MiRNA and Melanoma

Subjects: Biochemistry & Molecular Biology

Contributor: Nianhan Ma

Melanoma is the major skin cancer-related cause of death. The survival rate of meta-static melanoma is approximately 10–15%, even though many effective approaches, such as targeted therapy and immunotherapy, have gained the approval by the Food and Drug Administration (FDA) for the treatment of melanoma. Immunotherapy leads to praiseworthy benefits and improves overall survival by approximately 35–50% for the treatment of melanoma.

immunotherapy resistance miRNA biomarker

1. Introduction

Melanoma is the major skin cancer-related cause of death. The survival rate of metastatic melanoma is approximately 10–15%, even though many effective approaches, such as targeted therapy and immunotherapy, have gained the approval by the Food and Drug Administration (FDA) for the treatment of melanoma. Immunotherapy leads to praiseworthy benefits and improves overall survival by approximately 35–50% for the treatment of melanoma [1]. In the past decade, different kinds of immunotherapies have been developed. Interferon (IFN)-alpha and interleukin-2 are the early immunotherapies for advanced-stage melanoma patients [2][3][4]. However, the severe toxicity and a low percentage of long-term complete response have been observed [4]. Subsequent immunotherapy approaches have focused on monoclonal antibodies targeting immune checkpoint proteins. The first immune checkpoint inhibitors were anti-cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) agents, ipilimumab and tremelimumab. However, only ipilimumab was approved by the FDA in 2011 for melanoma treatment [5][6]. The anti-programmed death 1 (PD-1) agents, nivolumab (monotherapy or in combination with ipilimumab) and pembrolizumab (monotherapy), and the anti-programmed death ligand 1 (PD-L1) agent, atezolizumab, were rapidly developed and approved by the FDA [7][8][9][10]. The combination of ipilimumab and nivolumab has been shown to be more effective than single agents [11]. CTLA-4 and PD-1 are inhibitory receptors on activated T cells, and PD-L1 is a PD-1 ligand on tumor cells. Although immunotherapy has improved the clinical outcomes, approximately 50% of melanoma patients do not respond or acquire resistance to immune checkpoint inhibitors [8][12].

miRNAs are noncoding RNAs that regulate both transcription and translation. miRNAs modulate mRNAs by targeting 3′UTR of genes. Abnormal expression of miRNAs is commonly found in cancers. It has been shown that miRNAs mediate not only the biological functions of tumor cells but also of immune cells. Recent studies have reported the modulation of miRNAs in innate and adaptive immunity by regulating the differentiation and activation of immune cells [13]. Moreover, immunosuppressive tumor microenvironment-regulated miRNAs are related to

overall survival in melanoma patients [14]. It has been shown that miR-155 is involved in the activation or differentiation of immune cells including B cells, T cells, dendritic cells (DCs), natural killer (NK) cells, myeloid cells, and macrophages [15][16][17]. Some clinical trials involving certain miRNAs, such as miR-16 (NCT02369198), miR-29 (NCT03601052), and miR-34 (NCT01829971), or miRNA inhibitors, such as anti-miR-21 (NCT03373786), anti-miR-92a (NCT03603431), and anti-miR-122 (NCT01200420), have been conducted. Notably, a clinical trial of cobomarsen, an inhibitor of miR-155 (NCT02580552), has been designed to treat patients diagnosed with cutaneous T-cell lymphoma, chronic lymphocytic leukemia, diffuse large B-cell lymphoma, or adult T-cell leukemia/lymphoma. These findings indicated that miRNAs play a central role in tumor progression and the tumor microenvironment.

2. Mechanism of Immunotherapy Resistance in Melanoma

It was reported that loss of phosphatase and tensin homolog (PTEN) was associated with resistance to immunotherapy [18][19]. An analysis of metastatic melanoma patients treated with anti-PD-1 agents (pembrolizumab and nivolumab) showed that a lack of PTEN in melanoma patients increased tumor growth as compared to the PTEN-expressing patients by decreasing T cell functions [19]. Additionally, a PI3Kβ inhibitor enhanced the inhibitory effects of anti-PD-1 treatment in vivo. Of note, a recent study involved the collection of tumor samples from a metastatic melanoma patient over nine years. The results demonstrated that deletion of chromosome 15q, including B2M, caused loss of PTEN and cyclin-dependent kinase inhibitor 2A (CDKN2A) homozygous deletion was observed in the resistance to immunotherapy in melanoma patients [20].

It was reported that high expression of sphingosine kinase-1 (SK1), an important modulator of antitumor immunity, reduced the survival of melanoma patients (1 of mucosal, 30 of cutaneous, and 1 of other subtype) after anti-PD-1 treatment [21]. SK1-downregulated mice were more sensitive to anti-CTLA-4 or anti-PD-1 treatment than were control mice as a result of decreased infiltration of regulatory T (Treg) cells and rising CD8/Treg ratio.

The interaction between melanoma cells and the immune system is influenced by the genetic alteration of human leukocyte antigen class I (HLA-I) and antigen-processing machinery (APM) [22]. The expression of HLA-I APM components in biopsies of melanoma responsive to anti-CTLA-4 therapy was higher and survival was longer than among nonresponders [23]. Activation of the immunoreceptor RIG-I led melanoma cells to sensitize to CD8+ T cells by inducing HLA-I APM expression. Another study has shown that higher expression of IL-1R was observed in nonresponsive melanoma patients to anti-PD-1 therapy [24].

These findings indicated that melanoma cells induced different strategies to alter the IFN/STAT, PI3K/AKT, and WNT/β-catenin pathways, leading to survival under immunotherapy pressure. However, the interaction between these signaling pathways in melanoma resistance to immunotherapy is not yet understood and should be considered for further study.

