Global MicroRNA Profiling for Diagnostic Appraisal of Melanocytic Spitz Tumors

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Abstract

Background—Melanoma skin cancer remains the leading cause of skin cancer related deaths. Spitz lesions represent a subset of melanocytic skin lesions characterized by epitheloid or spindled melanocytes organized in nests. These lesions occupy a spectrum ranging from benign Spitz and atypical Spitz lesions all the way to malignant Spitz tumors. Appropriate management is reliant on accurate diagnostic classification, yet this effort remains challenging utilizing current light microscopic techniques. The discovery of novel biomarkers such as microRNAs (miR) may ultimately be a useful diagnostic adjunct for the evaluation of Spitz lesions. miR expression profiles have been suggested for non-Spitz melanomas but have yet to be ascribed to Spitz lesions.

We hypothesized that distinct miR expression profiles would be associated with different lesions along the Spitz spectrum.

Materials and Methods—RNA extracted from paraffin embedded, formalin fixed tissues of 11 resected skin lesions including benign nevi (n=2), benign Spitz lesions (n=3), atypical Spitz lesions (n=3), and malignant Spitz tumors (n=3) were analyzed by the NanoString platform for simultaneous evaluation of over 800 miRs in each patient sample.

Results—Benign Spitz lesions had increased expression of miR-21-5p and miR-363-3p compared to benign nevi. Malignant Spitz lesions exhibited over-expression of miR-21-5p, miR-155-5p, and miR-1283 relative to both benign nevi and benign Spitz tumors. Notably, atypical Spitz tumors had increased expression of miR-451a and decreased expression of miR-155-5p expression relative to malignant Spitz lesions. Conversely, atypical Spitz lesions had increased expression of miR-21-5p, miR-34a-5p, miR-451a, miR-1283, and miR-1260a relative to benign Spitz tumors.

Conclusions—Overall, distinct miR profiles are suggested among Spitz lesions of varying malignant potential with some similarities to non-Spitz melanoma tumors. This work demonstrates the feasibility of this analytic method and forms the basis for further validation studies.

Keywords

Spitz; Melanoma; MicroRNA

1. Introduction

Spitz melanocytic lesions were first described by Dr. Sophie Spitz in 1948 (1). These lesions are characterized by epithelioid or spindled shaped melanocytes arranged in nests (1). A spectrum exists, ranging from benign Spitz lesions to malignant Spitz melanomas (2). Herein, we refer to an intermediate category as atypical Spitz tumors although the terms indeterminate Spitz and melanocytic tumors of uncertain malignant potential (MELTUMP) have also been used interchangeably. There has been some difficulty surrounding the accurate diagnosis of these lesions based upon standard histopathologic analysis. Byrne *et al.* found discordance in the diagnosis of 36% of Spitz lesions when evaluated by different pathologists (3). Barnhill *et al.* suggested the rate may be higher than anticipated as their group reported dermatopathologists were unable to achieve diagnostic consensus in 16 of 17 Spitz lesions (4). The clinical ramifications of an inaccurate diagnosis can be immense. Improper pathologic designation of a benign Spitz tumor as an atypical Spitz lesion may promote a transition in clinical management from a non-operative approach to one involving surgical resection. Thereby, patients may unnecessarily be subjected to the morbidity of surgery. While the exact incidence of complete lymph node dissections for Spitz lesions in the United States is difficult to discern, a systematic review of 541 patients with atypical Spitz lesions revealed that sentinel lymph node biopsy was performed in 303 (56%) patients (5). 119 of these patients (39%) had a positive sentinel lymph node and the majority (97/119 patients) went on to receive a completion lymph node dissection (5). Importantly, 99% of patients with a positive sentinel lymph node were alive at a median follow-up of 59 months. This suggests that while sentinel lymph node biopsy in the setting of an atypical Spitz tumor

is more commonly involved with microscopic disease than conventional cutaneous malignant melanoma (10-16%), sentinel lymph node involvement does not appear to be prognostic for atypical Spitz lesions (5). Roatan *et al.* found a 57% complication rate in melanocytic skin lesions requiring completion lymph node dissections, most commonly from lymphedema and infections (6). Conversely, misdiagnosis of an atypical lesion as a benign Spitz tumor may allow for disease progression through omission of surgical intervention. Ultimately, diagnostic adjuncts using novel biomarkers may be a valuable tool for clinicians faced with diagnostic uncertainty.

MicroRNAs (miR) are 19-22 nucleotide, non-coding RNA molecules that inhibit protein translation. Over 800 different microRNAs are present within human cells and the expression profile of these miRs may vary across neoplastic lesions at different stages of malignancy (7). Our group has previously shown malignant, (non-Spitz) melanomas have over-expression of miR-21 and miR-155 compared to benign nevi (8). Similarly, Jiang *et al.* discovered a sequential increase in miR-21 expression between benign nevi to primary melanoma and from primary melanoma to metastatic melanoma (P<0.05) (9). Exploitation of differential miR expression patterns has been suggested as a diagnostic adjunct for other malignancies including gastric cancer, colon cancer, and hepatocellular carcinoma (10-12).

The miR profile of Spitz lesions remains unknown. We hypothesize distinct miR expression profiles are present within lesions along the Spitz spectrum. Discovery of a differential expression profile between benign Spitz, atypical Spitz, and malignant Spitz may serve as a diagnostic aid to standard histopathology. Important clinical benefits may result from the establishment of an accurate diagnosis. In this study, the first use of NanoString nCounter miR expression profiling for global analysis of Spitz lesions is described.

