate individual treatment.

1.1. miRNAs That Modulate Response to ICIs

According to **Table 1** and **Figure 1**, increases in let-7a, let-7b, miR-15b-5p, miR-16-5p, miR-20b-5p, miR-128a, miR-582, miR-708, and miR-4759 levels are positively correlated with increased effectiveness of ICI therapy, while miR-21 and miR-340 presented a reduced response to ICIs in the analyzed models. miR-424 and miR-155 produced opposing outcomes according to the tumor type studied.

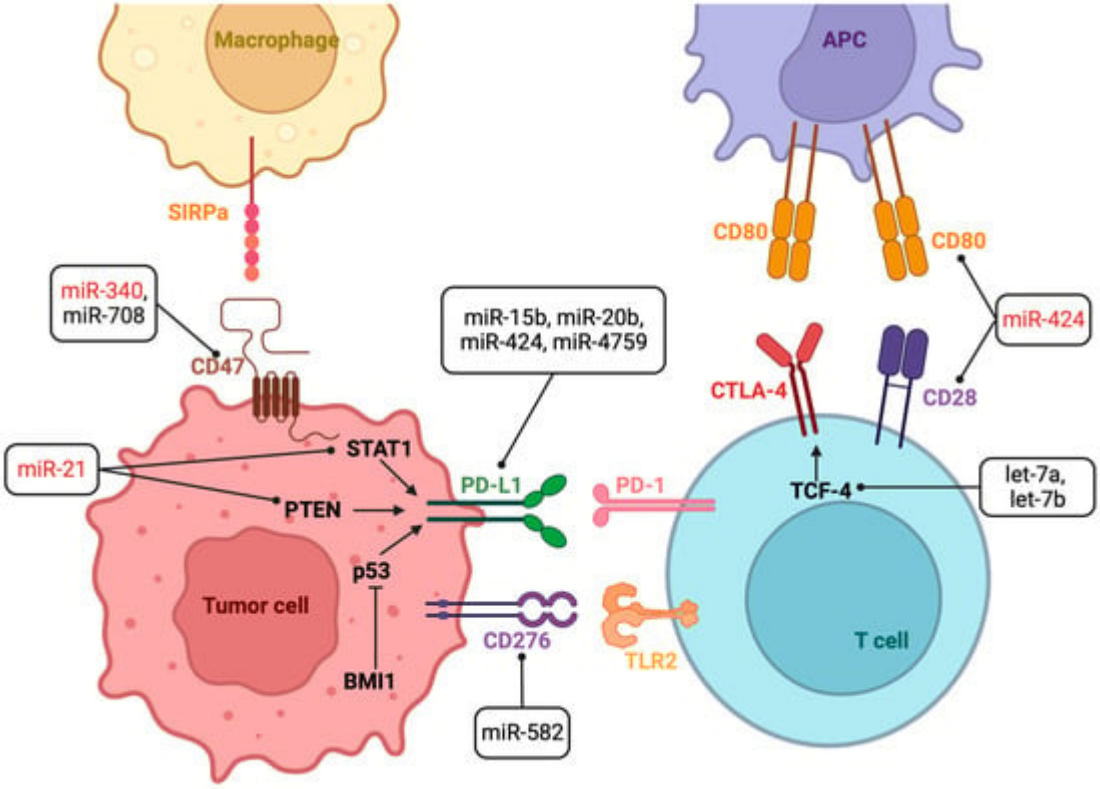

Figure 1. Scheme representing relevant miRNAs with the ability of modulating the effectiveness of immune checkpoint inhibitors (ICIs) by directly targeting ICs or other related modulators. Black: high miRNA levels enhanced ICI efficacy; Red: low miRNA levels enhanced ICI efficacy. APC: antigen-presenting cell; BMI1: BMI1 proto-oncogene, polycomb ring finger; CD47: cluster of differentiation 47; CD80: CD80 molecule (B7-1, CD28LG); CTLA-4: cytotoxic T lymphocyte-associated antigen-4 (CD152); p53: tumor protein p53; PD-1: programmed cell death protein 1 (CD279); PD-L1: programmed cell death protein 1 ligand (CD274; B7-H1); PTEN: phosphatase and tensin homolog; SIRPα: signal regulatory protein alpha; STAT1: signal transducer and activator of transcription 1; TCF-4: T Cell Factor 4; TLR2: toll-like receptor 2. Created with Bio.Render.com.

