ORIGINAL PAPER

The Potential of Circulating miR-193b, miR-146b-3p and miR-483-3p as Noninvasive Biomarkers in Cutaneous Melanoma Patients

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Abstract

Melanoma is a destructive skin disease with few therapeutic options in the developed stage and therefore there is a critical need for reliable biomarkers for early diagnosis. In this context, microRNAs could play an important role as diagnostic biomarkers. Three datasets with accession numbers GSE31568, GSE61741 and GSE20994 were downloaded from the Gene Expression Omnibus (GEO) database. MATLAB software was used to analyze differentially expressed miRNAs between cutaneous melanoma plasma samples and normal plasma samples (control). Plasma levels of miR-193b, miR-146b-3p and miR-483-3p were evaluated by the RT-PCR method. Furthermore, linear regression followed by receiver operating characteristic analyses was performed to estimate whether selected plasma miRNAs were able to distinguish between cases and controls. Finally, the data were analyzed by unpaired Mann–Whitney U test using Graph pad prism 8 computer software. Specifically, miR-193b and miR-146b-3p were downregulated in the plasma of melanoma patients compared with control groups which were decreased 5×10^6-fold in miR-193b and 58-fold in miR-146b-3p, while miR-483-3p was upregulated 3.5-fold. After receiver operating characteristic (ROC) curve analysis, miR-193b with the most area under the curve (AUC: 1.00, 95% confidence interval 1.00–1.00, $p < 0.0001$) had the best discriminatory power, and miR-146b-3p had the large area under the curve (AUC: 0.96, 95% confidence interval 0.96–1.00, $p < 0.0001$) and consequently the high discriminatory power. Between these three miRNAs, miR-193b and miR-146b-3p had a high capacity to distinguish between melanoma patients and control groups that are appropriate to be applied in melanoma diagnosis as an early and noninvasive method.

Keywords Melanoma · MiRNA · System biology · Plasma · Diagnosis · Biomarker

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Introduction

Melanoma is a malignant tumor that exhibits the most serious clinical symptoms and the highest mortality rate among skin cancers [1–3]. The incidence of melanoma cases and deaths increased in 204 countries from 1990 to 2019 [1]. Melanoma represents only 1–2% of all cancer cases in the world and less than 5% of skin cancers but is responsible for approximately 95% of skin cancer deaths [4].

There are three major types of skin cancer: basal cell carcinoma, squamous cell carcinoma and melanoma [5]. Melanocytes in the human skin are able to produce melanin, and altered melanocyte proliferation causes melanoma [6, 7]. The process of melanoma genesis is a linear progression of normal melanocytes through various precursor lesions and ultimately to melanoma [7].

Currently, the diagnosis of melanoma requires removal and analysis of primary melanoma and to determine the presence and stage of metastatic disease when high-risk markers are detected, a sentinel lymph node biopsy is required [8, 9]. This is invasive, costly and time-consuming. Consequently, given the clinical importance of melanoma, some laboratory methods are noninvasive, allowing economical, rapid and repetitive sampling and thus enabling early intervention and dynamic treatment management. Much effort has been put into identifying blood-based biomarkers [8, 9]. Circulating microRNAs (miRNAs) have emerged as potential noninvasive biomarkers for melanoma. To date, several efforts have been made to identify circulating miRNA biomarkers for the diagnostic and prognostic usefulness of melanoma [10].

MiRNAs are stable and easily detectable molecules in biological fluids that appear as potential candidate biomarkers [10]. MiRNAs are a type of small, single-stranded, non-coding RNAs with a length of 18–25 nucleotides that can play the role of oncogenes and tumor suppressors [11, 12]. MiRNAs can be released into the human serum or plasma and can be identified in melanoma cells. Studies evaluating serum or plasma miRNAs as biomarkers in skin melanoma have focused on discriminating melanoma patients from control groups [11, 13].

The aberrant expression of miRNA has been detected in tumors and biofluids [14]. Using molecular biomarkers will suggest the potential to determine melanoma before it is evidently visible or symptomatic, and alleviate easy detection without even minimum surgical operation [15, 16].

In our study, system biology and bioinformatics approaches were used to analyze gene expression data to determine differentially expressed microRNAs (DEMIRs) between control and cutaneous melanoma plasma samples.

Three datasets were acquired from the Gene Expression Omnibus (GEO) database of the National Center for Biotechnology Information (NCBI) to identify miRNAs that were differentially expressed that could serve as potential biomarkers for melanoma diagnosis.