3. miRNAs as Biomarkers to Predict the Response of Melanoma Patients to Immunotherapy

Immunotherapy is widely applied in the treatment of many cancers, but only a subset of patients derive benefit. Therefore, it is important to define who is suitable to receive immune checkpoint inhibitors. PD-L1 expression is the earliest and most promising predictive biomarker for anti-PD-1 therapy. Emerging evidence indicates that patients expressing high levels of PD-L1 experience an increased clinical benefit after treatment with anti-PD-1 agents. However, the definition of positive PD-L1 expression has been debated because different antibodies purified from different clones (clone 22C3 and clone 28-8) presented different evaluations. The cutoff value of PD-L1 staining was at least 5% and 1% of tumor cells by using clones 28-8 and clone 22C3, respectively [7][8]. Additionally, the expression of PD-L1 was dynamic during the course of clinical treatment [25][26]. The combination of PD-L1 status and the presence or absence of tumor-infiltrating lymphocytes has been considered as a promising biomarker for immunotherapy [27]. In addition, tumor mutation burden (TMB) has been considered to be a biomarker for immunotherapy [28][29][30]. Mutations in tumors may be translated to neoantigens that are recognized by T cells, leading to enhanced sensitivity to immunotherapy. Unfortunately, high cost and bioinformatic tools are the limitations of this assay. Moreover, the optimal cutoff value of TMB should be confirmed in different tumors. Several studies have been focused on microsatellite instability, mismatch-repair deficiency, somatic mutations, gut microbiome, human leukocyte antigen genotype, germline single-nucleotide polymorphisms, and circulating immune cells as predictive biomarkers for immunotherapy [25]. Recently, it was reported that greater aneuploidy in melanoma patients showed a poor response to immunotherapy [31]. These findings indicated that tumor aneuploidy could be considered as a potential prognostic marker for predicting the response of melanoma patients to immune checkpoint inhibitors.

Given that miRNAs have been widely studied as the biomarkers for many types of cancers, including melanoma, miR-199b-5p, miR-4488, and miR-524-5p could be considered as the predictive biomarkers for the response of melanoma patients to MAPK inhibitors [32][33]. In addition, miRNAs play a major role in the regulation of both the innate and adaptive immune systems. Therefore, it is possible to investigate miRNA-based biomarkers in response to immunotherapy. A previous study showed that adenosine deaminase acting on RNA 1 (ADAR1) was decreased in melanoma cells and that downregulation of ADAR1 supported melanoma cells to avoid tumor infiltrating lymphocyte-mediated killing by regulating intercellular adhesion molecule 1 (ICAM1) [34][35]. ICAM1 regulates the immune response through interaction with lymphocyte function-associated antigen-1 (LFA-1) that leads to T cell activation [36]. Overexpression of ADAR1 induced ICAM1 expression and blocking of ICAM1 reduced the functions of ADAR1 killing melanoma cells [35]. Overexpression of ADAR1 reduced the expression of miR-222 and miR-221, while ADAR1 knockdown increased miRNA levels. Moreover, miR-222, but not miR-221, directly targeted ICAM1. It indicated that ADAR1 regulated ICAM1 through miR-222. Importantly, miR-222 expression levels in tissues of nonresponders to ipilimumab (n = 23) were higher than those in the response group (n = 12). This result indicated that miR-222 could be considered as a prognostic marker for the response of melanoma to anti-CTLA-4 treatment (ipilimumab). Another study showed that the detection levels of five miRNAs, including let-7e, miR-99b, miR-125a, miR-125b, and miR-146b, in plasma could be predictive markers of the response of melanoma patients to ipilimumab and nivolumab [37]. Of note, higher expression of the miRNA cluster in relation to shorter progression-

free survival and overall survival was found in melanoma patients treated with ipilimumab and nivolumab. Detection of miRNAs in exosomes promises to be a beneficial cancer biomarker method. Exosomes extracted from the serum of melanoma patients (54% of cutaneous, 22.7% of mucosal, and 23.3% of other subtype) treated with pembrolizumab or from nontreated serum were used to differentiate the expression of miRNAs between the two groups [38]. The exo-miRNA serum panel, including miR-532-5p and miR-106b, was decreased in melanoma patients treated with pembrolizumab (n = 57) compared with that from the nontreated group (n = 38). The area under the curve (AUC) values of miR-532-5p and miR-106b were 0.629 and 0.682, respectively, and the combination of both miRNAs reached an AUC value of 0.735. A high population of CD8+ T cells is a prognostic marker and is related to clinical results in many kinds of cancers [39]. It was reported that CD8+ T cells showed elevated miR-155 expression in a melanoma mouse model [40]. miR-155 expression in CD8+ T cells was increased after anti-PD-1 treatment in vivo and in situ. High expression of miR-155 or low expression of its target, PTPN2, was associated with higher survival of melanoma patients. This result indicated that the miR-155 level may be considered as a biomarker of the response to immunotherapy. A microarray was used to analyze 2560 different miRNAs in the serum from melanoma patients who responded to anti-PD-1 treatment (n = 3) and from nonresponders (n = 3) [41]. Thirteen miRNAs (miR-1972, miR-4502, miR-7110-5p, miR-3064-5p, miR-4459, miR-7107-5p, miR-1180-3p, miR-6799-5p, miR-7114-5p, miR-6849-5p, miR-4701-3p, miR-4462, and miR-6875-3p) and six miRNAs (miR-451a, miR-17-5p, miR-16-5p, miR-20a-5p, miR-106a-5p, and miR-1180-5p) were selected as the nonresponse and response markers, respectively. miR-1972 and miR-4502 and miR-16-5p, miR-17-5p, miR-20a-5p, and miR-451a were chosen for further evaluation because their highest expression and their targets were related to immunity. Serum expression of miR-16-5p, miR-17-5p, and miR-20a-5p was found to be higher in melanoma responding to anti-PD-1 treatment (n = 10) than in nonresponders (n = 23). In addition, the entry reported that increased levels of miR-1972 and miR-4502 were detected in nonresponders. Another study investigated whether miR-615-3p, miR-1234-3p, and miR-4649-3p in serum were decreased in complete responders (n = 4) as compared to in partial responders (n = 4) among stage IV of melanoma patients; miR-3197 was used to distinguish stage III responders from nonresponders in pretreatment samples [42]. These findings highlighted the functions of miRNAs as prognostic biomarkers in response to immunotherapy in melanoma and are summarized in **Table 1** . In addition to melanoma, several miRNAs (miR-200b, miR-429, miR-93, miR-138-5p, miR-200, miR-27a, miR-424, miR-34a, miR-28, miR-106b, miR-193a-3p, miR-181a, miR-320d, miR-320c, and miR-320b) are also considered to be predictors of immunotherapy in lung cancer [43][44][45].