Table 1. miRNAs that modulate response to immune checkpoint inhibitors (ICIs).

miRNA	Cancer	miRNA Target Gene	ICI	Experimental Model	Effect on ICI Response	Refs.
let-7a and let-7b	Head and neck squamous cell carcinoma	*TCF-4* *	anti CTLA-4	Overexpressing let-7a/b tumor cells inoculated into mice + anti CTLA-4	H	[13]
miR-15b-5p	Colorectal cancer	*PD-L1* *	anti PD-1	Overexpressing miR-15b-5p tumor cells inoculated into mice + anti PD-1.	H	[14]
miR-16-5p	Lung cancer		anti PD-L1	Tumor cell + overexpressing miR-16-5p exosomes + anti PD-L1	H	[15]

miRNA	Cancer	miRNA Target Gene	ICI	Experimental Model	Effect on ICI Response	Refs.
miR-20b-5p	Lung cancer	*PD-L1* *	anti PD-1	Tumor cells transfected with miRNA mimic + Pembrolizumab	H	[16]
	Breast cancer			Tumor cells transfected with miRNA mimic + Pembrolizumab	H	
	Oral squamous cell carcinoma	*PTEN*	anti PD-L1	Tumor cells inoculated into mice + miR-21 knockdown tumor-derived exosomes + anti-PD-L1	L	[17]
miR-21	Melanoma	*STAT1* *	anti PD-1	Tumor cells and knocked down miR-21 tumor-associated macrophages (TAM) subcutaneously injected in mice + anti PD-1	L	[18]
miR-128a	Laryngeal squamous cell carcinoma	*BMI1* *	anti PD-1	Overexpressing miR-128a tumor cells + Pembrolizumab	H	[19]
miR-155	Metastatic melanoma		anti PD-1 + anti PD-L1 + anti CTLA-4	Tumor cells inoculated into modified mice for knockout of miR-155 in CD4/8 T cells + anti PD-1, anti PD-L1 and anti CTLA-4	L	[20]
	Diffuse large B-cell lymphoma	*PD-L1* *	anti PD-L1	Overexpressing miR-155 tumor cells inoculated into mice + anti PD-L1	L	[21]
	Breast cancer	*SOCS1*	anti PD-L1	Overexpressing miR-155 tumor cells inoculated into mice + anti PD-L1	H	[22]
	Melanoma	*PD-L1* *	anti PD-L1	Overexpressing miR-155 tumor cells co-cultured with peripheral blood mononuclear cells + anti PD-L1	H	[23]
miR-340	Pancreatic carcinoma	*CD47* *	anti CD47	Overexpressing miR-340 tumor cells inoculated into mice + anti CD47	L	[24]
miR-424	Colorectal cancer	*CD28* and *CD80* *	anti PD-1 + anti CTLA-4	Tumor cells inoculated into miR-424 knocked mice + anti PD-1 and anti CTLA-4	L	[25]

miRNA	Cancer	miRNA Target Gene	ICI	Experimental Model	Effect on ICI Response	Refs.
				Mouse cecum orthotopic colorectal cancer + miR-424 knocked tumor cell-derived extracellular vesicles + anti PD-1 and anti CTLA-4	L	
	Hepatocellular carcinoma	*PD-L1*	anti PD-L1	Tumor cells inoculated into mice + nanobubbles carrying miR-424 mimic and anti PD-L1	H	[26]
miR-582	B-cell precursor acute lymphoblastic leukemia	*CD276* *	anti CD276	Overexpressing miR-582 tumor cells co-cultured with NK cells + anti CD276	H	[27]
miR-708	T-acute lymphoblastic leukemia	*CD47* *	anti CD47	Overexpressing miR-708 tumor cells + anti CD47. [30]	H	[28]
miR-4759	Breast cancer	*PD-L1* *	anti PD-L1	Overexpressing miR-4759 tumor cells co-cultured with peripheral blood mononuclear cells + anti PD-L1	H	[29]