We aimed to study the expression of three miRNAs, miR-193b-3p, miR-146b-3p and miR-483-3p in melanoma cancer. We quantified the expression levels of these three miRNAs by stem-loop real-time PCR, which detects miRNA expression levels with higher specificity and sensitivity than conventional detection methods [17, 18]. Twenty-seven normal and paired melanoma cancer specimens were selected for this study. In addition, we analyzed differences in miRNA expression between melanoma patients and control groups.

Materials and Methods

Dataset Analysis

MiRNA expression microarray data related to cutaneous melanoma were downloaded from the Gene Expression Omnibus (GEO) database. The following keywords were used in the GEO database: cutaneous OR skin melanoma AND miRNA expression AND human [organism]. The microarray data platform was Febit Homo sapiens miRbase 13.0 (GPL9040) for three datasets.

We preprocessed and analyzed microarray raw data using MATLAB software (R2014b). First, preprocessing of miRNA expression raw data was performed by transferring all miRNA expression values to log2, and by indicating the normalization step (using the quantile norm function in MATLAB). Then, p-values for two different datasets (control vs. cancer) were estimated using the mat test function in MATLAB [18].

Finally, fold change values were derived based on expression data and p-value. This procedure was repeated for cancer and control sets of data.

Differentially expressed (DE) analysis was performed by comparing miRNA expression between melanoma and control plasma. A volcano plot was drawn to show fold change and p-values of DEMIRNAs between melanoma patients and control groups. Remarkably up- and downregulated miRNAs were described as those with an adjusted p-value < 0.05 and a fold change greater than 2, log2 (fold change) > 1 or < -1 for up- and downregulated miRNAs, respectively [19].

Study Population

The statistical population was obtained by the Cochran formula of 27 melanoma patients and 27 control groups.

Blood samples of melanoma patients and control groups were collected from Imam Khomeini hospital complex and

Razi hospital of Tehran. All procedures performed in studies involving human participants were in accordance with the ethical principles and the national norms and standards for conducting medical research in iran. Approval ID: IR.TUMS.MEDICINE.REC.1400.453, evaluated by Research Ethics Committees of School of Medicine—Tehran University of Medical Sciences. Samples were collected regardless of age and gender, melanoma patients are from stages I to IV. All patients had histological and pathological tests that confirmed melanoma (Table 1).

Samples were collected from the hospital in the form of blood in the EDTA tube. Then blood samples were quickly transferred from the hospital to the laboratory, centrifuged at 7000×g for 10 min, and then plasma isolated and stored at − 70 °C until further use.

RNA Extraction

Total RNA was extracted using the RNX plus™ kit (Cinna-Gen, Tehran, Iran. Cat No: EX6101) based on the manufacturer's recommendations. Then, 300 μL of the serum sample was homogenized with 200 μL of the RNX-PLUS solution and incubated at room temperature for 5 min. Chloroform (200 μL) was added to the solution and centrifuged at 4 °C at 10,000×g for 20 min. The supernatant was transferred to another tube and an equal volume of isopropanol was added. The mixture was incubated at − 20 °C for 30 min. Then the mixture was centrifuged at 4 °C at 10,000×g for 20 min and the resulting pellet was washed in ethanol (75%) and centrifuged at 4 °C at 7500×g for 8 min. The supernatant was discarded and the pellet was left to dry at room temperature for a few minutes then dissolved in 40 μL Diethyl pyro carbonate (DEPC)-treated water. The purity and the integrity of the extracted RNA were evaluated by optical density measurements and visual observation of sample electrophoresis on 2% agarose gel and a Nano Drop spectrophotometer [20].

Primers

Reverse primer The universal reverse primer was obtained from an article by Yang et al. [21] and ordered for synthesis by Pishgam Biotech Co., Ltd. (Tehran, IRAN). (Primer sequences are shown in Table 2).

Forward primer Forward primers for each miRNA were designed according to the Shi et al., Busk et al. and Esmaeili-bandboni et al. protocols [22–24], and ordered for synthesis by Pishgam Biotech Co., Ltd. (Tehran, IRAN). (Primer sequences are shown in Table 2).