2. Materials and Methods

2.1 Tissue Samples

Between 2006-2012, 11 adults with Spitz lesions or benign nevi that underwent biopsy or resection of primary lesions at The Ohio State University Wexner Medical Center were selected for analysis. 2-3 sections with sufficient paraffin embedded tumor were selected at random for further analysis. The diagnosis was confirmed using histopathologic evaluation by co-author SBP. Paraffin embedded tissue samples of lesions were obtained for further analysis. This work was conducted under the auspices of The Ohio State University IRB protocol 2007C0015 .

2.2 RNA Isolation and NanoString microRNA Profiling

RNA isolation from paraffin embedded samples was performed using RecoverAll Total Nucleic Acid Isolation Kit® (Ambion, Foster City, CA). 100 ng of isolated RNA was loaded onto a NanoString nCounter (NanoString Technologies, Seattle, WA) platform and miR quantification was carried out as previously described (13). Briefly, miRs were ligated with DNA tags to normalize melting temperatures and provide unique identification of each miR. Excess tags were removed and a panel of capture and reporter probes containing unique fluorescent signals corresponding to individual miRs were hybridized at 64°C for 18 hours.

Immobilization of hybridized probes onto a streptavidin coated cartridge was performed using nCounter Prep Station (NanoString Technologies, Seattle, WA). The fluorescence of each hybridized miR was measured by an nCounter Digital analyzer (NanoString Technologies, Seattle, WA) with a high density scan containing 600 fields of view. The investigation included 5 negative and 5 positive synthetic controls containing sequences not homologous to any known organism as well as 5 housekeeping genes supplied by NanoString Technologies (Seattle, WA).

2.3 Technical normalization of NanoString nCounter microRNA expression

Six positive controls at known concentrations supplied by NanoString Technologies (Seattle, WA) were included in each nCounter microRNA expression assay to calculate a Normalization Factor and control for inter-lane variation. For each lane, the counts for all 6 positive controls were summed to generate a value representing the "Sum of Positives." A Normalization Reference was calculated by taking the average of all Sum of Positives values. In order to generate a Normalization Factor for each lane, each Sum of Positives value was divided by the Normalization Reference. Normalized endogenous miR targets were obtained by multiplying each target count value by the Normalization Factor for that lane. The Normalization Factor is recommended to be between 0.3 and 3 (NanoString Technologies, Seattle, WA). One benign nevi sample that was outside of this range was removed from further analysis to yield 11 samples with reliable measurements.

2.4 Unsupervised Hierarchical Clustering of miRs

Broad Institute GENE-E software was used to conduct unsupervised hierarchical clustering across the four sample types using all miR expression features: http://www.broadinstitute.org/cancer/software/GENE-E/. Euclidean distance metric and the average linkage method was implemented for dendrogram construction.

2.5 Differential miR expression analysis and Statistics

edgeR software was used for differential expression in technically normalized miR expression data between sample groups at False Discovery Rate (FDR) <0.05. Implementation of edgeR analysis was conducted via the EDDA platform: http://edda.gis.a-star.edu.sg/dad/ (14).

2.6 Pathway enrichment analysis of miR target genes

Differentially expressed miRs discovered via edgeR analysis were mapped to their experimentally validated target genes contained within the miRTarBase, and the union of the enriched KEGG pathways were identified using a hypergeometric test and FDR-correction for p-values via the DIANA miRPath program (15-17). Dendrogram branches were generated to visualize clustered miRs and pathways.

3. Results

3.1 Clinical Results

10 females and 1 male were included in the sample and the average age of patients was 23.2 years old (Table 1). There were no local recurrences or deaths. Patient 7 had a right upper extremity lesion and was the only individual to undergo a sentinel lymph node biopsy. 0/6 nodes were positive for microscopic disease. She has been followed for 75 months during which she developed metachronous cutaneous lesions at different sites containing atypical foci of: vulva, left thigh, left arm, left arm and chest at 12, 54, 57, 68 and 70 months, respectively. Additionally, she developed malignant melanoma (T1b) of the left arm at 59 months and underwent a sentinel lymph node biopsy that revealed 0/2 nodes were positive for microscopic disease. She currently has no evidence of disease 16 months following resection of her malignant melanoma.

3.2. Unsupervised hierarchical clustering analysis of microRNA expression

In order to investigate how global patterns of miR expression could influence groupings of Spitz tumors and benign nevi, we performed unsupervised hierarchical clustering using all miR features across all samples (n=11) in this study. The resultant dendrogram from this analysis depicts the relative closeness of distinct patient samples (Figure 1). 4 out of 5 benign nevi and benign Spitz tumors are clustered together. Additionally, 2 of 3 atypical Spitz tumors are tightly clustered. Conversely, Spitz melanoma tumors do not appear to cluster together according to their global miR expression patterns, perhaps to due heterogeneous tumor biology among these select lesions.

3.3 MicroRNA pattern distinguishes benign Spitz lesions

To identify differentially expressed miRs between tumor samples, miR expression within patient samples (n=11) was quantified using the NanoString platform and analyzed using the edgeR program. Differentially expressed miRs were considered significant at an FDR < 0.05 (**Supplemental Tables S1-S6**). To determine the miR expression profile suggestive of a Spitz lesion, benign Spitz tumors (n=3) were compared to benign nevi (n=2). Benign Spitz lesions exhibited an up-regulation of miR-21-5p and miR-363-3p relative to benign nevi (Figure 2) (FDR<0.05).