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Table 2 shows miRNAs modulated after response to ICIs in patients. Good responders (complete response, partial response, or stable disease) to ICIs saw increased miR-22, miR-24, miR-99a, miR-194, miR-214, miR-335, miR-339, and miR-708 levels, while the overexpression of miR-4649-3p and miR-615-3p correlated with no response to ICIs.

Abbreviations: Effect of miRNA levels on ICI response: H, high miRNA levels enhanced ICI efficacy; L, low miRNA levels enhanced ICI efficacy. miRNA target gene (*) indicates that it has been validated by luciferase reporter assay in the work.

Table 2. miRNAs modulated after response to immune checkpoint inhibitors (ICIs) in patients.

ICI	Experimental Model	miRNA	Experimental Effect on miRNA	Refs.
anti PD-1	miRNA analysis in peripheral lymphocytes from 21 good responders (complete response, partial response, or stable disease) with metastatic renal cell carcinoma before and after a 4-weeks period (2 cycles) of nivolumab administration	miR-99a, miR-708, miR-655, miR-582-3p, miR-492, miR-487a, miR-485-3p, miR-449a, miR-433, miR-431, miR-429, miR-376c, miR-342-5p, miR-340, miR-339-5p, miR-335, miR-324-5p, miR-25, miR-24, miR-22, miR-221, miR-214, miR-194, miR-18b, miR-152, miR-143, miR-100, miR-let-7ev	High levels of expression in peripheral lymphocytes after treatment compared to before treatment in good responders.	[31]

ICI	Experimental Model	miRNA	Experimental Effect on miRNA	Refs.
	miRNA analysis in peripheral lymphocytes from 17 good long-responders (complete response, partial response or stable disease and progression-free survival (PFS) > 18 months) with metastatic renal cell carcinoma before and after a 4-weeks period (2 cycles) of nivolumab administration	miR-22, miR-24, miR-99a, miR-194, miR-214, miR-335, miR-339, miR-708	High expression levels in peripheral lymphocytes after treatment compared to before treatment in good responders.	
anti CTLA-4 + anti PD-1	Plasma from stage IV melanoma non-responders (13 patients), partial response (4 patients) and complete response (5 patients) before and after Ipilimumab and Nivolumab/Pembrolizumab treatment	miR-4649-3p and miR-615-3p	Increased levels in post- vs. pre-treatment in non-responders. No changes post- vs. pre-treatment in patients with partial response. Decreased levels post- vs. pre-treatment in patients with complete response.	[32]

RNAs that modulate response to ICIs" and "miRNAs modulated after response to ICIs in patients". Among them, miR-708 was the only one with the same results in the different studies analyzed. While miR-340 was related to reduced response to ICIs in some studies, in others, its expression levels (in peripheral lymphocytes) after treatment with ICIs was associated with a good response.