Table 1 Data of patients with melanoma

Sex	Age	Location	Size of tumor	Stage
Male	57	Left calf skin	2.5 mm	IV
Male	87	Right cheek	Variable	I
Female	44	Back	2.5 mm	II
Female	65	Right foot	5 mm	IV
Male	74	Left foot	1.5 cm	V
Male	90	Right foot	4 mm	IV
Female	73	Right foot	More than 4 mm	IV
Male	76	Face	35 mm	IV
Female	62	Lower limb and hip	5 mm	II
Female	53	Face	8 mm	III
Male	72	Scalp and neck	4 mm	III
Female	75	Scalp and neck	4 mm	III
Male	82	Face	10 mm	IV
Male	65	Back	5 mm	III
Male	77	Scalp and neck	3 mm	IV
Female	48	Upper limb and shoulder	30 mm	IV
Male	47	Lower limb and hip	3 mm	III
Female	53	Lower limb and hip	13 mm	IV
Male	54	Scalp and neck	6 mm	IV
Female	83	Face	6 mm	III
Female	55	Lower limb and hip	35 mm	III
Male	56	Lower limb and hip	30 mm	IV
Male	49	Upper limb and shoulder	4.5 mm	III
Male	61	Upper limb and shoulder	10 mm	IV
Male	57	Lower limb and hip	50 mm	IV
Male	60	Scalp and neck	3 mm	IV
Female	55	Face	3 mm	IV

Table 2 Primer sequences

Oligo name	Sequence 5′–3′	Tm (°C)	MW (Da)
Universal stem-loop	GAAAGAAGGCGAGGAGCAGATCGAGGAAGA AGACGGAAGAATGTGCGTCTCGCCTTCTTT CNNNNNNNN	90	21.49
Reverse primer	CGAGGAAGAAGACGGAAGAAT	59	6.586
miR146b-3p	GCCCTGTGGACTCAGTTC	58	5.467
miR-193b-3p	GAACTGGCCCTCAAAGTCC	59	5.758
miR-483-3p	TCACTCCTCTCCTCCCG	57	4.993
miR-16-5p	GGGTAGCAGCACGTAAATATTG	60	6.823

cDNA Synthesis

The universal stem-loop primer of the miRNAs was extracted from an article by Yang et al. [21] and ordered for synthesis by Pishgam Biotech co. Ltd. (Tehran, IRAN). (Primer sequences are shown in Table 2). For using stem-loop in cDNA synthesis, it was heated to 95 °C for 10 m. The heat was reduced slowly to 75 °C then the temperature was held at 75°, 68°, 65° and 62 °C for an hour each, held at 60 °C for several more hours and finally the primer was transferred to a new tube. This step should never need repeating if the primer is never heated above room temperature. The working stocks were kept at − 20 °C.

Following RNA extraction, each sample was reverse transcribed using thermos-resistant H minus M-MULV reverse transcriptase (Pars Tous, Mashhad, Iran. Cat No: C101031). For synthesizing cDNA of miRNA, 7 μL RNA, 5 μL stem-loop, 2 μL thermos-resistant RT enzyme, 4 μL 5×RT buffer and 2 μL dNTP were added together to make up a final volume of 20 μL reaction mix. The reaction mix was incubated in a thermocycler for 20 min at 16 °C, 10 min at 25 °C, 1 h at 47 °C and 10 min at 70 °C at last was chilled on ice. The cDNA was stored at − 70 °C [25].

Real-Time PCR

Each cDNA (2 μL) of each control and melanoma samples was diluted to a volume of 20 μL of PCR mix containing 3 μL of primer, 5 μL of DEPC water and 10 μL of SYBR Green master mix (Ampliqon, Denmark. Cat No: A190303). Primers were purchased from Pishgam Biotech Co (Tehran, Iran). For amplification, an initial denaturation step at 95 °C for 15 min was used. For 40 subsequent cycles, the conditions were denaturation for 30 s at 95 °C, annealing for 30 s at 60 °C and elongation for 30 s at 72 °C. The melting curve was at 60–95 °C [26]. For the amplification of miRNAs, the Corbet Rotor-Gene 3000 system was used. The expression of each miRNA was analyzed in plasma samples of 27 melanoma patients and 27 control groups.

The relative expression of miRNAs in plasma was normalized to the expression of miR-16. Data were analyzed with $\Delta\Delta CT$ method [27]. Relative expression of each person in melanoma patients and control groups was performed with mean ΔCT of control group.

Statistical Analysis

GraphPad Prism version 8.0 (Graph Pad software) and SPSS 26.0 software (IBM) were used for statistical analysis. The data were tested for normality and were found to be different, so the nonparametric Mann–Whitney U test (a nonparametric test that compares the distribution of two unmatched groups) was applied to compare two groups of melanoma patients and control groups. Receiving operating characteristic (ROC) curve analysis was then used to evaluate the discriminatory power of the combinations. Statistical significance was set at p-values < 0.05.

