A preliminary study of microRNA expression in different types of primary melanoma

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ABSTRACT

MicroRNAs (miRNAs) have been proven to regulate the development and progression of cancer through various mechanisms. The aim of the present study was to compare miRNA expression between primary melanomas from different sites. We analyzed the expression of 84 miRNAs in 27 primary melanoma and 5 nevus formalin-fixed paraffin-embedded (FFPE) samples using the Human Cancer PathwayFinder miScript miRNA PCR Array. The FFPE samples were obtained from the archives of the Municipal Clinical Emergency Hospital of Timisoara and included 10 cutaneous melanomas, 10 uveal melanomas, 7 mucosal melanomas, and 5 cutaneous nevi. Out of 84 miRNAs, 11 miRNAs showed altered expression in all types of melanoma compared with the nevi. Among these, miR-155-5p, miR-9-5p, miR-142-5p, miR-19a-3p, miR-134-5p, and miR-301a-3p were upregulated, while miR-205-5p, miR-203a-3p, miR-27b-3p, miR-218-5p, and miR-23b-3p were downregulated. The highest similarity in miRNA expression pattern was found between uveal and mucosal melanoma groups, i.e., 15 miRNAs had altered expression in both groups. Overall, we identified several miRNAs with significantly altered expression in primary melanomas, including those reported for the first time in this type of cancer. Among them, mir-9-5p, mir-203a-3p, mir-19a-3p, mir-27b-3p, and mir-218-5p showed altered expression in all three melanoma types vs. nevi. Further research should explore the potential of these miRNAs in melanoma.

KEYWORDS: microRNA; primary melanoma; miScript miRNA PCR Array

INTRODUCTION

Primary melanoma, a malignant neoplasm of melanocytes, can be highly aggressive and has an increasing incidence worldwide [1]. In 2016, 76,380 new cases of melanoma were estimated in the United States (US) from which approximately 10,130 cases would be fatal [2]. Romania and other countries from Central and Eastern Europe show a higher incidence of 111 primary melanomas at advanced stage compared with Western Europe, which may be due to the lack of proper health education in these countries, among other reasons [3]. Based on this information, we consider melanoma to be one of the most important research areas in our country.

Melanoma can occur in any tissue that contains melanocytes. These cells are predominantly present in the skin, but they can also be found in organs such as the eyes, inner ear, and brain (the substantia nigra and locus coeruleus) as well as in the mucosal lining of the leptomeninges, oral cavity, esophagus, rectum, anal canal, nasal cavity, paranasal sinuses, larynx, vagina, and cervix [4-13]. In each of these tissues melanocytes have different functions and are influenced by different local factors [13].

Primary melanomas originating from different sites show characteristic tumor mutational burden [14-20]. According to the site of involvement, primary melanomas are classified as: cutaneous melanoma (CM), uveal melanoma (UM), mucosal melanoma (MM), and melanoma of the internal organs.

Numerous genomic studies showed different mutational patterns in melanoma, which was followed by the investigation of epigenetic factors involved in melanoma development. MicroRNAs (miRNAs) are small noncoding RNAs (~22 nt in length) that can regulate the development and progression of cancer through various mechanisms. Different miRNAs have been shown to be upregulated or downregulated in melanoma, which suggests their use as diagnostic and prognostic biomarkers as well as therapeutic targets [21]. Furthermore,

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circulating miRNAs can be used for non-invasive diagnosis and prognosis of early metastatic disease, representing a more cost-effective method for monitoring patients and deciding about the treatment. These biomarkers have a higher sensitivity for detection in early stages of disease than the current imaging techniques (e.g., computed tomography [CT], positron emission tomography [PET] scan, etc.), and they are less invasive compared with tumor excision and sentinel lymph node biopsy, used for staging of melanoma [22].

Several studies investigated miRNA expression changes in melanomas, most notably in cutaneous and uveal types, however, there is little data on miRNA expression in primary mucosal melanoma. To the best of our knowledge, only two studies have investigated changes in miRNA expression in conjunctival melanomas [23,24] and none in mucosal melanomas involving other sites. Therefore, the current study is the first to analyze miRNA expression in primary mucosal melanomas involving mucosal surfaces other than the conjunctiva. Here, we compared miRNA expression among three primary melanoma types (CM, UM, and MM) and control cutaneous nevi. We identified several miRNAs with significantly altered expression in primary melanomas, including those reported for the first time in this type of cancer.

MATERIALS AND METHODS

Tissue samples

We obtained 27 primary melanoma and 5 nevus formalin-fixed paraffin-embedded (FFPE) samples from the archives of the Pathology Department at the Municipal Clinical Emergency Hospital of Timisoara. The FFPE samples included 10 cutaneous melanomas (stage III-IV), 10 uveal melanomas, 7 mucosal melanomas (2 intestinal mucosa, 2 genital mucosa, 1 nasal mucosa, and 2 oral mucosa) and 5 cutaneous nevi (Table 1). To reduce genomic and transcriptomic changes that occur because of environmental factors, we collected the control nevus samples from younger individuals.

All participants signed informed consent to participate in the study, and the study was approved by the Institutional Ethics Committee (approval number 1-015922/2019). Patients did not receive any treatment prior to tumor excision.

Real-time polymerase chain reaction (real-time PCR)

MiRNAs were purified from FFPE samples using a miR-Neasy FFPE Kit (Qiagen, MD, US), according to the manufacturer's instructions. We analyzed the expression of 84 miRNAs using the Human Cancer PathwayFinder miScript miRNA PCR Array (Qiagen, MD, US), on an ABI 7900HT real-time PCR instrument (Thermo Fisher Scientific, MA, US).