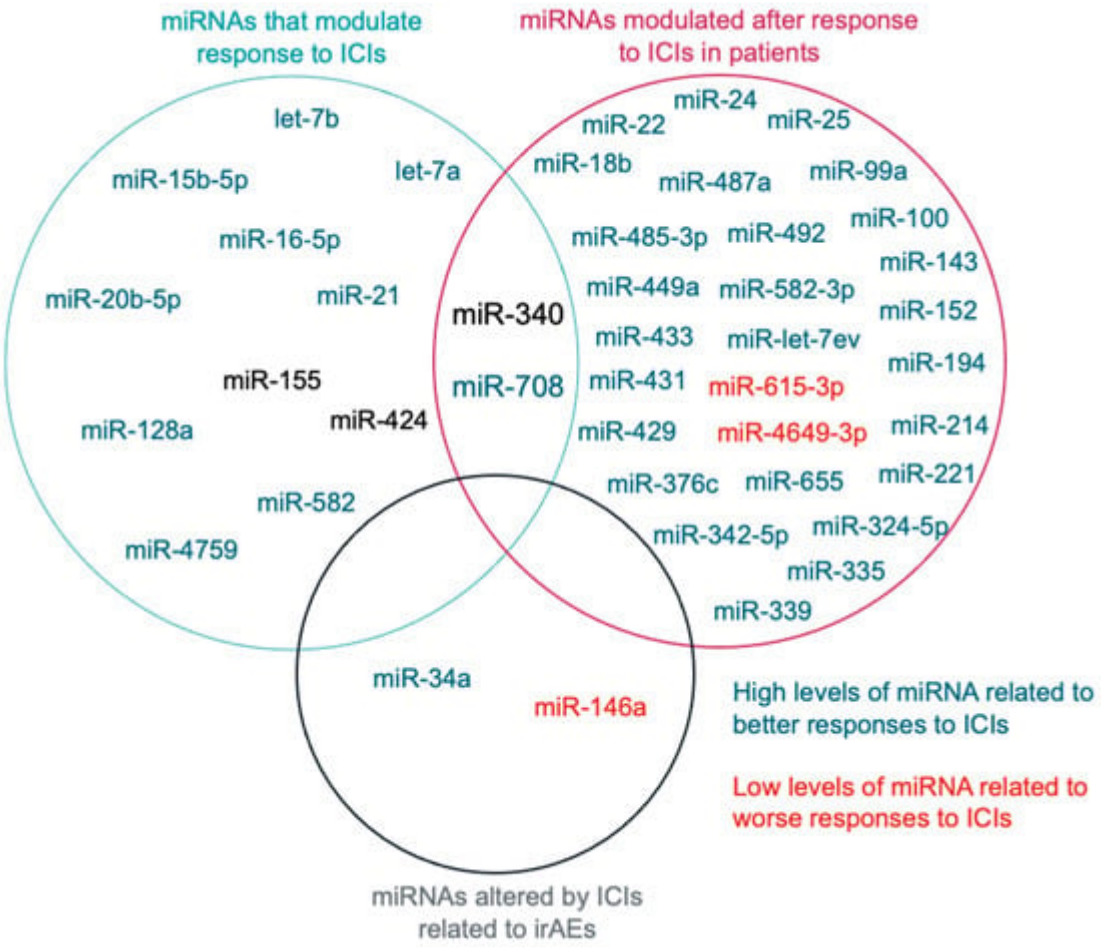

Figure 2. Venn diagram showing miRNAs that were identified as miRNAs able to modulate responses to ICI, miRNAs which were modulated after response to ICIs in patients, as well as miRNAs that can regulate irAEs.

2. miRNAs Related to Immune Checkpoint Inhibitor Response

2.1. miRNAs That Modulate Response to ICIs

Several miRNAs directly or indirectly regulate PD-L1 expression (**Table 1**). The let-7 family (let-7a, let-7b, let-7c, let-7d and let-7e) is thought to mediate tumor suppression in cancer by inhibiting tumor cell proliferation, promoting cell death evasion or metastasis [33] but also alterations to immunity. Let-7 was significantly downregulated in tissue from head and neck squamous cell carcinoma patients compared to healthy tissue [13]. Let-7 downregulation has been observed in other types of cancer associated with reduced copy numbers, such as melanoma [34], with an upregulation of LIN28A/LIN28B, which is an RNA binding protein that inhibits Drosha or Dicer binding during let-7 biogenesis (breast cancer) [35], and with DNA hypermethylation (epithelial ovarian cancer) [36].