Results

Identification of DEmiRs

Analysis of the GSE61741 dataset identified 141 DEMIRs between melanoma patients and control groups; these included 85 upregulated and 56 downregulated miRNAs (Fig. 1a), analysis of the GSE20994 dataset identified 273 DEMIRs between melanoma patients and control groups; these included 145 upregulated and 128 downregulated miR-NAs (Fig. 1b). Analysis of the GSE31568 dataset identified 11 DEMIRs between melanoma patients and control groups; these included five upregulated and six downregulated miR-NAs (Fig. 1c).

Remarkable Downregulation of miR-146b-3p and miR-193b

It was observed that miR-146b-3p and miR-193b expression levels decreased in the melanoma patients contrasted with control groups. After statistical analysis by unpaired Mann–Whitney U test, significant downregulation was observed, $p < 0.0001$. Furthermore, to contrast mean fold changes of expression levels of miR-146b-3p and miR-193b, among melanoma patients and control groups, it was shown that there was a decreased level of expression in the melanoma patients with the control groups, which was decreased 5×10^6-fold in miR-193b (This amount of decreased expression means close to zero expression in melanoma) and 58-fold in miR-146b-3p, respectively (Fig. 2).

For miR-483-3p, the statistical analysis by unpaired Mann–Whitney U test demonstrated that the melanoma patients had a higher level of expression compared to the control groups. Furthermore, the mean fold changes demonstrated that the melanoma patients had 3.5-fold higher levels of expression contrasted to the control groups (Fig. 2).

Great Distinguishing Power of microRNA Biomarkers

To measure the distinguishing power of the three microRNAs biomarkers for melanoma diagnosis, receiver operating characteristic (ROC) curve analysis for each of the patients was performed.

As displayed in Fig. 3, miR-193b had the best distinguishing power. For this marker, area under the curve (AUC) in comparison among the melanoma patients and control
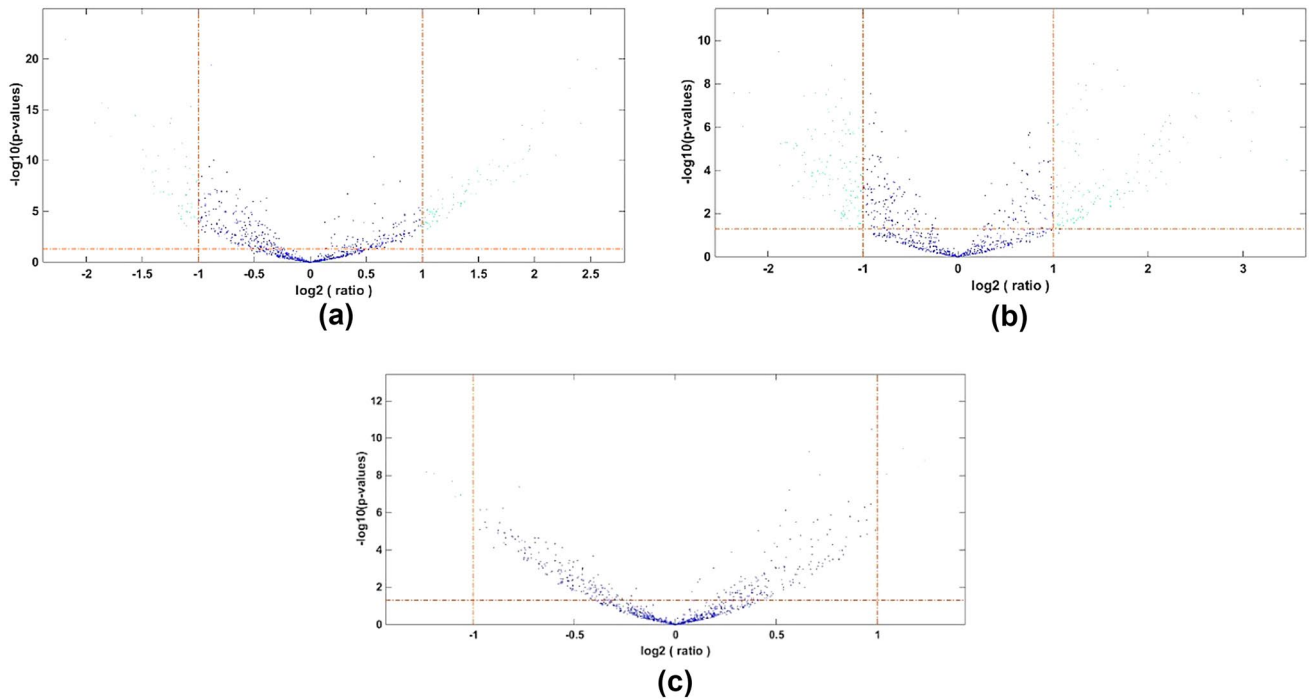
Fig. 1 Expression of differentially expressed miRNAs (DEMIRs) in the three datasets. **a** Volcano plot of DEMIRs between melanoma patients and control groups in GSE61741. **b** Volcano plot of DEMIRs between melanoma patients and control groups in GSE20994. **c** Vol-cano plot of DEMIRs between melanoma patients and control groups in GSE31568. (*X* axis represents fold change and *Y* axis represents *p*-value)