3.4 MicroRNA pattern distinguishing malignant Spitz lesions

To characterize the miR profile of a malignant Spitz lesion, malignant Spitz tumors (n=3) were first compared to benign nevi (n=2). Similar to benign Spitz lesions, malignant Spitz tumors were also distinguished from benign nevi by up-regulation of miR-21-5p (Figure 3) (FDR<0.05). Additionally, malignant Spitz tumors had up-regulation of miR-155-5p and miR-1283 relative to benign nevi by (FDR<0.05). To confirm these findings, malignant Spitz melanomas (n=3) were compared to benign Spitz tumors (n=3). This comparison also revealed an increase in miR-21-5p, miR-155-5p, and miR-1283 expression associated with malignant Spitz tumors (Figure 4) (FDR<0.05).

3.5 MicroRNA pattern distinguishing Atypical Spitz Lesions

We next explored potential differences in the miR pattern between atypical Spitz lesions and other categories of Spitz lesions. Atypical Spitz lesions were compared separately to benign and malignant Spitz lesions. A comparison of atypical Spitz lesions to malignant Spitz lesions revealed increased miR-451a and decreased miR-155-5p expression associated with atypical Spitz lesions (Figure 5). In contrast, a comparison of atypical Spitz lesions (n=3) to benign Spitz lesions (n=3) revealed increased expression of miR-21-5p, miR-34a-5p, miR-451a, miR-283, and miR-1260a associated with atypical lesions. These results suggest a potential incremental increase in miR-21 expression from a benign nevi towards a malignant Spitz lesion (Figure 6).

3.6 Downstream targets of dysregulated microRNAs

In order to understand the functional impact of differentially expressed miRs on the various Spitz lesions, the downstream mRNA targets of differentially expressed miRs and their involvement within biological pathways were explored. It is widely recognized that individual miRs can control dozens of genes, and many other miRs have been shown to synchronize their activity in targeting a diverse array of cellular and molecular processes (18). In order to decipher the biological pathways affected the complex cross-talk among differentially expressed miRs, we used a computational approach to determine significantly enriched pathways. Differentially expressed miRs were mapped to their experimentally validated target genes via miRTarBase, and enriched biological pathways were identified using the DIANA miRPath program (hypergeometric distribution). Clustered miRs and pathways were visualized in a heatmap in order to demonstrate sets of significantly enriched pathways. As an example, the results for 5 differentially expressed miRs (miR-21-5p, miR-34a-5p, miR-451a, miR-1283, and miR-1260a) derived from comparing benign Spitz vs. atypical Spitz tumors are shown in **Figure 7**. This comparison yielded 25 significantly enriched target pathways (FDR<0.05, **Supplemental Table S8**). miR-34a-5p and miR-21-5p showed the highest degree of enriched biological pathways in Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways (http://www.genome.jp/kegg/). Interestingly, the pattern of significantly enriched pathways appeared to be mutually exclusive between miR-34a-5p and miR-21-5p, suggesting these miRs may regulate specific biological functions in a non-redundant manner. For example, miR-34a-5p appears to be enriched for a set of pathways involved in DNA processes, including DNA replication, pyrimidine metabolism, transcriptional misregulation in cancer and cell cycle. Highly significantly enriched pathways for miR-21-5p target genes appear to be disease-specific, including colorectal cancer, bladder cancer, pancreatic cancer, hepatitis B and pathways in cancer. The full list of enriched target pathways are provided in **Supplemental Tables S7-12**. The results of these biological pathway enrichment studies may be used to inform future functional studies that seek to confirm the roles of specific miRs on the development of Spitz lesions.

4. Discussion

The current report describes the first use of the NanoString platform for characterization of the miR transcriptome in melanocytic Spitz lesions. Specifically, benign Spitz lesions were characterized by an up-regulation of miR-21-5p and miR-363-3p relative to benign nevi,

whereas malignant Spitz lesions are differentiated from both benign nevi and benign Spitz tumors by up-regulation of miR-21-5p, miR-155-5p, and miR-1283. Atypical Spitz tumors have increased miR-451a and decreased miR-155-5p expression relative to malignant Spitz lesions, while increased expression of miR-21-5p, miR-34a-5p, miR-451a, miR-1283, and miR-1260a was present in atypical Spitz lesions relative to benign Spitz tumors. A summary of key results is shown in **Figure 8**. Together, these results suggest distinct miR expression profiles may be present across Spitz lesions of varying malignant potential and could serve as a basis for further validation studies.

Atypical Spitz lesions remain a diagnostic challenge (4). As such, new biomarkers have been explored in an attempt to augment diagnostic light microscopy. Investigations into BRAF mutations reveal the presence of V600E mutations in benign Spitz, atypical Spitz, and malignant Spitz thereby, precluding its usefulness as a means for determining malignant potential (19). Da Forno *et al.* made similar findings when employing NRAS and BRAF mutational analysis across the Spitz spectrum (20). Immunohistochemical methods focused on Ki-67, S-100, and HMB-45 levels have also been investigated as a means to distinguish Spitz lesions however, these efforts have been only partially useful for diagnostic differentiation (21). Fluorescent in situ hybridization (FISH) and comparative genomic hybridization (CHG) of DNA copy changes across Spitz lesions have been explored. Gaiser *et al.* found no clinical usefulness for FISH in a series of 22 melanocytic lesions containing probes against chromosome 6p25, centromere 6, 6q23, and 11q13 (22). The present study provides a rationale for an alternative approach using miRs for diagnostic evaluation of Spitz lesions given the ongoing development of the aforementioned methodologies.