Table 1. miRNAs as predicted biomarkers for immunotherapy.

miRNA	Sample Source	Expression	Target Genes	Ref.
miR-222	Tissue	Low in clinical benefit melanoma tissues received anti-CTLA-4 (ipilimumab)	ICAM1	[35]
let-7e miR-99b miR-125a	Plasma EVs	High expression of miRNA cluster reduced the overall survival and progression-free survival of the patients treated with anti-CTLA-4 (ipilimumab) and anti-PD-1 (nivolumab)		[37]

miRNA	Sample Source	Expression	Target Genes	Ref.
miR-125b miR-146b				
miR-106b miR-532-5p	Serum	Decrease in melanoma treated with anti-PD-1 (pembrolizumab)		[38]
miR-155	Peripheral blood	Increase after treatment with anti-PD-1	PTPN2	[40]
miR-16-5p miR-17-5p miR-20a-5p	Serum	High in serum from melanoma responded to anti-PD-1 (nivolumab or pembrolizumab)		[41]
miR-1972 miR-4502	Serum	Increase in non-responders treated with anti-PD-1 (nivolumab or pembrolizumab)		[41]
miR-615-3p miR-1234-3p miR-4649-3p	Serum	Decrease in responders received anti-PD-1 (nivolumab or pembrolizumab) and anti-CTLA-4 (ipilimumab) or combination of ipilimumab and nivolumab		[42]

The above evidence demonstrates that miRNAs could be considered as effective predictors to indicate the response of cancers to immunotherapy. Circulating miRNAs would be a better choice due to noninvasive methods. However, a larger sample size should be conducted to investigate the sensitivity and specificity of these miRNAs.

4. Role of miRNAs in the Tumor Microenvironment

Tumor microenvironments are involved in the action of BRAF inhibitors including the efficacy and resistance [46][47]. It is indicated that the tumor microenvironment plays an important role in progression of melanoma. Cancer-associated fibroblasts (CAFs), DCs, T cells, macrophages, MDSCs, and NK cells are common cell populations in tumor immunity. Immune cells interact with cancer cells to regulate the tumor microenvironment including hypoxia and inflammation. In this section, we summarize the contribution of miRNAs to the major immune cells of the tumor microenvironment (**Figure 2** and **Table 2**).

DCs are the key regulators of the antitumor immune response and induce the activation and differentiation of naïve T cells by presenting antigens to naïve T cells. miR155-deficient DCs failed to activate T cells by reducing antigen presentation and cytokine production [48][49]. The p38 MAPK pathway plays a key role in regulating the maturation of DC cells via IL-10 [50]. Inhibition of miR-22 or overexpression of miR-128 enhances the tumor-suppressing role of DC cells by targeting p38 [51][52]. Melanoma tumor growth was decreased in DC-inhibited miR-22 or DC-overexpressed miR-128. Elevated expression of miR-9 was found both in bone marrow-derived dendritic cells

(BMDCs) and conventional DC1s (cDC1s), modulators of the antitumor immune response through NF-κB signaling [53]. Overexpression of miR-9 in BMDCs not only promotes DC activation and functions by targeting polycomb group ring finger 6 (PCGF6), an inhibitor of DC activation, but also activates CD4 + and CD8 + T cells. Additionally, miR-9 overexpression in DCs reduced tumor progression in a melanoma mouse model. miR-192-5p- and miR-148a-3p-derived hypoxic melanoma cells were transferred into DCs by the Cx43 channel and miR-192-5p was delivered to both DCs and T cells to inhibit their functions [54].