Fig. 2 Comparative expression of miR-193b, miR-146b-3p and miR-483-3p, comparisons were performed using the Mann–Whiney *U* test; *Y* axis represents fold changes using the $\Delta\Delta CT$ method. The levels of miR-193b and miR-146b-3p decreased in melanoma patients versus control groups. However, all of these findings were significant ($p < 0.0001$ for miR-193b and miR-146-3p, $p < 0.04$ for miR-483-3P, *p*-values less than 0.0001 are summarized with four asterisks and *p*-values less than 0.05 are summarized with one asterisk)
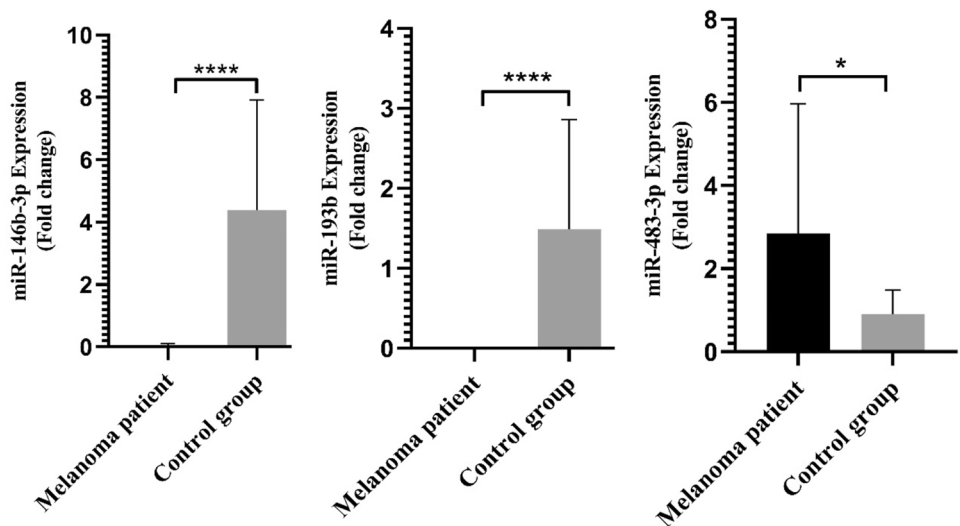
groups was 1.00 (95% confidence interval [CI] 1.00–1.00; $p < 0.0001$). In addition, miR-146b-3p had high distinguishing power, for this marker, AUC was 0.96 (95% confidence interval [CI] 0.96–1.00, $p < 0.0001$). However, miR-483-3p had low distinguishing power, for this marker, AUC was 0.31 (95% confidence interval [CI] 0.31–1.00, $p = 0.04$). Consequently, miR-193b had the highest and the best distinguishing power for melanoma diagnosis in this study.