Real-time PCR data were analyzed using the online QIAGEN GeneGlobe Data Analysis Center, with a threshold of 2 for fold changes of miRNA expression in melanoma samples. Afterwards, we compared these results with miRNA expression in control nevus samples. We corrected the results using the Benjamini-Hochberg procedure to account for any possible false discovery rate. Moreover, we

TABLE 1. Characteristics of patients with primary melanoma (n = 27) and controls (n = 5)

Characteristic	Cutaneous melanoma	Uveal melanoma	Mucosal melanoma	Cutaneous nevi
Mean age (years)	61	60	66	33
Number of samples	10	10	7	5
Male	7	5	2	3
Female	3	5	5	2
Melanocyte type				
Epithelioid	8	3	4	Medium size, round nevus cells
Spindle-shaped	1	3	0	
Mixed (epithelioid and spindle-shaped)	1	4	3	

TABLE 2. MicroRNAs (miRNAs) with most significant changes in their expression in primary melanoma samples (n = 27) compared with cutaneous nevi (n = 5)

Melanoma type	Upregulated miRNAs	Downregulated miRNAs
Cutaneous	miR-155-5p, miR-9-5p, miR-19a-3p, miR-142-5p, miR-134-5p, miR-127-5p, miR-372-3p, miR-15a-5p, miR-301a-3p	miR-205-5p, miR-203a-3p, miR-130a-3p, miR-218-5p, miR-146a-5p, miR-27b-3p, miR-125b-5p, miR-146b-5p, miR-23b-3p, miR-92a-3p
Uveal	miR-206, miR-184, miR-130a-3p, miR-29a-3p, miR-9-5p, miR-29b-3p, miR-378a-3p, miR-155-5p, miR-183-5p, miR-142-5p, miR-124-3p, miR-181d-5p, miR-96-5p, miR-373-3p, miR-335-5p, miR-1-3p, miR-146b-5p, miR-134-5p, miR-7-5p, miR-133b, miR-19a-3p, miR-138-5p, miR-34a-5p, miR-301a-3p, miR-135b-5p	miR-10b-5p, miR-200c-3p, miR-203a-3p, miR-205-5p, miR-100-5p, miR-196a-5p, miR-27b-3p, miR-149-5p, miR-193b-3p, miR-10a-5p, miR-214-3p, miR-143-3p, miR-23b-3p, miR-215-5p, miR-218-5p
Mucosal	miR-206, miR-1-3p, miR-155-5p, miR-18a-5p, miR-21-5p, miR-9-5p, miR-373-3p, miR-142-5p, miR-17-5p, miR-196a-5p, miR-7-5p, miR-138-5p, miR-301a-3p, miR-127-5p, miR-20b-5p, miR-215-5p, miR-133b, miR-19a-3p, miR-134-5p, miR-143-3p, miR-135b-5p, miR-132-3p	miR-200c-3p, miR-203a-3p, miR-183-5p, miR-205-5p, miR-10b-5p, miR-125b-5p, miR-149-5p, miR-146a-5p, miR-27b-3p, miR-193b-3p, miR-218-5p, miR-23b-3p, miR-140-5p, miR-125a-5p, miR-122-5p

used the Qiagen Ingenuity pathway analysis (IPA) tool to identify pathways and diseases associated with miRNAs with altered expression.

RESULTS

The distribution of upregulated and downregulated miRNAs in the three primary melanoma types compared with control nevi is presented in Figure 1-3. MiRNAs with most significant changes in their expression in the primary melanoma samples compared with nevus group are presented in Table 2. We used these results to generate a cluster dendrogram that highlights the segregation of miRNA expression according to the 4 studied groups (Figure 4). The samples clustered together for each melanoma type and for nevi, showing similar RNA expression patterns in all three repetitions of miRNA expression analysis.

Out of 84 miRNAs, 11 miRNAs showed altered expression in all types of melanoma compared with the nevi. Among these, miR-155-5p, miR-9-5p, miR-142-5p, miR-19a-3p, miR-134-5p, and miR-301a-3p were upregulated, while miR-205-5p, miR-203a-3p, miR-27b-3p, miR-218-5p, and miR-23b-3p were downregulated. The highest similarity in miRNA expression pattern was found between uveal

and mucosal melanoma groups, i.e., 15 miRNAs had altered expression in both groups.

Using the IPA, we determined the diseases, pathways, and biological functions associated with the miRNAs with altered expression. The IPA revealed that the dysregulated miRNAs are involved in many physiological and pathological processes. Table S1 shows tumor stages, tumor sites, and diseases associated with the dysregulated miRNAs for each melanoma type. It is worth noting that both primary and metastatic disease are common to all three melanoma types. We used IPA for each data set, identifying the targets of the miRNAs that were significantly overexpressed or underexpressed in our study ($p < 0.05$). Matching our results against the IPA data sets we generated pathway networks for each data set and, by identifying shared molecules, we created merged networks for each type of melanoma (Figure S1-S3). The targets common to all three types of melanoma were Smad2/3, insulin, sirtuin 1 (SIRT1), and tumor protein p53. The other identified targets were either unique to each type of melanoma (35 targets in MM, 29 targets in CM, and 38 targets in UM) or common to at least 2 of the analyzed melanoma types (MM and CM: Dickkopf WNT signaling pathway inhibitor 1 [DKK1], G protein signaling modulator 2 [GPSM2], one cut homeobox 2 [ONECUT2], protein
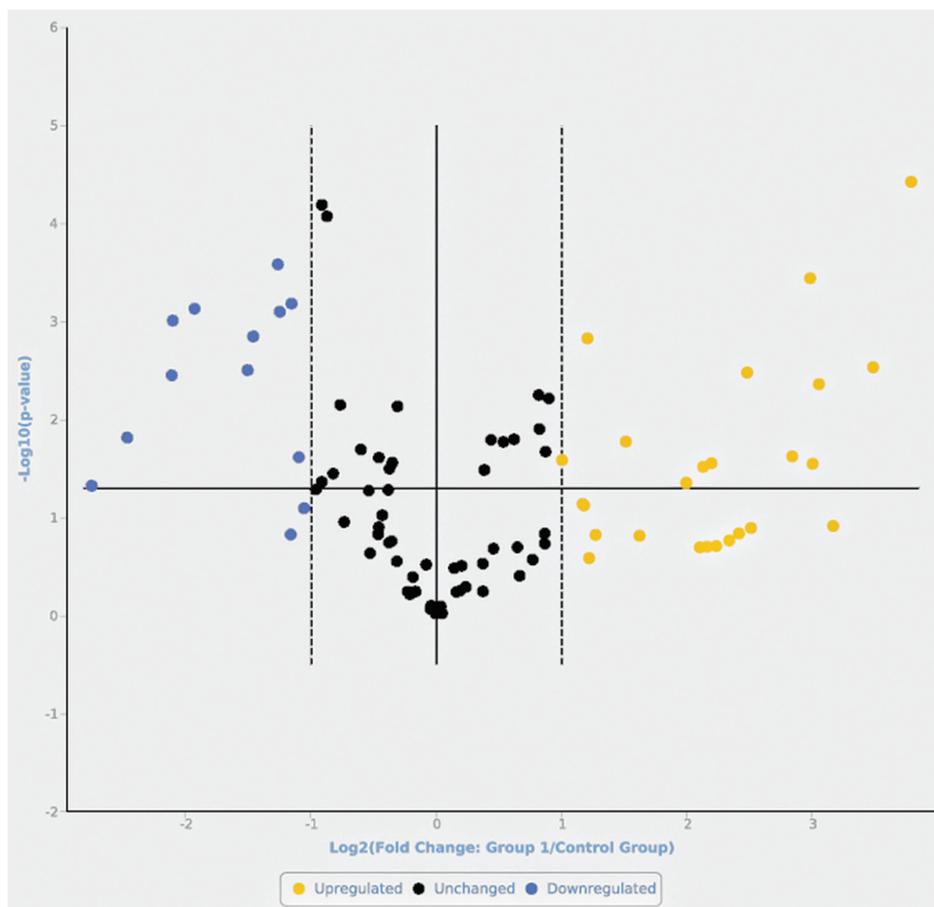