Several studies have focused on the roles of miRNAs in melanoma response to immunotherapy by impacting on tumor cells or immune cells. CD8+ T cells express antitumor functions and, therefore, are a top candidate for immunotherapy. It has been reported that CTLA-4, PD-1, and PDL-1 are regulated by miRNAs [55]. Growing evidence has revealed the functions of miRNAs in T cells through the regulation of key pathways and molecules related to the activation of T cells. miR-23a acts as a negative mediator of CD8+ T cells [56]. miR-23a directly targets B lymphocyte-induced maturation protein-1 (BLIMP-1), a key transcription regulator of T cell function, to attenuate the antitumor response of cytotoxic T cells by reducing granzyme B, and IFN-γ. It has been reported that PD-1 is a target of miR-28 in T cells [57]. Low levels of miR-28 increased the expression of inhibitory receptors, including PD-1, T cell immunoglobulin domain and mucin domain 3 (TIM3), and B and T lymphocyte attenuator (BTLA), which enable tumor cells to evade immune control in a melanoma mouse model. Importantly, high expression of miR-28 rescued the exhausted T cells by recovering the ability of T cells to induce cytokine production, including IL-2 and TNF-α. miR-21-deficient mice had reduced tumor size as compared to that of wild-type mice [58]. Moreover, tumor-associated macrophages (TAMs) shifted to the M1 phenotype in miR-21-/- mice and repaired the function of T cells to induce proinflammatory cytokines and cytolytic granules. miR-21 negatively regulated the IFN-γ pathway by directly targeting STAT1 and indirectly targeting JAK2. The combination of miR-21-/- TAMs and anti-PD1 significantly reduced tumor growth compared to the single treatment. miR-146 expression is known to be increased in melanoma tissues as compared to that in nevi and healthy tissues [59]. miR-146a-/- mice had longer survival than wild-type mice by inducing IFN-γ-producing T cells through activation of STAT1. Moreover, elevated PD-L1 levels were observed in miR-146a-/- mice and in melanoma cells treated with IFN-γ. The combination of anti-miR-146a and anti-PD-1 reduced melanoma tumors and prolonged the survival of the mice compared to treatment with anti-PD-1 alone. miR-146a-/- mice treated with anti-PD-1 induced immune-related adverse events (irAEs) as opposed to the wild-type group [60]. It has been shown that a better clinical response to immune checkpoint inhibitors correlates with the induction of irAEs [61][62]. Enhanced accumulation of CD4+ and CD8+ T cells and increased inflammatory cytokines are involved in irAEs [63]. More activated T cells, CD4+ T cells, and neutrophil recruitment have been observed in miR-146a-/- mice treated with anti-PD-1. It has been demonstrated that miR-155 is a core modulator of the immune response [48]. miR-155 expression is high in T cells, B cells, DCs, and macrophages [64]. Overexpression of miR-155 in CD8+ T cells enhances the antitumor response by reducing the expression of signal transducer and activator of transcription 5 (STAT5), Src homology-2 domain-containing inositol 5-phosphatase 1 (SHIP1), SOCS1, and PTPN2 [65]. Loss of miR-155 in T cells leads to reduced antitumor immunity in a melanoma mouse model by decreasing the activated T cell response and increasing the population of myeloid cells [66][67][68]. Moreover, miR-155 T cell-conditional knockout mice exhibit enhanced tumor growth and reduced IFN-γ-expressing CD4+ and CD8+ T cells [67]. Additionally, high expression of miR-155

prolongs the survival of skin cutaneous melanoma [66]. Moreover, miR-155 acts as a tumor suppressor in melanoma [69][70][71]. Interestingly, immunotherapy rescues the deficient antitumor response caused by the lack of miR-155 in T cells by reducing miR-155 target genes, including SOCS1, BTB domain and CNC homolog 1 (BACH1), CCAAT enhancer binding protein beta (CEBPB), and interleukin 7 receptor (IL7R) [67]. It has been reported that T cell receptor signaling induces NF-κB and activator protein 1 (AP-1), which binds to the miR-155 promoter to increase its expression [72][73]. A previous study demonstrated that miR-498 and miR-3187-3p in melanoma-derived exosomes reduce the functions of CD8+ T cells by targeting IFN-α and protein tyrosine phosphatase receptor type C (PTPRC), the coding gene for CD45, respectively [74]. Hypoxic melanoma cells deliver miR-192-5p to cytotoxic T cells via Connexin-43 (Cx43)-constituted gap junctions to reduce the T cell functioning [54].

References

1. Weiss, S.A.; Wolchok, J.D.; Sznol, M. Immunotherapy of Melanoma: Facts and Hopes. Clin. Cancer Res. 2019, 25, 5191–5201.
2. Kirkwood, J.M.; Ibrahim, J.G.; Sosman, J.A.; Sondak, V.K.; Agarwala, S.S.; Ernstoff, M.S.; Rao, U. High-dose interferon alfa-2b significantly prolongs relapse-free and overall survival compared with the GM2-KLH/QS-21 vaccine in patients with resected stage IIB-III melanoma: Results of intergroup trial E1694/S9512/C509801. J. Clin. Oncol. 2001, 19, 2370–2380.
3. Kirkwood, J.M.; Strawderman, M.H.; Ernstoff, M.S.; Smith, T.J.; Borden, E.C.; Blum, R.H. Interferon alfa-2b adjuvant therapy of high-risk resected cutaneous melanoma: The Eastern Cooperative Oncology Group Trial EST 1684. J. Clin. Oncol. 1996, 14, 7–17.
4. Coit, D.G.; Andtbacka, R.; Bichakjian, C.K.; Dilawari, R.A.; Dimaio, D.; Guild, V.; Halpern, A.C.; Hodi, F.S.; Kashani-Sabet, M.; Lange, J.R.; et al. Melanoma. J. Natl. Compr. Cancer Netw. 2009, 7, 250–275.
5. Hodi, F.S.; O'Day, S.J.; McDermott, D.F.; Weber, R.W.; Sosman, J.A.; Haanen, J.B.; Gonzalez, R.; Robert, C.; Schadendorf, D.; Hassel, J.C.; et al. Improved survival with ipilimumab in patients with metastatic melanoma. N. Engl. J. Med. 2010, 363, 711–723.
6. Mansh, M. Ipilimumab and cancer immunotherapy: A new hope for advanced stage melanoma. Yale J. Biol. Med. 2011, 84, 381–389.
7. Larkin, J.; Chiarion-Sileni, V.; Gonzalez, R.; Grob, J.J.; Cowey, C.L.; Lao, C.D.; Schadendorf, D.; Dummer, R.; Smylie, M.; Rutkowski, P.; et al. Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. N. Engl. J. Med. 2015, 373, 23–34.
8. Schachter, J.; Ribas, A.; Long, G.V.; Arance, A.; Grob, J.J.; Mortier, L.; Daud, A.; Carlino, M.S.; McNeil, C.; Lotem, M.; et al. Pembrolizumab versus ipilimumab for advanced melanoma: Final

overall survival results of a multicentre, randomised, open-label phase 3 study (KEYNOTE-006). Lancet 2017, 390, 1853–1862.