Discussion

The prevalence of melanoma is expanding in the world. This illness is treatable when diagnosed in preliminary stages and it might be fatal when detected in developed stages. Though recently new and extremely impressive treatments came into clinical use, the fatality rate of stage
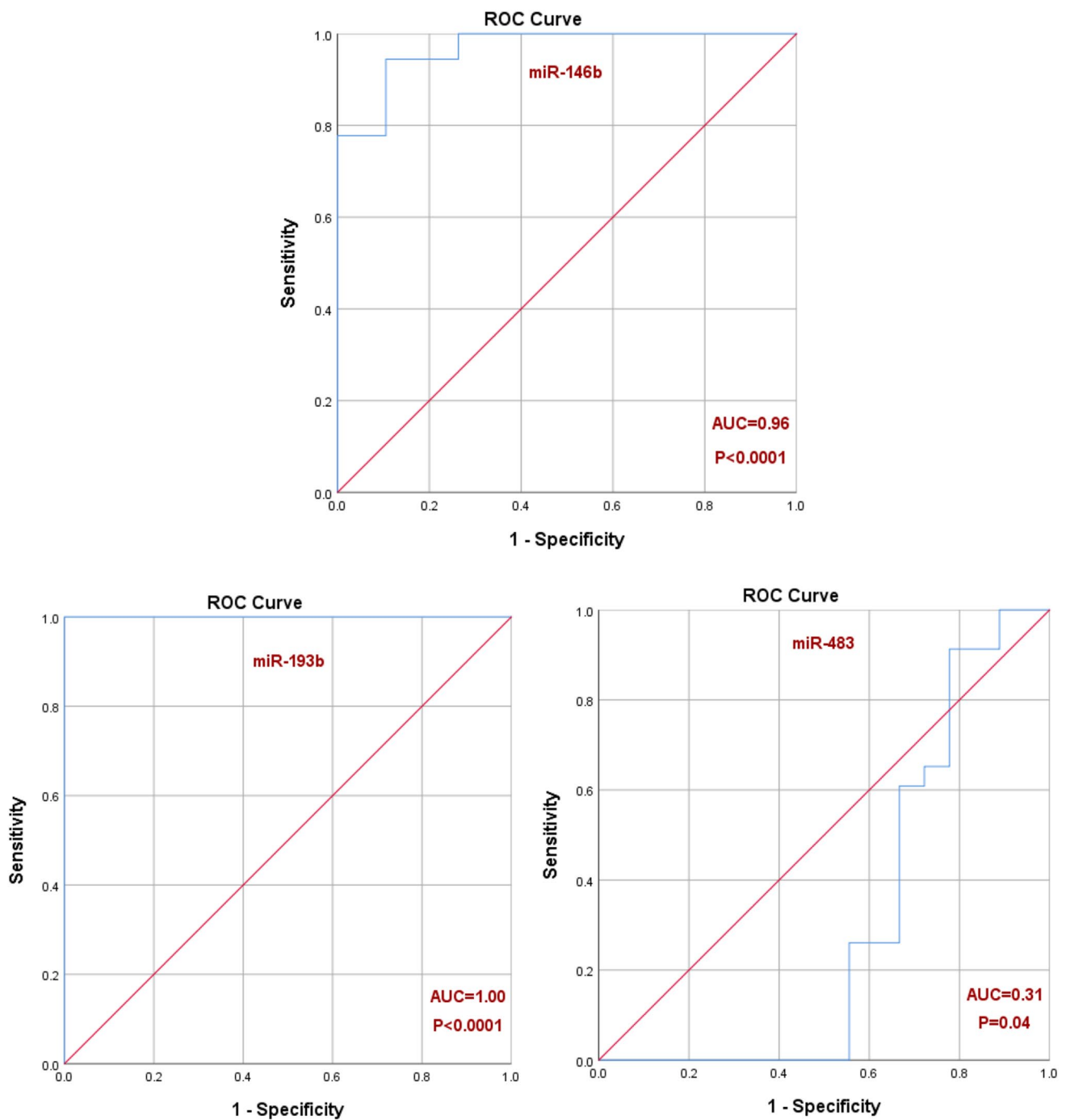
Fig. 3 ROC curves analysis of miRNAs; comparison between melanoma patients and control groups. AUC represents the "area under curve". As perceived, AUC for the miR-193b is more than other microRNAs

IV melanoma patients is still high [28, 29]. Melanoma should be treated in preliminary stages to amplify the possibilities of patient survival. The capability to determine preliminary symptoms of melanoma progression would be very valuable in a clinical setting. Several studies have indicated that miRNA expression is correlated with cell proliferation, metastasis, invasion and response to therapy. Circulating miRNAs, are resistant, extremely specific, sensitive and easily detectable molecules in biological fluids revealed as potential noninvasive biomarkers for diagnosing cancers. These are major characteristics to look for in a potential circulating biomarker [30]. To date, trustworthy biomarker has been distinguished as sensitive or specific enough to be useful for the preliminary

discovery of melanoma in each of the stages. Trustworthy circulating biomarker to identify melanoma with distant or regional spread prior to clinical evidence of metastasis might develop therapy and result for melanoma patient.