FIGURE 1. Volcano plot representing the microRNAs (miRNAs) in cutaneous melanomas compared with cutaneous nevi. Upregulated (yellow) and downregulated (blue) miRNAs with a fold change >2 vs. nevi, and miRNAs that were not significantly differently expressed (black, fold change <2 vs. nevi).
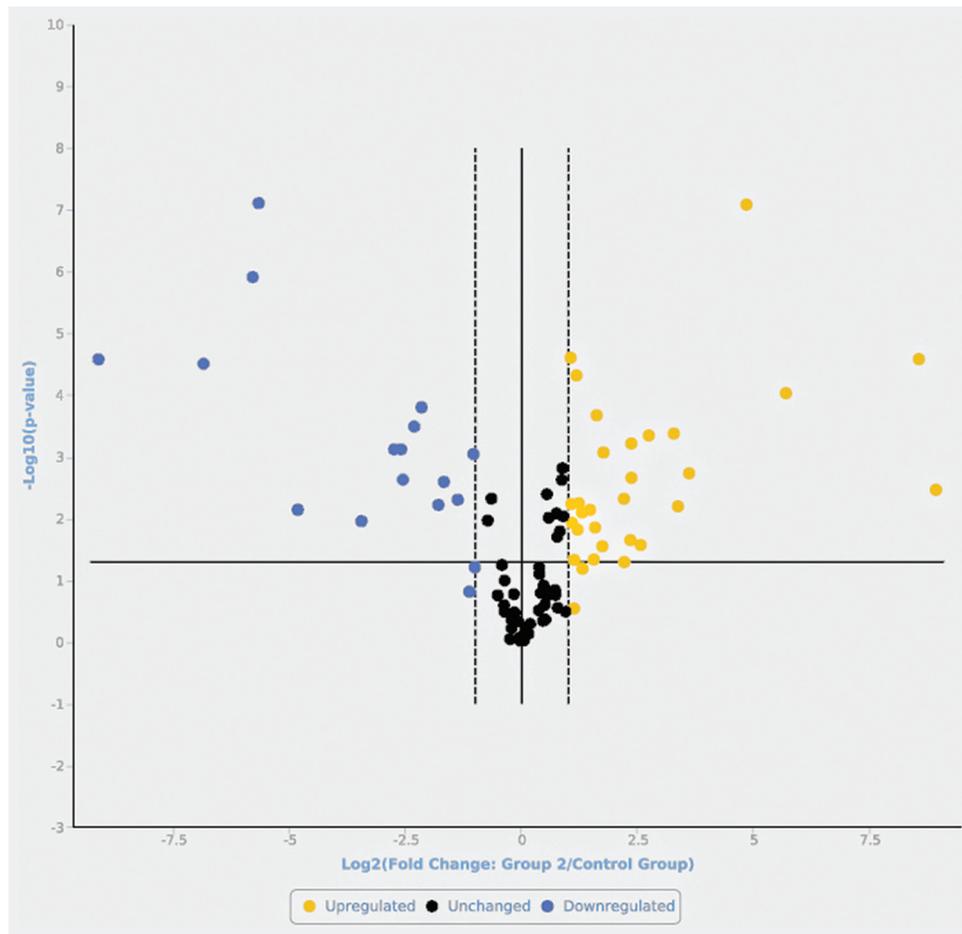
FIGURE 2. Volcano plot representing the microRNAs (miRNAs) in uveal melanomas compared with cutaneous nevi. Upregulated (yellow) and downregulated (blue) miRNAs with a fold change >2 vs. nevi, and miRNAs that were not significantly differently expressed (black, fold change <2 vs. nevi).

regulator of cytokinesis 1 [PRC1], reversion-inducing-cyste-ine-rich protein with kazal motifs [RECK], and secretory carrier membrane protein 1 [SCAMP1]; CM and UM: protein kinase B [PKB or Akt], and insulin receptor [INSR]; UM and MM: interferon alpha [IFN-α], pro-inflammatory cytokines, parathyroid hormone like hormone [PTHLH], retinoblastoma [Rb] protein, and telomerase reverse transcriptase [TERT]).

DISCUSSION

In this study, we analyzed the expression of 84 miRNAs in cutaneous, uveal, and mucosal melanomas compared with miRNA expression in control cutaneous nevi. Out of 84 miRNAs, 11 miRNAs showed altered expression in all types of melanoma compared with the nevi. Among these miRNAs, only miR-155-5p, miR-142-5p, miR-205-5p, miR-23b-3p, miR-134-5p, and miR-301a-3p were previously studied in melanoma. Thus, for the first time, here we reported altered expression of miR-9-5p, miR-203a-3p, miR-19a-3p, miR-27b-3p, and miR-218-5p in the three melanoma types vs. nevi.