9. Luke, J.J.; Flaherty, K.T.; Ribas, A.; Long, G.V. Targeted agents and immunotherapies: Optimizing outcomes in melanoma. Nat. Rev. Clin. Oncol. 2017, 14, 463–482.
10. Herbst, R.S.; Soria, J.C.; Kowanetz, M.; Fine, G.D.; Hamid, O.; Gordon, M.S.; Sosman, J.A.; McDermott, D.F.; Powderly, J.D.; Gettinger, S.N.; et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. Nature 2014, 515, 563–567.
11. Hodi, F.S.; Chiarion-Sileni, V.; Gonzalez, R.; Grob, J.J.; Rutkowski, P.; Cowey, C.L.; Lao, C.D.; Schadendorf, D.; Wagstaff, J.; Dummer, R.; et al. Nivolumab plus ipilimumab or nivolumab alone versus ipilimumab alone in advanced melanoma (CheckMate 067): 4-year outcomes of a multicentre, randomised, phase 3 trial. Lancet Oncol. 2018, 19, 1480–1492.
12. Wolchok, J.D.; Chiarion-Sileni, V.; Gonzalez, R.; Rutkowski, P.; Grob, J.J.; Cowey, C.L.; Lao, C.D.; Wagstaff, J.; Schadendorf, D.; Ferrucci, P.F.; et al. Overall Survival with Combined Nivolumab and Ipilimumab in Advanced Melanoma. N. Engl. J. Med. 2017, 377, 1345–1356.
13. Paladini, L.; Fabris, L.; Bottai, G.; Raschioni, C.; Calin, G.A.; Santarpia, L. Targeting microRNAs as key modulators of tumor immune response. J. Exp. Clin. Cancer Res. 2016, 35, 103.
14. Jorge, N.A.N.; Cruz, J.G.V.; Pretti, M.A.M.; Bonamino, M.H.; Possik, P.A.; Boroni, M. Poor clinical outcome in metastatic melanoma is associated with a microRNA-modulated immunosuppressive tumor microenvironment. J. Transl. Med. 2020, 18, 56.
15. Mashima, R. Physiological roles of miR-155. Immunology 2015, 145, 323–333.
16. Trotta, R.; Chen, L.; Ciarlariello, D.; Josyula, S.; Mao, C.; Costinean, S.; Yu, L.; Butchar, J.P.; Tridandapani, S.; Croce, C.M.; et al. miR-155 regulates IFN-gamma production in natural killer cells. Blood 2012, 119, 3478–3485.
17. Tili, E.; Croce, C.M.; Michaille, J.J. miR-155: On the crosstalk between inflammation and cancer. Int. Rev. Immunol. 2009, 28, 264–284.
18. Trujillo, J.A.; Luke, J.J.; Zha, Y.; Segal, J.P.; Ritterhouse, L.L.; Spranger, S.; Matijevich, K.; Gajewski, T.F. Secondary resistance to immunotherapy associated with beta-catenin pathway activation or PTEN loss in metastatic melanoma. J. Immunother. Cancer 2019, 7, 295.
19. Peng, W.; Chen, J.Q.; Liu, C.; Malu, S.; Creasy, C.; Tetzlaff, M.T.; Xu, C.; McKenzie, J.A.; Zhang, C.; Liang, X.; et al. Loss of PTEN Promotes Resistance to T Cell-Mediated Immunotherapy. Cancer Discov. 2016, 6, 202–216.
20. Liu, D.; Lin, J.R.; Robitschek, E.J.; Kasumova, G.G.; Heyde, A.; Shi, A.; Kraya, A.; Zhang, G.; Moll, T.; Frederick, D.T.; et al. Evolution of delayed resistance to immunotherapy in a melanoma responder. Nat. Med. 2021, 27, 985–992.

21. Imbert, C.; Montfort, A.; Fraisse, M.; Marcheteau, E.; Gilhodes, J.; Martin, E.; Bertrand, F.; Marcellin, M.; Burlet-Schiltz, O.; Peredo, A.G.; et al. Resistance of melanoma to immune checkpoint inhibitors is overcome by targeting the sphingosine kinase-1. Nat. Commun. 2020, 11, 437.

22. Anichini, A.; Mortarini, R.; Nonaka, D.; Molla, A.; Vegetti, C.; Montaldi, E.; Wang, X.; Ferrone, S. Association of antigen-processing machinery and HLA antigen phenotype of melanoma cells with survival in American Joint Committee on Cancer stage III and IV melanoma patients. Cancer Res. 2006, 66, 6405–6411.

23. Such, L.; Zhao, F.; Liu, D.; Thier, B.; Le-Trilling, V.T.K.; Sucker, A.; Coch, C.; Pieper, N.; Howe, S.; Bhat, H.; et al. Targeting the innate immunoreceptor RIG-I overcomes melanoma-intrinsic resistance to T cell immunotherapy. J. Clin. Investig. 2020, 130, 4266–4281.

24. Singh, S.; Xiao, Z.; Bavisi, K.; Roszik, J.; Melendez, B.D.; Wang, Z.; Cantwell, M.J.; Davis, R.E.; Lizee, G.; Hwu, P.; et al. IL-1alpha Mediates Innate and Acquired Resistance to Immunotherapy in Melanoma. J. Immunol. 2021, 206, 1966–1975.

25. Tray, N.; Weber, J.S.; Adams, S. Predictive Biomarkers for Checkpoint Immunotherapy: Current Status and Challenges for Clinical Application. Cancer Immunol. Res. 2018, 6, 1122–1128.