In this study, we recognized three miRNAs with desirable diagnostic power in the plasma of melanoma patients at distinct stages of the disease. Two of them, miR-146b-3p and miR-193b were significantly downregulated, while one of them, miR-483-3p was significantly upregulated in the plasma of melanoma patients contrasted with control groups. While we observed low considerable levels of miR-193b from plasma samples of melanoma patients compared with control groups, Kardab et al. obtained lower miR-193b levels in melanoma cell lines [29]. Furthermore, other authors observed decreased miR-193b expression in melanoma cell lines and tissue samples [31, 32]. This suggests that miR-193b has a functional role as a tumor suppressor in human melanoma. Concerning miR-193b, there is some evidence that suggests that in some cancer varieties other than melanoma, it may operate as a tumor suppressor. For instance, miR-193b expression was reduced in human ovarian cancer tissues and cell lines compared with controls, miR-193b might be nearly associated with the metastatic potential of ovarian cancer cells via increasing uPA expression [33, 34]. Furthermore, Wu et al. revealed that the expression of miR-193b was notably downregulated in endometrioid adenocarcinoma [35]. Rauhala et al. investigated that miR-193b is a tumor suppressor in prostate cancer [36]. The same authors described the decreased expression of miR-193b in melanoma cell lines and tissues [37]. Chen et al. identified that Mcl-1, an antiapoptotic protein associated with the Bcl-2 family was upregulated in malignant melanoma. They presented that the expression of miR-193b was related conversely to the expression of Mcl-1 in melanoma tissue samples. So overexpression of miR-193b suppresses Mcl-1 levels in some melanoma cell lines [31, 32]. Chen et al. reported that miR-193b overexpression decreases cell proliferation in melanoma cell lines and recommended that miR-193b may regulate cell cycle advancement by downregulating the expression of CCND1 protein [31]. Leivonen et al. identified extinguishing some targets of miR-193b such as AKR1C2 and AKR1C1 suppresses breast cancer cell growth in the MCF-7 cell line [38]. In addition, it was determined that miR-193b instantly targets estrogen receptor a (ERa) and ultimately represses cancer cell growth [38]. It was identified that miR-193b is downregulated in breast cancer tissues and has been illustrated as a tumor suppressor associated with clinical metastasis. Significantly there was a contrary relation among miR-193b and it is a direct target dimethylaminohydrolase (DDAH1) expression in breast cancer cell lines that miR-193b regulates cell proliferation and migration in breast cancer cell line, while DDAH1 knock down represses cell migration [39, 40]. A latter study verified that silencing

of miR-193b in colorectal cancer cells (CRC) remarkably reduces cell proliferation and induced apoptosis. In detail, the downregulation of miR-193b evidently activated the expression of SMAD3 and TGF-β in CRC cells [41, 42]. A prior study determined miR-193b was downregulated importantly in hepatocellular carcinoma (HCC) tissues contrasted with normal liver tissues. Furthermore, the expression level of miR-193b was reduced in hepatic cirrhosis [43]. It was determined that miR-193b may have a tumor suppressor role in hepatocellular carcinoma (HCC) progression by direct targeting of Cyclin D1 (CCND1) and ETS1, which causes cell cycle arrest and prevents the invasion and migration of hepatoma cells [44].

Evidently, the tumorigenesis function of miR-193b is associated with the upregulation or downregulation of particular targets, which leads to cancer cell growth, migration, invasion and metastasis in the origination and development of various cancers (Fig. 4).

In this study, miR-146b-3p was downregulated in the plasma of melanoma patients contrasted with control groups. Although, upregulation of miR-146a has been discovered in preliminary and metastatic cutaneous melanoma, and it may be particular for metastatic disease. It was obvious that miR-146a and miR-146b have oncogenic functions in cutaneous melanoma whereas miR-146a promotes both initiation and progression of BRAF/NRAS-mutated cutaneous melanoma by promoting activation of the NOTCH protein and NOTCH1 is implicated in melanoma formation and may improve the metastatic potential of primary melanoma cells through activation of the mitogen-activated protein kinase (MAPK) pathway or the PI3K/Akt pathway. Upregulation of
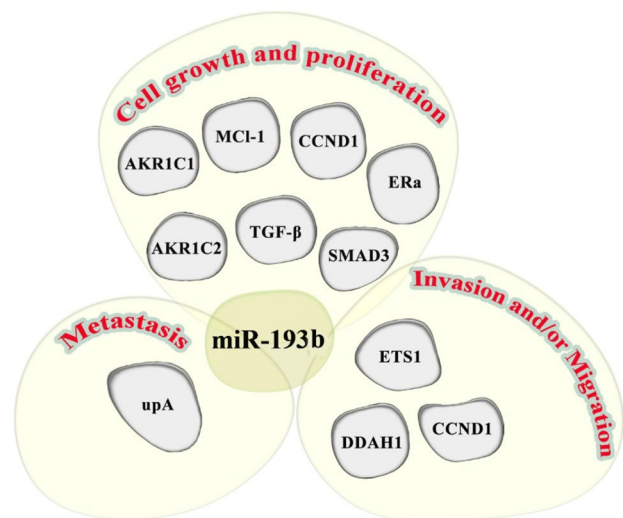