We found that miR-155-5p was upregulated in all three types of melanoma. Similarly, a previous study reported miR-150

and miR-155 to be upregulated in primary and metastatic melanoma compared with nevi [25]. In addition, miR-155-5p was suggested to play a role in the development of other solid and hematopoietic cancers [26-29]. Nevertheless, in melanoma cell lines, ectopic expression of miR-155-5p had an anti-proliferative and pro-apoptotic effect [30], and higher miR-155-5p expression in metastatic melanoma could predict longer post-recurrence survival [25,31]. The expression of miR-155-5p increases during inflammatory response, especially during lymphocyte proliferation, and some authors suggest this to support the role of miR-155 in melanoma progression [32,33].

In this study, miR-205-5p was downregulated in melanoma vs. nevus samples, which is consistent with previous studies [34,35]. MiR-205 was shown to act as a tumor suppressor, inhibiting melanoma cell proliferation and inducing apoptosis by targeting vascular endothelial growth factor (VEGF) and transcription factor E2F1 *in vitro* as well as *in vivo* [35-37].

MiR-142-5p was upregulated in our melanoma samples compared with control nevi, and previous research indicated that miR-142 is one of five miRNAs with important clinical implications and a high prognostic value in metastatic melanoma [38].
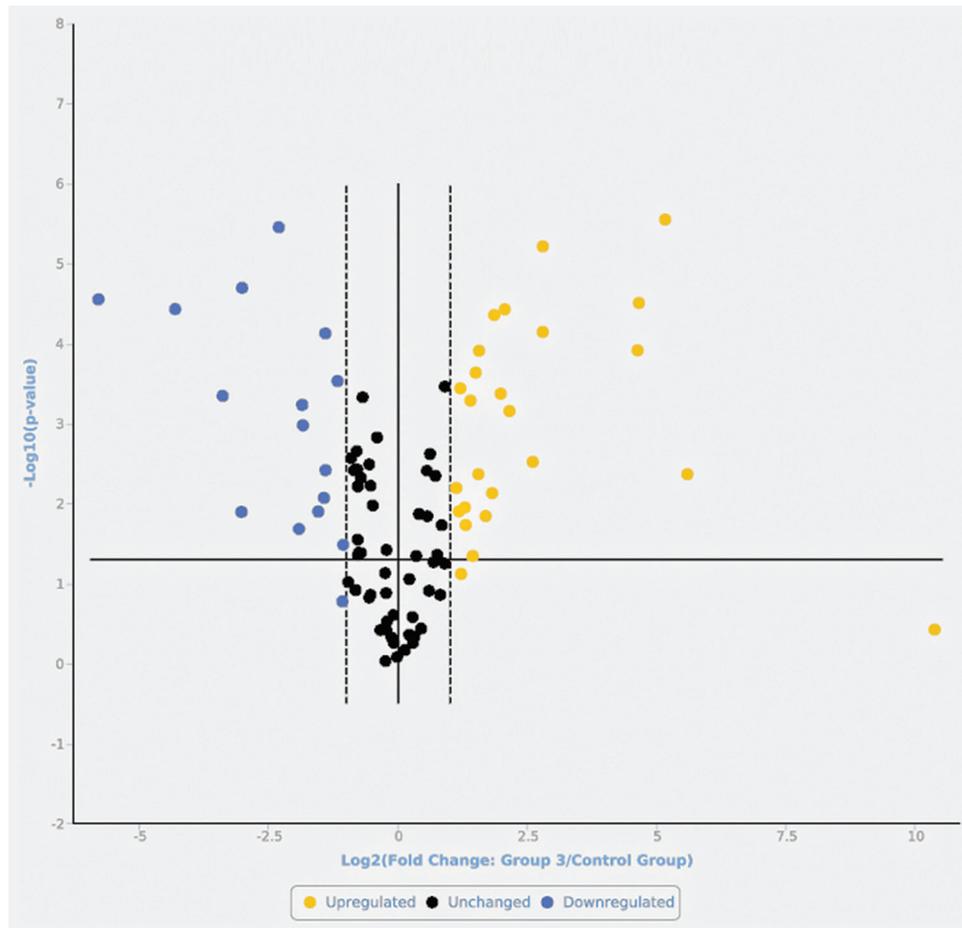
FIGURE 3. Volcano plot representing the microRNAs (miRNAs) in mucosal melanomas compared with cutaneous nevi. Upregulated (yellow) and downregulated (blue) miRNAs with a fold change >2 vs. nevi, and miRNAs that were not significantly differently expressed (black, fold change <2 vs. nevi).

MiR-23b-3p was downregulated in our melanoma samples vs. control nevi. This miRNA was shown to have altered expression in different malignant tumors, including melanoma, with clinical, therapeutic, and prognostic significance [34,39].

We showed for the first time that miR-9-5p is upregulated in melanoma compared with nevi. MiR-9-5p promotes cell proliferation and metastasis in non-small cell lung cancer (NSCLC) and colorectal cancer [40,41]. It is involved in the differentiation of B lymphocytes into plasma cells by the negative regulation of the transcription factor PR domain zinc finger protein 1 (PRDM1/BLIMP-1), showing lower levels as the lymphocytes differentiate. This explains high levels of miR-9-5p in primary large-B cell lymphomas [42]. On the other hand, miR-9 is downregulated in human ovarian cancer compared with normal ovary, and the overexpression of miR-9 inhibits cell growth in ovarian cancer *in vitro* through the negative regulation of nuclear factor NF-kappa-B p105 (NFκB1) [42,43]. MiR-9-5p may represent a new prognostic marker in melanoma and possibly a new therapeutic target.

We found that miR-19a-3p is upregulated in melanoma vs. nevi, which is another novel finding in this cancer type. In

gastric cancer, miR-19a-3p had a negative prognostic impact and promoted cell malignancy [44].

MiR-134-5p was upregulated in our melanoma samples compared with nevi. Previous studies showed that miR-134-5p is downregulated in NSCLC cells [45] and nasopharyngeal carcinoma cells [46] and that it has a role in inhibiting tumor progression. Interestingly, another study on melanoma showed that miR-134-5p is downregulated in melanoma patients compared with healthy controls [47], and this discrepancy with our results should be further investigated.