26. Vilain, R.E.; Menzies, A.M.; Wilmott, J.S.; Kakavand, H.; Madore, J.; Guminski, A.; Liniker, E.; Kong, B.Y.; Cooper, A.J.; Howle, J.R.; et al. Dynamic Changes in PD-L1 Expression and Immune Infiltrates Early During Treatment Predict Response to PD-1 Blockade in Melanoma. Clin. Cancer Res. 2017, 23, 5024–5033.

27. Teng, M.W.; Ngiow, S.F.; Ribas, A.; Smyth, M.J. Classifying Cancers Based on T-cell Infiltration and PD-L1. Cancer Res. 2015, 75, 2139–2145.

28. Snyder, A.; Makarov, V.; Merghoub, T.; Yuan, J.; Zaretsky, J.M.; Desrichard, A.; Walsh, L.A.; Postow, M.A.; Wong, P.; Ho, T.S.; et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. N. Engl. J. Med. 2014, 371, 2189–2199.

29. Van Allen, E.M.; Miao, D.; Schilling, B.; Shukla, S.A.; Blank, C.; Zimmer, L.; Sucker, A.; Hillen, U.; Foppen, M.H.G.; Goldinger, S.M.; et al. Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. Science 2015, 350, 207–211.

30. Rizvi, N.A.; Hellmann, M.D.; Snyder, A.; Kvistborg, P.; Makarov, V.; Havel, J.J.; Lee, W.; Yuan, J.; Wong, P.; Ho, T.S.; et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science 2015, 348, 124–128.

31. Davoli, T.; Uno, H.; Wooten, E.C.; Elledge, S.J. Tumor aneuploidy correlates with markers of immune evasion and with reduced response to immunotherapy. Science 2017, 355.

32. Fattore, L.; Ruggiero, C.F.; Pisanu, M.E.; Liguoro, D.; Cerri, A.; Costantini, S.; Capone, F.; Acunzo, M.; Romano, G.; Nigita, G.; et al. Reprogramming miRNAs global expression

orchestrates development of drug resistance in BRAF mutated melanoma. Cell Death Differ. 2019, 26, 1267–1282.

33. Nguyen, M.T.; Lin, C.H.; Liu, S.M.; Miyashita, A.; Ihn, H.; Lin, H.; Ng, C.H.; Tsai, J.C.; Chen, M.H.; Tsai, M.S.; et al. miR-524-5p reduces the progression of the BRAF inhibitor-resistant melanoma. Neoplasia 2020, 22, 789–799.
34. Nemlich, Y.; Greenberg, E.; Ortenberg, R.; Besser, M.J.; Barshack, I.; Jacob-Hirsch, J.; Jacoby, E.; Eyal, E.; Rivkin, L.; Prieto, V.G.; et al. MicroRNA-mediated loss of ADAR1 in metastatic melanoma promotes tumor growth. J. Clin. Investig. 2013, 123, 2703–2718.
35. Galore-Haskel, G.; Nemlich, Y.; Greenberg, E.; Ashkenazi, S.; Hakim, M.; Itzhaki, O.; Shoshani, N.; Shapira-Fromer, R.; Ben-Ami, E.; Ofek, E.; et al. A novel immune resistance mechanism of melanoma cells controlled by the ADAR1 enzyme. Oncotarget 2015, 6, 28999–29015.
36. Bachmann, M.F.; McKall-Faienza, K.; Schmits, R.; Bouchard, D.; Beach, J.; Speiser, D.E.; Mak, T.W.; Ohashi, P.S. Distinct roles for LFA-1 and CD28 during activation of naive T cells: Adhesion versus costimulation. Immunity 1997, 7, 549–557.
37. Huber, V.; Vallacchi, V.; Fleming, V.; Hu, X.; Cova, A.; Dugo, M.; Shahaj, E.; Sulsenti, R.; Vergani, E.; Filipazzi, P.; et al. Tumor-derived microRNAs induce myeloid suppressor cells and predict immunotherapy resistance in melanoma. J. Clin. Investig. 2018, 128, 5505–5516.
38. Tengda, L.; Shuping, L.; Mingli, G.; Jie, G.; Yun, L.; Weiwei, Z.; Anmei, D. Serum exosomal microRNAs as potent circulating biomarkers for melanoma. Melanoma Res. 2018, 28, 295–303.
39. Fridman, W.H.; Pages, F.; Sautes-Fridman, C.; Galon, J. The immune contexture in human tumours: Impact on clinical outcome. Nat. Rev. Cancer 2012, 12, 298–306.
40. Martinez-Usatorre, A.; Sempere, L.F.; Carmona, S.J.; Carretero-Iglesia, L.; Monnot, G.; Speiser, D.E.; Rufer, N.; Donda, A.; Zehn, D.; Jandus, C.; et al. MicroRNA-155 Expression Is Enhanced by T-cell Receptor Stimulation Strength and Correlates with Improved Tumor Control in Melanoma. Cancer Immunol. Res. 2019, 7, 1013–1024.
41. Nakahara, S.; Fukushima, S.; Okada, E.; Morinaga, J.; Kubo, Y.; Tokuzumi, A.; Matsumoto, S.; Tsuruta-Kadohisa, M.; Kimura, T.; Kuriyama, H.; et al. MicroRNAs that predict the effectiveness of anti-PD-1 therapies in patients with advanced melanoma. J. Dermatol. Sci. 2020, 97, 77–79.
42. Bustos, M.A.; Gross, R.; Rahimzadeh, N.; Cole, H.; Tran, L.T.; Tran, K.D.; Takeshima, L.; Stern, S.L.; O'Day, S.; Hoon, D.S.B. A Pilot Study Comparing the Efficacy of Lactate Dehydrogenase Levels Versus Circulating Cell-Free microRNAs in Monitoring Responses to Checkpoint Inhibitor Immunotherapy in Metastatic Melanoma Patients. Cancers 2020, 12, 3361.
43. Grenda, A.; Krawczyk, P.; Blach, J.; Chmielewska, I.; Kubiatowski, T.; Kieszko, S.; Wojas-Krawczyk, K.; Kucharczyk, T.; Jarosz, B.; Pasnik, I.; et al. Tissue MicroRNA Expression as a Predictor of Response to Immunotherapy in NSCLC Patients. Front. Oncol. 2020, 10, 563613.