Fig. 4 Tumorigenesis function of miR-193b. Various gene targets of miR-193b which lead to cancer cell growth (AKR1C1, AKR1C2, SMAD3, CCND1, MCl-1, ERa and TGF-β), migration, invasion (CCND1, DDAH1 and ETS1), and metastasis (upA)

miR-146b expression has been revealed in cutaneous melanoma and emerges to be related to melanoma evolution. MiR-146a-5p was significantly upregulated in fresh frozen tissue samples from conjunctival melanoma and these miRNAs may have the same function in conjunctival melanoma [45]. This suggests that miR-146a and miR-146b may have a similar function in a particular tumor. The mature sequences for miR-146a and miR-146b are extremely preserved across species. Furthermore, the two miRNAs are different in two nucleotides on the 3′-end of the mature strand, not within the seed region [46]. Li et al. found that miR-146b-5p has been revealed as aberrantly downregulated in gliomas, the correlation between its expression level and the grade of tumor malignity remains to be clarified, the consequences showed that the expression of miR-146b-5p reduced with the upgrading of the tumors malignity grade, demonstrating that miR-146b-5p downregulation might play a crucial role in the incidence and malignant advancement of gliomas [47]. Furthermore, Yu et al. established that miR-146b-5p and miR-146b-3p expression were remarkably upregulated in thyroid cancer tissues and cell lines compared with normal thyroid tissue and cells [48]. Mature miRNA-3p and miRNA-5p are created from the identical pre-miRNA progenitor, but more often, one of them is degenerated, though the co-existence of these two species has also been reported [49]. Su et al. showed that miR-146b-3p altered expression is nearly associated with inflammation and thrombosis. Inflammatory factors can cause thrombosis via the miR-146b-3p/P38MAPK/COX-2 pathway [50]. These clear diversities may be due to the complicated functional role of miR-146b-3p in tumor biology.

The third miRNA found upregulated in this study is miR-483-3p, that level was significantly elevated in the plasma of melanoma patients than in control groups. This study investigated the relationship between miR-483-3p and melanoma for the first time. Regarding miR-483-3p, there is some evidence that suggests that it may be upregulated in certain cancers other than melanoma. For instance, miR-483-3p is overexpressed in 30% of the colon, breast and liver cancers tissues and 100% of Wilms' tumors. Veronese et al. determined an association between miR-483-3p, a miRNA placed within the IGF2 location at chromosome 11p15.5, and human tumorigenesis. This indicated that a critical oncogenic function was correlated with the miR-483-3p within the IGF2 location [51]. Upregulation of miR-483-3p was also found in pancreatic cancers. miR-483-3p expression was conversely coordinated with Dpc4/Smad4 in pancreatic cancer, and upregulated miR-483-3p in pancreatic cancer suppressed the expression levels of Dpc4/Smad4, which in turn increases carcinogenesis [52]. In apparent contradiction with these findings but in according with those of this study, in some cancers, including gastric, pancreatic, and hepatocellular carcinomas, miR-483-3p levels were discovered to be decreased in tissues of patients, and it could have antitumor features in those cancers [53]. Accumulated evidence suggested that the roles of miR-483-3p in carcinogenesis were complicated. Furthermore, these data demonstrate that different functions of miR-483-3p in various kinds of cancer depend on cancer types and cellular context.

Conclusions

In conclusion, our study determined three miRNAs, which were first distinguished in the plasma of melanoma patients. miR-193b, miR-146b-3p and miR-483-3p are appropriate diagnostic biomarkers in human melanoma and deserve validation in future prospective clinical trials. The most important limitation of this study was the small and rare number of melanoma cases, which led to spending a lot of time to reach the desired sample size in terms of statistics. According to the research result, miR-193, through particular targets and biological pathways, play remarkable roles in the origination and development of pivotal biological and pathological procedures in a broad range of significant human diseases, especially different cancers. More significantly, miR-193 can be contemplated to be a therapeutic biomarker in cancer treatment by improving or suppressing particular molecular procedures. Therefore, miR-193b is the best one of these three miRNAs with the best discriminatory power in melanoma diagnosis. However, according to the limitations of the study, these three biomarkers should be investigated in other cancers to show the specificity of these biomarkers in melanoma diagnosis with more certainty.

Declarations

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