We reported in this study upregulation of miR-301a-3p in melanoma vs. nevi. MiR-301a was previously reported to be upregulated in melanoma samples compared to benign melanocytic lesions [48]. In addition, in hepatocellular carcinoma cell lines, miR-301a-3p overexpression was shown to stimulate cell proliferation, invasion, and chemoresistance [49].

For the first time, we showed that miR-203a-3p was downregulated in melanoma compared with nevi. The overexpression of miR-203a-3p in colorectal cancer cell lines inhibited cell proliferation and reduced chemoresistance [50] and similarly, in nasopharyngeal carcinoma, overexpressed miR-203a-3p inhibited cell proliferation, migration, and invasion *in vitro* as well as
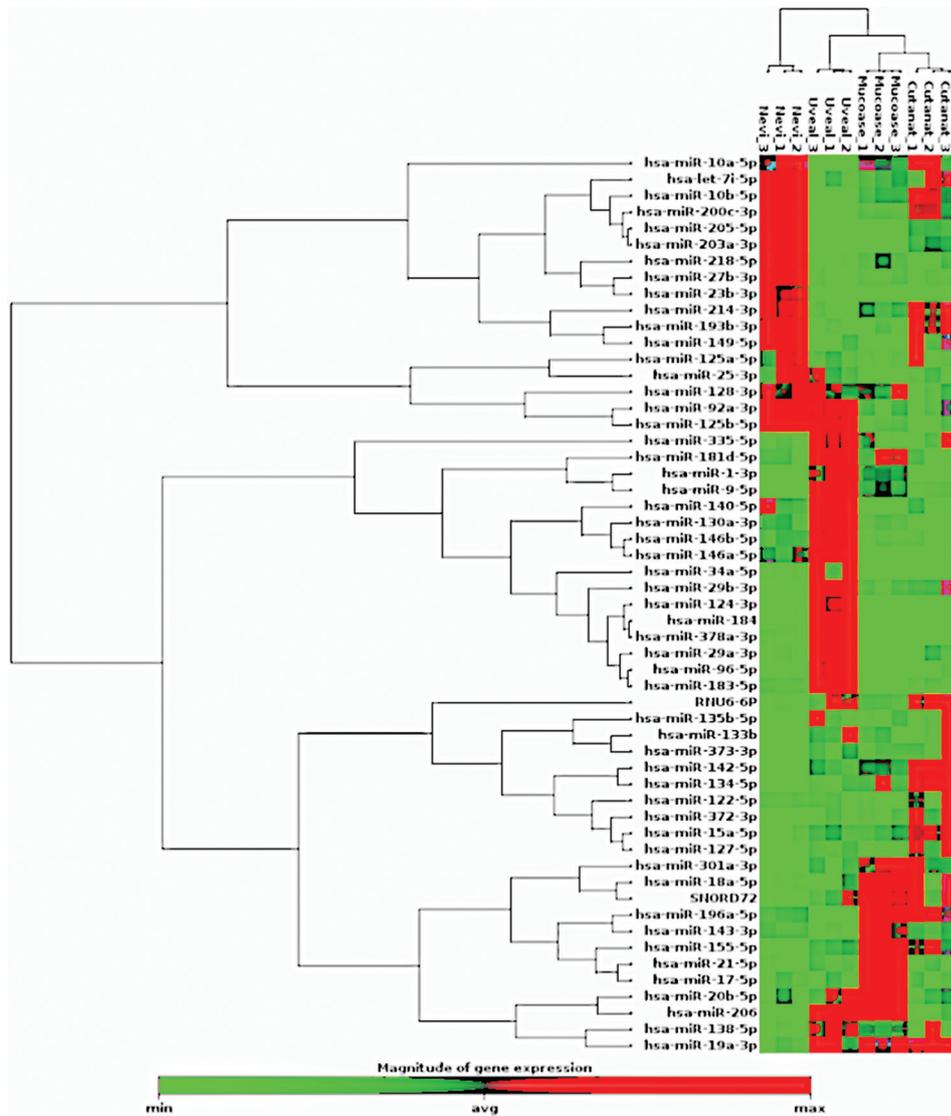
FIGURE 4. Cluster analysis of the changes in microRNA (miRNA) expression in 4 analyzed groups (cutaneous nevi, cutaneous melanoma, uveal melanoma, and mucosal melanoma). The samples (the top of the figure) clustered together for each melanoma type and for nevi, showing similar RNA expression patterns (the right of the figure) in all three repetitions of miRNA expression analysis.

TABLE 3. MicroRNAa (miRNAs) with altered expression in at least one group of the studied melanoma types (n = 27) that were or were not investigated before

	Previously studied in melanoma	Not yet studied in melanoma
miRNA	miR-127-5p, miR-142-5p, miR-15a-5p, miR-206, miR-184, miR-130a-3p, miR-29a-3p, miR-29b-3p, miR-378a-3p, miR-181d-5p, miR-96-5p, miR-373-3, miR-146b-5p, miR-7-5p, miR-34a-5p, miR-301a-3p, miR-18a-5p, miR-21-5p, miR-17-5p, miR-20b-5p, miR-143-3p, miR-132-3p, miR-127-5p, miR-15a-5p, miR-335-5p, miR-205-5p, miR-146a-5p, miR-125b-5p, miR-23b-3p, miR-10b-5p, miR-100-5p, miR-149-5p, miR-193b-3p, miR-10a-5p, miR-214-3p, miR-200c-3p, miR-140-5p, miR-125a-5p, miR-122-5p, miR-134-5p	miR-372-3p, miR-183-5p, miR-124-3p, miR-1-3p, miR-133b, miR-138-5p, miR-135b-5p, miR-196a-5p, miR-218-5p, miR-27b-3p, miR-92a-3p, miR-215-5p

xenograft tumor growth and lung metastasis *in vivo* [51]. On the other hand, in hepatocellular carcinoma cells, miR-203a-3p.1 overexpression was reported to be oncogenic [52].