The use of miRs as a diagnostic aid for distinguishing Spitz lesions of varying malignant potentials has not been previously reported. Similar approaches have been performed in non-Spitz melanomas with some overlapping miR patterns being identified. Our group has reported miR-21 up-regulation in primary melanomas relative to benign and dysplastic nevi (8, 9, 23). miR-21 up-regulation has also been found in borderline primary lesions that contain atypical cellular features (8). Similar to trends identified in non-Spitz melanoma by Jiang *et al.*, this study suggests a sequential increase in miR-21 expression from benign nevi to benign Spitz and benign Spitz compared to atypical Spitz and malignant Spitz lesions (9). miR-21 up-regulation in melanoma may promote oncogenesis through a variety of mechanisms, including alteration of cellular pathways involved with proliferation, migration, invasion and apoptosis (9, 24, 25). The mechanism of miR-21 mediated oncogenesis may involve inhibition of metalloprotinease-3 (TIMP3), BTG family member 2 (BTG2), programmed cell death 4 (PDCD4), or phosphate and tensin homologue (PTEN) (24, 25). Specifically, miR-21 over-expression in melanoma cells *in vitro* has been shown by our group to inhibit TIMP-3 expression and can promote increased cellular invasion (24).

These results also suggest miR-155-5p up-regulation in malignant Spitz lesions relative to benign and atypical Spitz lesions. miR-155 expression has previously been observed in malignant melanoma relative to benign nevi (8). Likewise, Segura *et al.* also found elevated miR-155 in primary and metastatic melanoma samples compared to benign nevi (P<0.02) (26). The mechanism of miR-155 oncogenesis in melanoma is incompletely understood but

may involve negative regulation of v-ski avian sarcoma viral oncogene homolog (SKI) gene, which is implicated in cell growth and invasion (27).

Herein, miR-34a-5p, miR-451a, miR-1283, and miR-1260a up-regulation was present in atypical Spitz lesions relative to benign Spitz tumors. miR-1260 up-regulation has previously been described in cutaneous malignant melanoma (28). Contrary to our findings, miR-34a down-regulation has been reported in melanoma cell lines and leads to inhibition of cellular proliferation, migration, invasion, and motility which may be mediated, in part, through inhibition of c-Met (29-32). Advantages of a global approach to analysis of miR transcriptome across Spitz lesions includes the potential to explore unfamiliar miR in melanocytic lesions. Notably, this is the first study implicating miR-363, miR-451a, and miR-1283 as potential players in melanocytic skin tumors including Spitz lesions as well as cutaneous melanomas.

While our current efforts were geared toward diagnostic differentiation, miRs have the capacity to potentially address other pertinent challenges related to Spitz lesions. In particular, the determination of prognosis for Spitz lesions remains difficult and sentinel lymph biopsies are performed to assist in predictions and guidance of treatment (33, 34). Indeed, miRs have been used in prognostication of other neoplastic settings with promising results. Tian *et al.* found miR-206 downregulation in malignant melanoma correlated with poorer 5 year survival and disease free survival (35). Investigation into the relationship of miRs for prognostication of Spitz lesions may be of interest and could be explored given current pitfalls of management.

The drawback of this study includes the limited number of patients analyzed. NanoString platforms have the capacity to hold up to 12 samples per analysis. Thus, the potential for Type II error is present when comparing multiple groups given inherent structural limitations. The female predominance of individuals within the sample may limit the generalizability of these findings. Additionally, validation of NanoString findings using PCR is necessary to control for potential Type I errors. In spite of these setbacks, the ability to analyze miR transcriptome without the need for amplification and reverse transcription has made NanoString an attractive platform (36). Furthermore, as in this investigation, minute quantities of RNA input are necessary while the capability of single miR discrimination per cell is retained (36, 37). In a recent study using fresh, frozen and formalin-fixed paraffin-embedded (FFPE) clinical samples from a wide array of tissue types, the NanoString platform was shown to be highly sensitive and reproducible across replicates (38). NanoString remains advantageous relative to other high throughput modalities given lower relative costs and less complex analysis relative to RNA-seq while providing greater sensitivity and the ability to overcome saturation issues seen with microarray techniques (39).

Validation of these miR findings in additional samples is the next step in evaluating the role of miRs Spitz lesions. Future NanoString studies should include multiple assays with balanced numbers of group samples (n=24 per group; power 0.5, alpha 0.05) to account for inter-assay variability. In particular, it will be important to evaluate the miR features individually and combinations thereof with the other histopathological features in order to

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determine the sensitivity, specificity and area under the curve (AUC) values for the potential diagnostic classifiers, as has been done for miR biomarkers in other cancers (39, 40). More definitive studies will be needed to evaluate these potential diagnostic miR biomarkers for additional prognostic and predictive value with regards to surgical management (41, 42).

We contend analyses utilizing Spitz lesions with unfavorable biology that progress to distant metastasis would be of great interest however the feasibility of this approach might be challenged by the rarity of such events. For instance, Ludgate *et al.* identified 67 patients with atypical Spitz lesions at the University of Michigan over a 13 year period and only 1 individual within this series developed recurrent disease and distant metastasis (33). To overcome these limitations, utilization of institutions with high volume of experience with melanocytic lesions and/or multi-institutional collaborations would be necessary to identify individuals with poor biology that could be used for robust comparison.