44. Fan, J.; Yin, Z.; Xu, J.; Wu, F.; Huang, Q.; Yang, L.; Jin, Y.; Yang, G. Circulating microRNAs predict the response to anti-PD-1 therapy in non-small cell lung cancer. Genomics 2020, 112, 2063–2071.

45. Peng, X.X.; Yu, R.; Wu, X.; Wu, S.Y.; Pi, C.; Chen, Z.H.; Zhang, X.C.; Gao, C.Y.; Shao, Y.W.; Liu, L.; et al. Correlation of plasma exosomal microRNAs with the efficacy of immunotherapy in EGFR / ALK wild-type advanced non-small cell lung cancer. J. Immunother. Cancer 2020, 8, e000376.

46. Proietti, I.; Skroza, N.; Michelini, S.; Mambrin, A.; Balduzzi, V.; Bernardini, N.; Marchesiello, A.; Tolino, E.; Volpe, S.; Maddalena, P.; et al. BRAF Inhibitors: Molecular Targeting and Immunomodulatory Actions. Cancers 2020, 12, 1823.

47. Proietti, I.; Skroza, N.; Bernardini, N.; Tolino, E.; Balduzzi, V.; Marchesiello, A.; Michelini, S.; Volpe, S.; Mambrin, A.; Mangino, G.; et al. Mechanisms of Acquired BRAF Inhibitor Resistance in Melanoma: A Systematic Review. Cancers 2020, 12, 2801.

48. Rodriguez, A.; Vigorito, E.; Clare, S.; Warren, M.V.; Couttet, P.; Soond, D.R.; van Dongen, S.; Grocock, R.J.; Das, P.P.; Miska, E.A.; et al. Requirement of bic/microRNA-155 for normal immune function. Science 2007, 316, 608–611.

49. Lu, C.; Huang, X.; Zhang, X.; Roensch, K.; Cao, Q.; Nakayama, K.I.; Blazar, B.R.; Zeng, Y.; Zhou, X. miR-221 and miR-155 regulate human dendritic cell development, apoptosis, and IL-12 production through targeting of p27kip1, KPC1, and SOCS-1. Blood 2011, 117, 4293–4303.

50. Sato, K.; Nagayama, H.; Tadokoro, K.; Juji, T.; Takahashi, T.A. Extracellular signal-regulated kinase, stress-activated protein kinase/c-Jun N-terminal kinase, and p38mapk are involved in IL-10-mediated selective repression of TNF-alpha-induced activation and maturation of human peripheral blood monocyte-derived dendritic cells. J. Immunol. 1999, 162, 3865–3872.

51. Liang, X.; Liu, Y.; Mei, S.; Zhang, M.; Xin, J.; Zhang, Y.; Yang, R. MicroRNA-22 impairs anti-tumor ability of dendritic cells by targeting p38. PLoS ONE 2015, 10, e0121510.

52. Liang, X.; Shangguan, W.; Zhang, M.; Mei, S.; Wang, L.; Yang, R. miR-128 enhances dendritic cell-mediated anti-tumor immunity via targeting of p38. Mol. Med. Rep. 2017, 16, 1307–1313.

53. Cordeiro, B.; Jeon, P.; Boukhaled, G.M.; Corrado, M.; Lapohos, O.; Roy, D.G.; Williams, K.; Jones, R.G.; Gruenheid, S.; Sagan, S.M.; et al. MicroRNA-9 Fine-Tunes Dendritic Cell Function by Suppressing Negative Regulators in a Cell-Type-Specific Manner. Cell Rep. 2020, 31, 107585.

54. Tittarelli, A.; Navarrete, M.; Lizana, M.; Hofmann-Vega, F.; Salazar-Onfray, F. Hypoxic Melanoma Cells Deliver microRNAs to Dendritic Cells and Cytotoxic T Lymphocytes through Connexin-43 Channels. Int. J. Mol. Sci. 2020, 21, 7567.

55. Omar, H.A.; El-Serafi, A.T.; Hersi, F.; Arafa, E.A.; Zaher, D.M.; Madkour, M.; Arab, H.H.; Tolba, M.F. Immunomodulatory MicroRNAs in cancer: Targeting immune checkpoints and the tumor microenvironment. FEBS J. 2019, 286, 3540–3557.

56. Lin, R.; Chen, L.; Chen, G.; Hu, C.; Jiang, S.; Sevilla, J.; Wan, Y.; Sampson, J.H.; Zhu, B.; Li, Q.J. Targeting miR-23a in CD8+ cytotoxic T lymphocytes prevents tumor-dependent immunosuppression. J. Clin. Investig. 2014, 124, 5352–5367.

57. Li, Q.; Johnston, N.; Zheng, X.; Wang, H.; Zhang, X.; Gao, D.; Min, W. miR-28 modulates exhaustive differentiation of T cells through silencing programmed cell death-1 and regulating cytokine secretion. Oncotarget 2016, 7, 53735–53750.

58. Xi, J.; Huang, Q.; Wang, L.; Ma, X.; Deng, Q.; Kumar, M.; Zhou, Z.; Li, L.; Zeng, Z.; Young, K.H.; et al. miR-21 depletion in macrophages promotes tumoricidal polarization and enhances PD-1 immunotherapy. Oncogene 2018, 37, 3151–3165.