MiR-27b-3p and miR-218-5p were another two down-regulated miRNAs in our case study that are reported for the first time in melanoma. MiR-27b-3p was also downregulated in lung cancer tissues [53] and in breast cancer tissues and cell lines, where it was associated with chemoresistance [54]. However, miR-27b-3p expression was increased

in Dox-resistant anaplastic thyroid cancer (ATC) cells [55]. MiR-218-5p expression was reduced in gastric cancer [56], NSCLC [57], and gallbladder cancer (GBC) tissue [58]; moreover, miR-218-5p overexpression reversed the resistance of GBC cells to gemcitabine [58].

Among miRNAs with altered expression in at least one type of melanoma (Table 3), we focused on those previously studied in melanoma. Although some studies reported that miR-127-5p and miR-15a-5p were not significantly differently

expressed in melanoma compared to melanocytic nevi [59,60], our results showed these miRNAs to be overexpressed in both cutaneous and mucosal melanomas vs. nevi. In addition, miR-335-5p was reported to be significantly differentially expressed in the melanoma cell line MML-1 compared to benign nevi [33], but our results showed a significant overexpression of miR-335-5p only in uveal melanoma group compared to control nevi.

The main limitation to our study is the small sample size. Thus, although we consider some of our findings to be groundbreaking in melanoma research, they still need to be confirmed in large-scale studies. To this end, we propose analyzing miRNAs in each melanoma sample separately and in combination with follow-up data, so to determine changes in miRNA expression specific to each cell type and site of origin, the impact of local factors, and the prognostic potential of miRNAs in melanoma. To achieve this, we plan to conduct a prospective study in the future.

CONCLUSION

Overall, we identified several miRNAs with significantly altered expression in primary melanomas, including those reported for the first time in this type of cancer. Mir-9-5p, miR-203a-3p, miR-19a-3p, miR-134-5p, miR-301a-3p, miR-155-5p, miR-142-5p, miR-205-5p, miR-23b-3p, miR-27b-3p, and miR-218-5p had altered expression in all three melanoma types. Further research should explore the potential of these miRNAs in melanoma. The primary contribution of this study is to demonstrate that despite originating from the same cell type, the three melanoma types are still separate entities characterized by different miRNA expression patterns.

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SUPPLEMENTAL DATA

TABLE S1. Tumor stages, tumor sites, and diseases associated with dysregulated microRNAs (miRNAs) for each melanoma type, according to the Qiagen Ingenuity pathway analysis (IPA)