In conclusion, the present study has suggested a distinct miR transcriptome for different Spitz lesions. This effort should form the framework for further investigation of miRs in Spitz lesions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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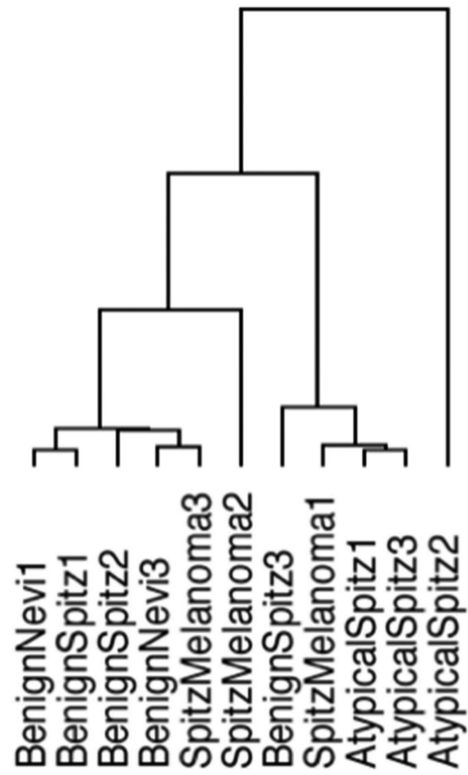
**Figure 1. Unsupervised hierarchical clustering of Spitz tumors and benign nevi across 805 miRNAs**