Let-7-5p-family miRNAs have been described as negative regulators of the pro-inflammatory and tumoricidal activity of “M1” macrophage tumor-associated macrophage (TAM) phenotypes (in contrast with the M2 phenotype with immunosuppressive and tumor-promoting activity), suppressing interferon (IFN)-γ-induced and immunostimulatory macrophage programming in cancer [37], and a luciferase reporter assay has demonstrated that T Cell Factor 4 (TCF-4) was a target of let-7a/b in head and neck squamous cell carcinoma [38]. TCF-4 forms a complex with β-catenin that activates N-glycosyltransferase STT3 transcription, maintaining PD-L1 glycosylation (critical for stability), binding to PD-1 and increasing PD-L1 expression [13]. Subcutaneous mice models were created with normal or let-7a overexpressing SCC7 cells, a mouse HNSCC line, and treated with anti CTLA-4. Let-7a/b overexpression in combination with anti CTLA-4 obtained the best results in terms of higher tumor lymphocyte infiltration and reduction in tumor volume [13]. Let-7 reduced the formation of the PD-1/PD-L1 complex, which allowed a role of tumor suppressor. Other authors have shown that treatment with a LIN28 inhibitor could re-establish let-7 biogenesis, reactivating T cell activity [39].

The miR-15 family (comprising miR-15a, miR-15b, miR-16, miR-195, miR-424, miR-497 and miR-503) targeted PD-1 [40] and presented an inverse correlation with PD-L1 in pleural mesothelioma [41]. In lung adenocarcinoma, miR-16-5p serum exosomes increased in a progression-dependent manner. Therefore, treating cells with high miR-16-5p-bearing exosomes in combination with anti PD-L1 increased tumor cell death. van Zandwijk and colleagues have proposed the use of TargomiRs, miR-16 mimic loaded epidermal growth factor receptor (EGFR)-targeted minicells, to improve ICI response, but this hypothesis has not yet been tested [42].

The overexpression of miR-15b-5p in tumor cells enhanced anti PD-1 sensitivity in colorectal cancer cell lines inoculated into mice [14]. In addition, miR-15b-5p was downregulated by nuclear respiratory factor 1 (NRF1), a transcription factor enriched in colorectal cancer, which is promoted by the elevated levels of interleukin IL-17A.

Therefore, the authors proposed the co-treatment of anti-IL-17A (to reduce PD-L1 expression) and anti-PD-1 therapy to improve ICI efficacy [14][43].

miR-128a targeted Bmi1 (BMI1 proto-oncogene, polycomb ring finger), which is a major component of the polycomb group complex 1 (PRC1) and a factor that stimulates the degradation of p53, which is a gene found to be altered in a wide variety of tumors and which participates in response to immunotherapy [19]. Laryngeal cancer cells overexpressing miR-128a were treated with Pembrolizumab, resulting in greater cytotoxicity than in cells that do not overexpress miR-128a [19].

miR-4759 directly targeted and blocked PD-L1 expression, asvalidated by luciferase reporter assay. When human triple-negative breast cancer cells (MDA-MB-231 and BT-549) were transfected with miR-4759, treated with anti-PD-L1 and co-cultured with peripheral blood mononuclear cells, the in vitro cell killing assays showed enhanced tumor cytotoxicity [29].

Finally, the blockade of CD47 with miR-708 produced phagocytosis and promoted apoptosis in a human CCRF-CEM leukemic T cell line and in human T-acute lymphoblastic leukemia Jurkat cells after transfection with miR-708 mimic. In another experiment, miR-708-overexpressed CCRF-CEM cells were incubated with macrophages derived from THP-1 (human leukemia monocytic cell line) and with anti CD47 antibody. Compared with treatment with anti CD47 only, miR-708 overexpression increased phagocytosis [28].