Diseases and function annotations	p	miRNAs
	Cutaneous melanoma	
Primary melanoma	5.84E-30	miR-125b-5p, miR-130a-3p, miR-142-5p, miR-146a-5p, miR-155-5p, miR-203a-3p, miR-205-5p, miR-218-5p, miR-23a-3p, miR-27a-3p, miR-291a-3p, miR-9-5p, miR-92a-3p
Primary neoplasm	1.79E-21	miR-125b-5p, miR-128-3p, miR-130a-3p, miR-142-5p, miR-146a-5p, miR-155-5p, miR-19b-3p, miR-203a-3p, miR-205-5p, miR-218-5p, miR-23a-3p, miR-27a-3p, miR-291a-3p, miR-9-5p, miR-92a-3p
Primary solid tumor	6.14E-21	miR-125b-5p, miR-130a-3p, miR-142-5p, miR-146a-5p, miR-155-5p, miR-19b-3p, miR-203a-3p, miR-205-5p, miR-218-5p, miR-23a-3p, miR-27a-3p, miR-291a-3p, miR-9-5p, miR-92a-3p
Early-stage invasive cervical squamous cell carcinoma	7.59E-21	miR-130a-3p, miR-142-5p , miR-155-5p, miR-16-5p, miR-19b-3p, miR-203a-3p, miR-218-5p, miR-3118, miR-9-5p, miR-92a-3p
Primary tumor	2.4E-20	miR-125b-5p, miR-130a-3p, miR-142-5p, miR-146a-5p, miR-155-5p, miR-19b-3p, miR-203a-3p, miR-205-5p, miR-218-5p, miR-23a-3p, miR-27a-3p, miR-291a-3p, miR-9-5p, miR-92a-3p
Cervical squamous cell carcinoma	2.01E-18	miR-128-3p, miR-130a-3p, miR-142-5p, miR-155-5p, miR-16-5p, miR-19b-3p, miR-203a-3p, miR-218-5p, miR-291a-3p, miR-3118, miR-9-5p, miR-92a-3p
Early-stage solid tumor	4.04E-18	miR-125b-5p, miR-130a-3p, miR-142-5p, miR-155-5p, miR-16-5p, miR-19b-3p, miR-203a-3p, miR-218-5p, miR-3118, miR-9-5p, miR-92a-3p
Metastatic melanoma	3.35E-17	miR-125b-5p, miR-130a-3p, miR-142-5p, miR-146a-5p, miR-155-5p, miR-203a-3p, miR-205-5p, miR-23a-3p, miR-27a-3p, miR-92a-3p
Metastatic solid tumor	6.69E-14	miR-125b-5p, miR-130a-3p, miR-142-5p, miR-146a-5p, miR-155-5p, miR-203a-3p, miR-205-5p, miR-218-5p, miR-23a-3p, miR-27a-3p, miR-9-5p, miR-92a-3p
Non-obstructive azoospermia	5.77E-12	miR-128-3p, miR-130a-3p, miR-19b-3p, miR-205-5p , miR-23a-3p, miR-291a-3p, miR-9-5p, miR-92a-3p
	Mucosal melanoma	
Primary melanoma	1.61E-37	miR-10a-5p, miR-125b-5p, miR-130a-3p, miR-135a-5p, miR-142-5p, miR-146a-5p, miR-149-5p, miR-155-5p, miR-183-5p, miR-196a-5p, miR-200b-3p, miR-203a-3p, miR-205-5p, miR-218-5p, miR-23a-3p, miR-27a-3p, miR-291a-3p, miR-9-5p
Early-stage invasive cervical squamous cell carcinoma	8.29E-29	miR-10a-5p, miR-130a-3p, miR-133a-3p, miR-135a-5p, miR-142-5p, miR-149-5p, miR-155-5p, miR-17-5p, miR-192-5p, miR-19b-3p, miR-203a-3p, miR-21-5p, miR-218-5p, miR-3118, miR-9-5p
Metastatic melanoma	2.01E-27	miR-1-3p, miR-125b-5p, miR-130a-3p, miR-133a-3p, miR-135a-5p, miR-138-5p, miR-142-5p, miR-146a-5p, miR-149-5p, miR-155-5p, miR-183-5p, miR-196a-5p, miR-200b-3p, miR-203a-3p, miR-205-5p, miR-23a-3p, miR-27a-3p
Primary solid tumor	2.63E-27	miR-10a-5p, miR-125b-5p, miR-130a-3p, miR-135a-5p, miR-142-5p, miR-146a-5p, miR-149-5p, miR-155-5p, miR-17-5p, miR-183-5p, miR-196a-5p, miR-19b-3p, miR-200b-3p, miR-203a-3p, miR-205-5p, miR-21-5p, miR-218-5p, miR-23a-3p, miR-27a-3p, miR-291a-3p, miR-9-5p
Early-stage solid tumor	2.7E-25	miR-10a-5p, miR-125b-5p, miR-130a-3p, miR-133a-3p, miR-135a-5p, miR-142-5p, miR-149-5p, miR-155-5p, miR-17-5p, miR-192-5p, miR-19b-3p, miR-200b-3p, miR-203a-3p, miR-21-5p, miR-218-5p, miR-3118, miR-9-5p
Primary tumor	1.42E-24	miR-10a-5p, miR-125b-5p, miR-130a-3p, miR-135a-5p, miR-142-5p, miR-146a-5p, miR-149-5p, miR-155-5p, miR-183-5p, miR-196a-5p, miR-19b-3p, miR-200b-3p, miR-203a-3p, miR-205-5p, miR-21-5p, miR-218-5p, miR-23a-3p, miR-27a-3p, miR-291a-3p, miR-9-5p
Squamous cell cancer of the hypopharynx	3.58E-23	miR-1-3p, miR-10a-5p, miR-125b-5p, miR-130a-3p, miR-140-5p, miR-143-3p, miR-183-5p, miR-18a-5p, miR-196a-5p, miR-203a-3p, miR-21-5p, miR-218-5p, miR-7a-5p
Metastatic solid tumor	2.15E-22	miR-1-3p, miR-122-5p, miR-125b-5p, miR-130a-3p, miR-133a-3p, miR-135a-5p, miR-138-5p, miR-142-5p, miR-146a-5p, miR-149-5p, miR-155-5p, miR-183-5p, miR-196a-5p, miR-200b-3p, miR-203a-3p, miR-205-5p, miR-21-5p, miR-218-5p, miR-23a-3p, miR-27a-3p, miR-9-5p
Invasive carcinoma	5.79E-22	miR-10a-5p, miR-130a-3p, miR-133a-3p, miR-135a-5p, miR-142-5p, miR-149-5p, miR-155-5p, miR-17-5p, miR-183-5p, miR-192-5p, miR-19b-3p, miR-200b-3p, miR-203a-3p, miR-21-5p, miR-218-5p, miR-3118, miR-9-5p
Cervical squamous cell carcinoma	1.07E-20	miR-10a-5p, miR-130a-3p, miR-133a-3p, miR-135a-5p, miR-142-5p, miR-149-5p, miR-155-5p, miR-17-5p, miR-192-5p, miR-19b-3p, miR-203a-3p, miR-21-5p, miR-218-5p, miR-291a-3p, miR-3118, miR-9-5p
	Uveal meanoma	
Primary melanoma	3.49E-41	let-7a-5p, miR-100-5p, miR-10a-5p, miR-130a-3p, miR-135a-5p, miR-142-5p, miR-146a-5p, miR-149-5p, miR-155-5p, miR-183-5p, miR-196a-5p, miR-200b-3p, miR-203a-3p, miR-205-5p, miR-218-5p, miR-23a-3p, miR-27a-3p, miR-291a-3p, miR-29b-3p, miR-9-5p
Early-stage invasive cervical squamous cell carcinoma	7.76E-35	let-7a-5p, miR-10a-5p, miR-130a-3p, miR-133a-3p, miR-135a-5p, miR-142-5p, miR-149-5p, miR-155-5p, miR-181a-5p, miR-192-5p, miR-19b-3p, miR-203a-3p, miR-214-3p, miR-218-5p, miR-29b-3p, miR-3118, miR-34a-5p, miR-9-5p
Metastatic melanoma	3.78E-30	let-7a-5p, miR-1-3p, miR-130a-3p, miR-133a-3p, miR-135a-5p, miR-138-5p, miR-142-5p, miR-146a-5p, miR-149-5p, miR-155-5p, miR-183-5p, miR-196a-5p, miR-200b-3p, miR-203a-3p, miR-205-5p, miR-23a-3p, miR-27a-3p, miR-34a-5p, miR-96-5p

(Contd...)

TABLE S1. (*Continued*)