Unsupervised hierarchical clustering was performed on microRNA expression data of all samples using the Euclidean distance metric and average linkage method.
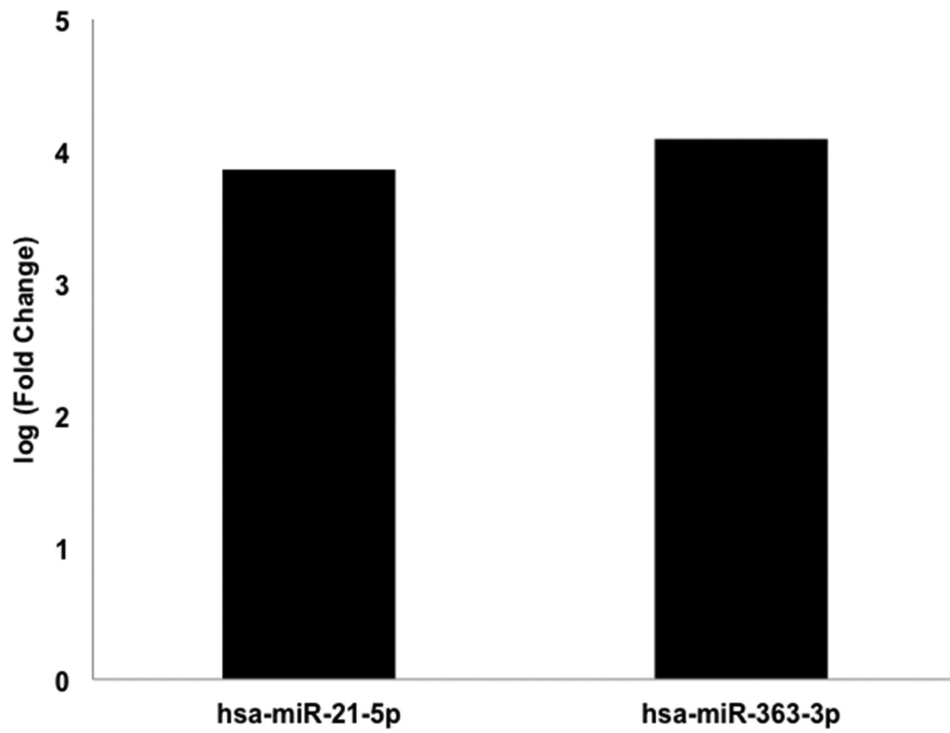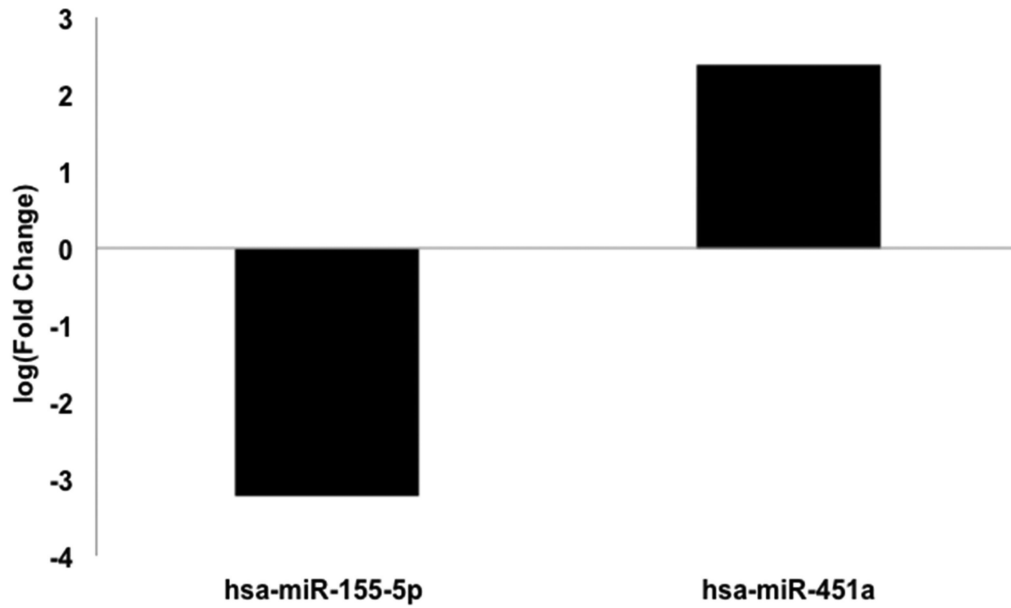
Figure 2. Differentially expressed microRNAs between benign Spitz and benign Nevi
microRNA expression of benign Spitz (n=3) compared to benign nevi (n=2) was analyzed for 805 microRNAs by NanoString nCounter. Differential expression of microRNAs with False Discovery Rate (FDR) <0.05 are shown.
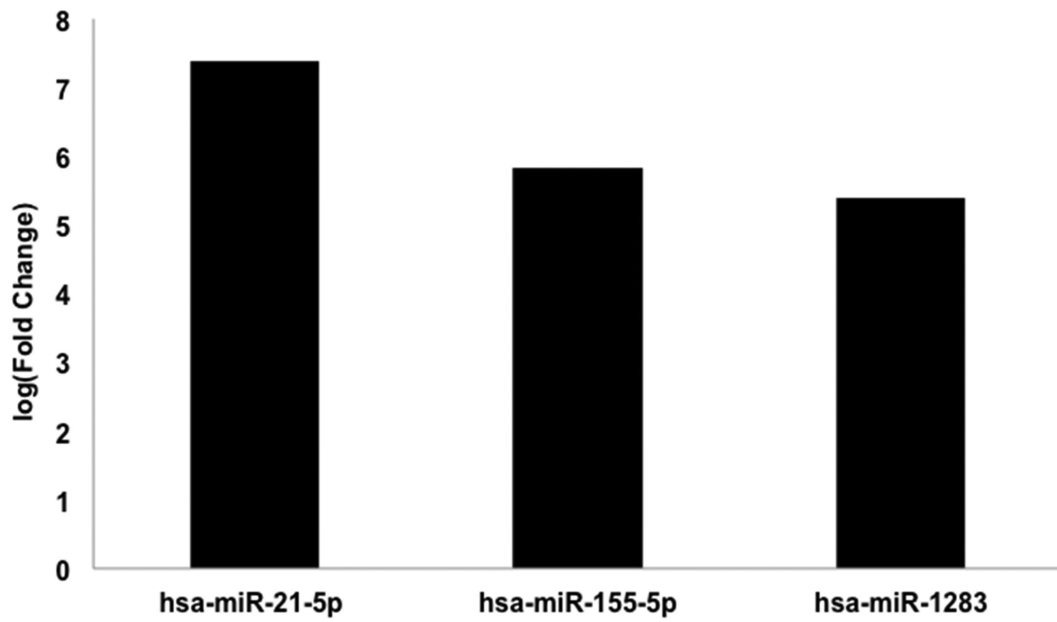
Figure 3. Differentially expressed microRNAs between Spitz melanoma and benign Nevi
microRNA expression of Spitz melanoma (n=3) compared to benign nevi (n=2) was analyzed for 805 microRNAs by NanoString nCounter. Differential expression of microRNAs with False Discovery Rate (FDR) <0.05 are shown.
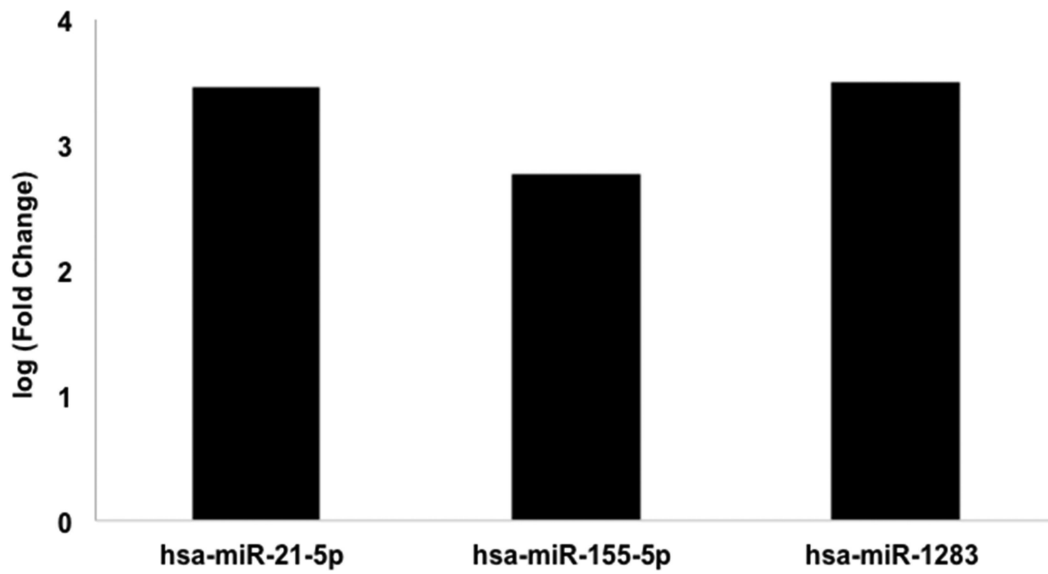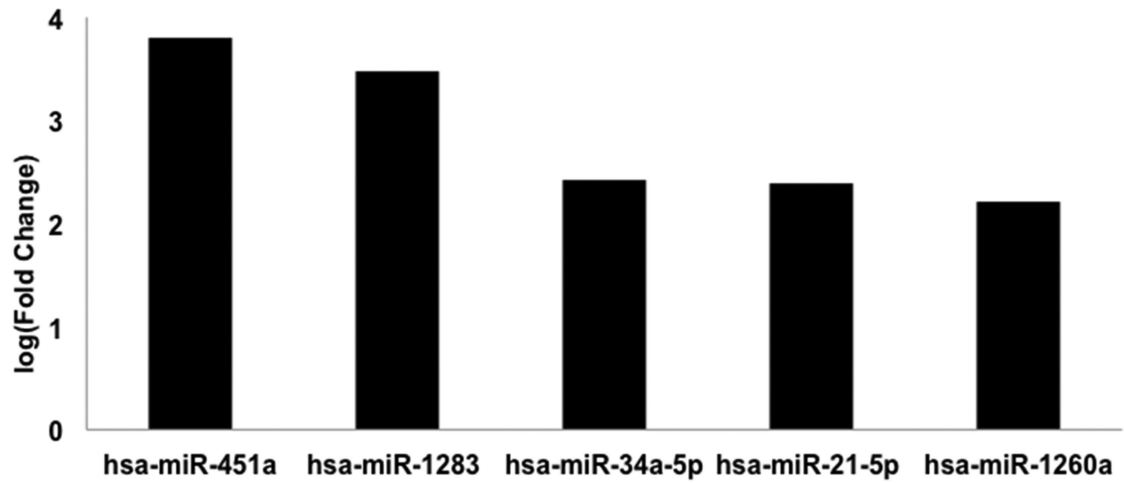
Figure 4. Differentially expressed microRNAs between Spitz melanoma and benign Spitz
microRNA expression of Spitz melanoma (n=3) compared to benign Spitz (n=3) was
analyzed for 805 microRNAs by NanoString nCounter. Differential expression of
microRNAs with False Discovery Rate (FDR) <0.05 are shown.