2.2. miRNAs Modulated after Response to ICIs

Metastatic renal cell carcinoma patients with good response (complete, partial response or stable disease) to Nivolumab treatment showed increased levels of 28 miRNAs in response to treatment. Among these, the overexpression of miR-22, miR-24, miR-99a, miR-194, miR-214, miR-335, miR-339, and miR-708 was associated with progression-free survival of over 18 months [31]. In contrast, miR-4649-3p and miR-615-3p overexpression was observed in stage IV melanoma patients who progressed after anti-CTLA-4 (Ipilimumab), anti-PD-1 (Nivolumab or Pembrolizumab), or the combination of Ipilimumab and Nivolumab [32]. miR-4649-3p has been identified as a tumor suppressor in triple-negative breast cancer by targeting PIP5K1C, an Akt activator [44], while miR-615 targets the NK group 2 (NKG2) family receptor or TNF-α from immune cells, leading to diminished cytotoxic activity against tumor cells [45].

miR-708 is a miRNA with the ability to modulate response to ICIs but is also modulated by ICIs. In both cases, its overexpression improved response to ICIs, although in contrast, it exhibited a tumor-suppressor role in both renal cancer cells [46] and T-acute lymphoblastic leukemia [47] unlike what is observed in other types of acute lymphoblastic leukemia (pre-B, pro-B, common). This miRNA can thus be considered a double-edged sword (oncomiR or tumor-suppressor role depending on tumor type).

2.3. miRNAs That Regulate Immune-Related Adverse Events (irAEs)

Xia et al. [48] published two interesting papers on the role of miRNAs in irAEs. One finding was an observed increase in miR-34a levels, which targeted and degraded Krüppel-like factor 4 (KLF4), an inhibitor of the M1 genetic program, and a significant increase in M1 macrophages and pro-inflammatory cytokines after treating mice with anti PD-1. This could be a cause of reduction in left ventricular ejection fraction and left ventricular fractional shortening (the fraction of the left ventricle shortens during a cardiac cycle).

The same authors have demonstrated that macrophages secreted exosomes enriched with miR-43a after exposure to anti PD-1. These exosomes transferred miR-34a to cardiomyocytes, targeting the serine/threonine-protein phosphatase 1 regulatory subunit 10 (PNUTS) to induce cardiomyocyte senescence [49].

Genetic polymorphism rs2910164 in the *MIR146A* gene has been shown to reduce inflammamiR-146a expression and correlated with an increased risk of developing severe irAEs after ICI therapy [50][51]. In addition, miR-146a-5p induced myocardial inflammation and cardiomyocyte dysfunction in non-ICI therapy [52]. Furthermore, therapy with ICIs has been associated to increased NF-kB activity [43]. It is known that NF-kB pathway regulation is fine-tuned by miR-146, which in turn is a target of NF-kB. Interestingly, miR-146 downregulates tumor necrosis factor receptor-associated factor 6 (TRAF6) and IL-1 receptor-associated kinase 1 (IRAK1), leading to a signaling pathway involved in activating the regulatory subunit of IkB kinase (IKK), which in turn activates the expression of inflammatory genes regulated by NF-kB [53].

2.4. miRNAs Related to ICIs Response

miRNAs are molecules that change dynamically when the microenvironment changes, and they are sensitive to therapies and regulate a large number of cellular processes [54][55]. In addition, miRNAs are very stable in biological samples, which make them potential markers to be measured with several methods (i.e., small RNA-sequencing), which allow for identifying new associated miRNAs. Importantly, miRNAs can be also measured using more conventional techniques such as RT-PCR, making these molecules potential biomarkers to predict the response to therapy [56][57]. However, the fact that they are involved in many processes and miRNAs are upstream regulators of key transcriptional networks, acting as oncomiRs or tumor suppressors in different tumor types has open new avenues to use these miRNAs as potential markers in cancer.

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