| Diseases and function annotations	*p*	miRNAs
	Cutaneous melanoma	
Early-stage solid tumor	9.25E-28	let-7a-5p, miR-10a-5p, miR-130a-3p, miR-133a-3p, miR-135a-5p, miR-142-5p, miR-149-5p, miR-155-5p, miR-181a-5p, miR-192-5p, miR-19b-3p, miR-200b-3p, miR-203a-3p, miR-214-3p, miR-218-5p, miR-29b-3p, miR-3118, miR-34a-5p, miR-9-5p
Invasive carcinoma	7.76E-26	let-7a-5p, miR-10a-5p, miR-130a-3p, miR-133a-3p, miR-135a-5p, miR-142-5p, miR-149-5p, miR-155-5p, miR-181a-5p, miR-183-5p, miR-192-5p, miR-19b-3p, miR-200b-3p, miR-203a-3p, miR-214-3p, miR-218-5p, miR-29b-3p, miR-3118, miR-34a-5p, miR-9-5p
Primary solid tumor	2.36E-25	let-7a-5p, miR-100-5p, miR-10a-5p, miR-130a-3p, miR-135a-5p, miR-142-5p, miR-146a-5p, miR-149-5p, miR-155-5p, miR-183-5p, miR-196a-5p, miR-19b-3p, miR-200b-3p, miR-203a-3p, miR-205-5p, miR-218-5p, miR-23a-3p, miR-27a-3p, miR-291a-3p, miR-29b-3p, miR-9-5p
Cervical squamous cell carcinoma	1.2E-24	let-7a-5p, miR-10a-5p, miR-130a-3p, miR-133a-3p, miR-135a-5p, miR-142-5p, miR-149-5p, miR-155-5p, miR-181a-5p, miR-192-5p, miR-19b-3p, miR-203a-3p, miR-214-3p, miR-218-5p, miR-291a-3p, miR-29b-3p, miR-3118, miR-34a-5p, miR-9-5p
Primary tumor	1.8E-24	let-7a-5p, miR-100-5p, miR-10a-5p, miR-130a-3p, miR-135a-5p, miR-142-5p, miR-146a-5p, miR-149-5p, miR-155-5p, miR-183-5p, miR-196a-5p, miR-19b-3p, miR-200b-3p, miR-203a-3p, miR-205-5p, miR-218-5p, miR-23a-3p, miR-27a-3p, miR-291a-3p, miR-29b-3p, miR-9-5p
Squamous cell cancer of the hypopharynx	2.39E-24	let-7a-5p, miR-1-3p, miR-100-5p, miR-10a-5p, miR-130a-3p, miR-143-3p, miR-183-5p, miR-196a-5p, miR-203a-3p, miR-214-3p, miR-218-5p, miR-29b-3p, miR-378a-3p, miR-7a-5p
Advanced malignant solid tumor	1.2E-20	let-7a-5p, miR-1-3p, miR-130a-3p, miR-133a-3p, miR-135a-5p, miR-138-5p, miR-142-5p, miR-146a-5p, miR-149-5p, miR-155-5p, miR-183-5p, miR-196a-5p, miR-200b-3p, miR-203a-3p, miR-205-5p, miR-218-5p, miR-23a-3p, miR-27a-3p, miR-29b-3p, miR-34a-5p, miR-9-5p, miR-96-5p
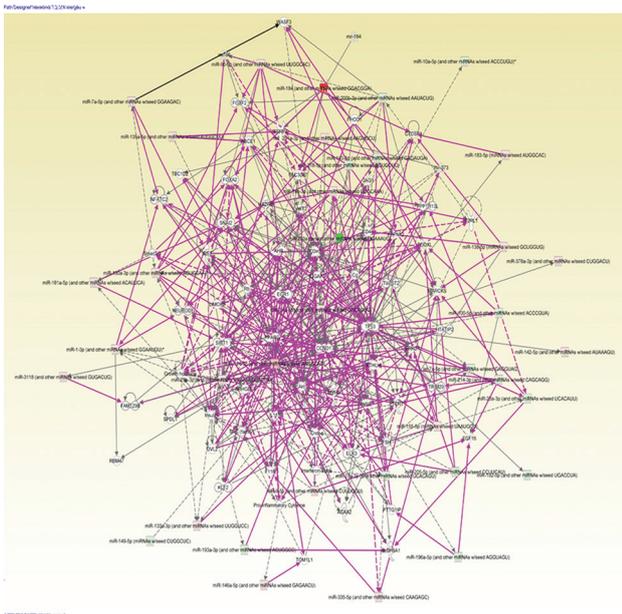
FIGURE S1. The Qiagen Ingenuity pathway analysis (IPA) in cutaneous melanoma (n = 10)
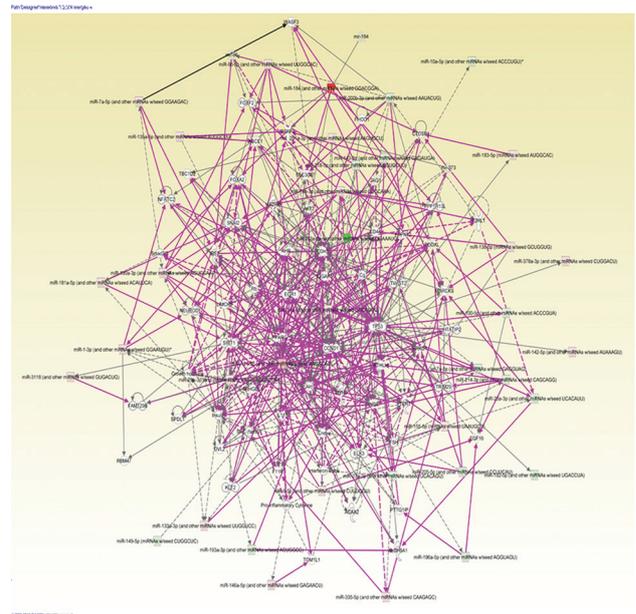

FIGURE S3. The Qiagen Ingenuity pathway analysis (IPA) in uveal melanoma (n = 10)
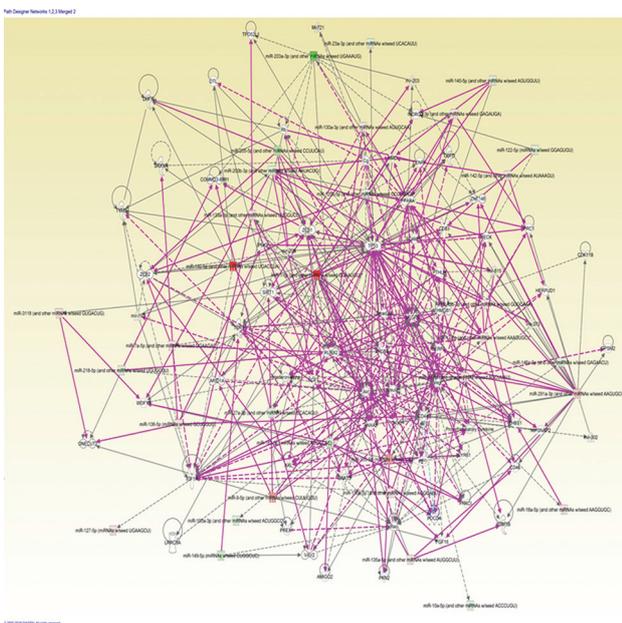

FIGURE S2. The Qiagen Ingenuity pathway analysis (IPA) in mucosal melanoma (n = 7)