Figure 5. Differentially expressed microRNAs between atypical Spitz and malignant Spitz
microRNA expression of atypical Spitz lesions (n=3) compared to malignant Spitz (n=3) was analyzed for 805 microRNAs by NanoString nCounter. Differential expression of microRNAs with False Discovery Rate (FDR) <0.05 are shown.
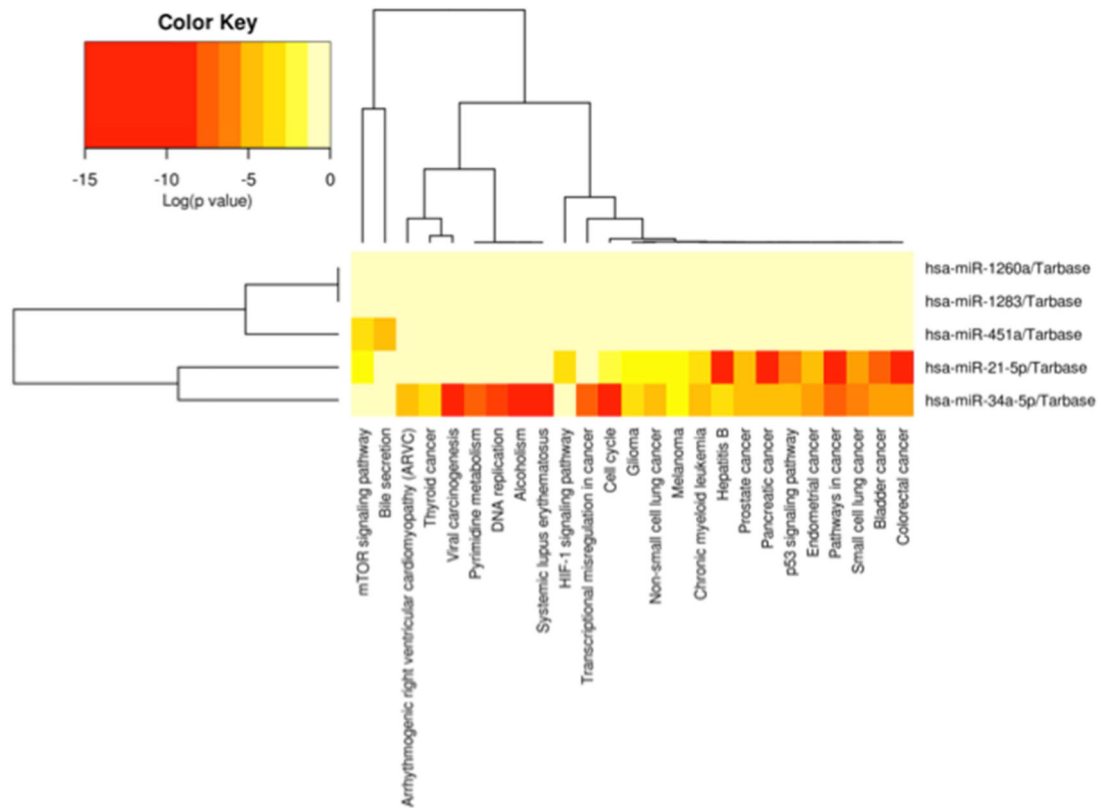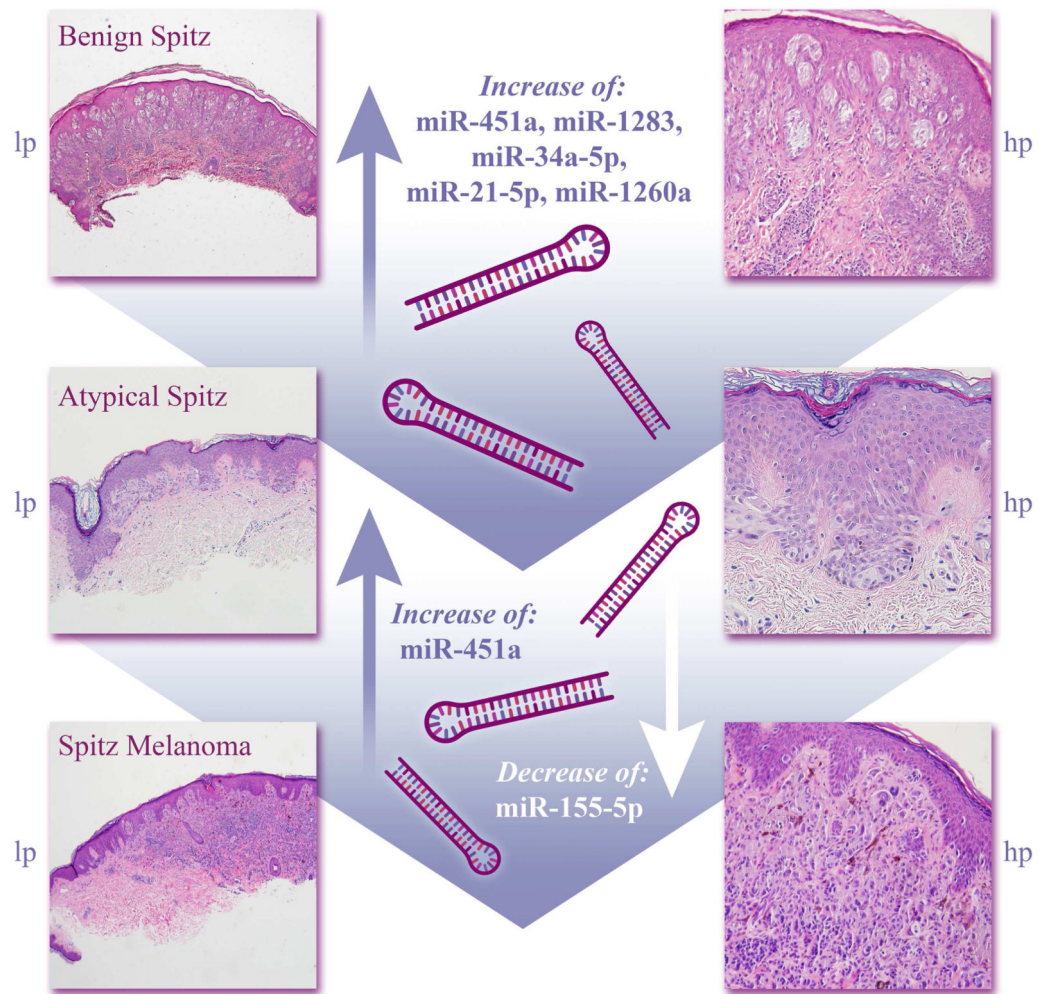
Figure 6. Differentially expressed microRNAs between atypical Spitz and benign Spitz
microRNA expression of atypical Spitz lesions (n=3) compared to benign Spitz (n=3) was analyzed for 805 microRNAs by NanoString nCounter. Differential expression of microRNAs with False Discovery Rate (FDR) <0.05 are shown.