59. Mastroianni, J.; Stickel, N.; Andrlova, H.; Hanke, K.; Melchinger, W.; Duquesne, S.; Schmidt, D.; Falk, M.; Andrieux, G.; Pfeifer, D.; et al. miR-146a Controls Immune Response in the Melanoma Microenvironment. Cancer Res. 2019, 79, 183–195.

60. Marschner, D.; Falk, M.; Javorniczky, N.R.; Hanke-Muller, K.; Rawluk, J.; Schmitt-Graeff, A.; Simonetta, F.; Haring, E.; Dicks, S.; Ku, M.; et al. MicroRNA-146a regulates immune-related adverse events caused by immune checkpoint inhibitors. JCI Insight 2020, 5, e132334.

61. Ricciuti, B.; Genova, C.; De Giglio, A.; Bassanelli, M.; Dal Bello, M.G.; Metro, G.; Brambilla, M.; Baglivo, S.; Grossi, F.; Chiari, R. Impact of immune-related adverse events on survival in patients with advanced non-small cell lung cancer treated with nivolumab: Long-term outcomes from a multi-institutional analysis. J. Cancer Res. Clin. Oncol. 2019, 145, 479–485.

62. Indini, A.; Di Guardo, L.; Cimminiello, C.; Prisciandaro, M.; Randon, G.; De Braud, F.; Del Vecchio, M. Immune-related adverse events correlate with improved survival in patients undergoing anti-PD1 immunotherapy for metastatic melanoma. J. Cancer Res. Clin. Oncol. 2019, 145, 511–521.

63. Kaehler, K.C.; Piel, S.; Livingstone, E.; Schilling, B.; Hauschild, A.; Schadendorf, D. Update on immunologic therapy with anti-CTLA-4 antibodies in melanoma: Identification of clinical and biological response patterns, immune-related adverse events, and their management. Semin. Oncol. 2010, 37, 485–498.

64. Hsin, J.P.; Lu, Y.; Loeb, G.B.; Leslie, C.S.; Rudensky, A.Y. The effect of cellular context on miR-155-mediated gene regulation in four major immune cell types. Nat. Immunol. 2018, 19, 1137–1145.

65. Ji, Y.; Wrzesinski, C.; Yu, Z.; Hu, J.; Gautam, S.; Hawk, N.V.; Telford, W.G.; Palmer, D.C.; Franco, Z.; Sukumar, M.; et al. miR-155 augments CD8+ T-cell antitumor activity in lymphoreplete hosts by enhancing responsiveness to homeostatic gammac cytokines. Proc. Natl. Acad. Sci. USA 2015, 112, 476–481.

66. Ekiz, H.A.; Huffaker, T.B.; Grossmann, A.H.; Stephens, W.Z.; Williams, M.A.; Round, J.L.; O'Connell, R.M. MicroRNA-155 coordinates the immunological landscape within murine

melanoma and correlates with immunity in human cancers. JCI Insight 2019, 4, e126543.

67. Huffaker, T.B.; Lee, S.H.; Tang, W.W.; Wallace, J.A.; Alexander, M.; Runtsch, M.C.; Larsen, D.K.; Thompson, J.; Ramstead, A.G.; Voth, W.P.; et al. Antitumor immunity is defective in T cell-specific microRNA-155-deficient mice and is rescued by immune checkpoint blockade. J. Biol. Chem. 2017, 292, 18530–18541.
68. Dudda, J.C.; Salaun, B.; Ji, Y.; Palmer, D.C.; Monnot, G.C.; Merck, E.; Boudousquie, C.; Utzschneider, D.T.; Escobar, T.M.; Perret, R.; et al. MicroRNA-155 is required for effector CD8+ T cell responses to virus infection and cancer. Immunity 2013, 38, 742–753.
69. Levati, L.; Pagani, E.; Romani, S.; Castiglia, D.; Piccinni, E.; Covaciu, C.; Caporaso, P.; Bondanza, S.; Antonetti, F.R.; Bonmassar, E.; et al. MicroRNA-155 targets the SKI gene in human melanoma cell lines. Pigment. Cell Melanoma Res. 2011, 24, 538–550.
70. DiSano, J.A.; Huffnagle, I.; Gowda, R.; Spiegelman, V.S.; Robertson, G.P.; Pameijer, C.R. Loss of miR-155 upregulates WEE1 in metastatic melanoma. Melanoma Res. 2019, 29, 216–219.
71. Li, H.; Song, J.B.; Chen, H.X.; Wang, Q.Q.; Meng, L.X.; Li, Y. MiR-155 inhibits proliferation, invasion and migration of melanoma via targeting CBL. Eur. Rev. Med. Pharmacol. Sci. 2019, 23, 9525–9534.
72. Yin, Q.; Wang, X.; McBride, J.; Fewell, C.; Flemington, E. B-cell receptor activation induces BIC/miR-155 expression through a conserved AP-1 element. J. Biol. Chem. 2008, 283, 2654–2662.
73. Yang, J.; Zhang, P.; Krishna, S.; Wang, J.; Lin, X.; Huang, H.; Xie, D.; Gorentla, B.; Huang, R.; Gao, J.; et al. Unexpected positive control of NFkappaB and miR-155 by DGKalpha and zeta ensures effector and memory CD8+ T cell differentiation. Oncotarget 2016, 7, 33744–33764.
74. Vignard, V.; Labbe, M.; Marec, N.; Andre-Gregoire, G.; Jouand, N.; Fonteneau, J.F.; Labarriere, N.; Fradin, D. MicroRNAs in Tumor Exosomes Drive Immune Escape in Melanoma. Cancer Immunol. Res. 2020, 8, 255–267.