Figure 7. Heatmap of significantly enriched biological pathways targeted by miRs from atypical Spitz vs. benign Spitz comparison

Differentially expressed miRs discovered via edgeR analysis of atypical Spitz vs. benign Spitz were mapped to their experimentally validated target genes contained within the miRTarBase, and the union of the enriched KEGG pathways were identified using a hypergeometric test and FDR-correction for p-values via the DIANA miRPath program. Dendrogram branches are shown for clustered miRs and pathways. The corresponding pathways and their significance values are listed in Supplemental Table S8.

Figure 8. Summary of select differentially expressed miRs
Increased expression of miR-451a, miR-1283, miR-34a-5p, miR-21-5p and miR-1260a distinguishes atypical Spitz relative to benign Spitz. Increased expression of miR-451a and decreased expression of miR-155-5p distinguishes Spitz melanoma relative to atypical Spitz.

Table 1

Clinical characteristics of patients.

Patient	Age at Diagnosis	Sex	Primary Tumor Site	Sentinel Lymph Node Biopsy[*]	Local Recurrence	Survival	Follow-up (months)
Benign nevi							
1	18	F	head	No	-	-	-
2	36	F	arm	No	none	alive	26
Benign Spitz							
3	18	F	buttock	No	none	alive	75
4	19	M	back	No	-	-	-
5	17	F	face	No	-	-	-
Atypical Spitz							
6	20	F	chest	No	-	-	-
7	35	F	arm	Yes- 0/6 positive	none	alive	68
8	16	F	leg	No	-	-	-
Spitzoid Melanoma							
9	26	F	arm	No	none	alive	92
10	20	F	axilla	No	-	-	-
11	30	F	face	No	none	alive	50

Clinical parameters of patients containing benign nevi, benign Spitz, atypical Spitz, and Spitzoid melanomas are shown.

(-) Indicates no data available.

[*] Total number of sentinel lymph nodes containing tumor to total number of lymph nodes biopsied.