Signature of microRNA dysregulation in spitzoid melanocytic lesions.

A Senior Honors Thesis

Presented in Partial Fulfillment of the Requirements for Graduation with distinction in Molecular Genetics in the undergraduate colleges of The Ohio State University

By

Jennifer Clark

The Ohio State University
May 2012

Project Advisors:

Dr. William Carson III, Department of Surgery

Dr. Harold Fisk, Department of Molecular Genetics

Abstract:

Background: Expression of microRNA has been found to be dysregulated among breast, hepatocellular, papillary thyroid, gastric, and colon carcinomas, as well as many other cancers. Several studies have used microRNA signatures as biomarkers to determine the diagnosis and prognosis of patient tumors. Although several studies have identified dysregulated microRNAs in malignant melanoma, dysregulated microRNAs in spitzoid melanocytic lesions have yet to be investigated. The purpose of this study is to develop a microRNA signature to be used in determining the diagnoses and prognoses of spitzoid melanocytic lesions.

Methods: A literature review was conducted to collate a list of microRNAs that were found to have significant dysregulation in primary malignant melanomas. The expression of the microRNAs was quantified by qPCR in benign nevi (n=10), benign Spitz tumors (n=8), atypical Spitz tumors (n=12), and spitzoid melanomas (n=4) tissue samples, using RNU48 (housekeeping snRNA) as an internal control.

Results: Nine microRNAs were found to be differentially expressed between the tissue types. For example, let-7a, miR-22, miR-125b, and miR-148b were found to be dysregulated in spitzoid melanomas when compared to benign Spitz tumors. The expression of let-7a, miR-125b, miR-148b, and miR-211 exhibited general trends across the tissue types as the architectural and cytological characteristics of malignancy increased.

Conclusion: These results may develop a specific microRNA signature that could distinguish one tissue type from another tissue type in a patient sample. Future studies will also include applying the signature to patient samples, verifying it with previously established histopathological methods, and following individual patient outcomes.

Introduction

Malignant melanoma is considered the most aggressive type of skin cancer. Melanoma has the fastest rising global incidence of any malignancy, as its incidence has doubled in the past 20 years[1,2]. Melanocytes, which are pigmented cells present in the basal layer of the epidermis, give rise to the malignant cells of melanoma after malignant transformation[3]. Although only 4% of all skin cancers are diagnosed as melanoma, it accounts for 74% of skin cancer deaths[4]. Early identification of a primary lesion as melanoma is critical. There is a 90% chance of survival when melanoma is diagnosed at stage I. However, the five-year survival rate decreases to 60% when it is diagnosed at stage II and then 10% at stage III. The steady decrease of survival rate following delayed diagnosis reflects a lack of effective treatments as the disease progresses[5].

Recently, studies have investigated the importance of molecular markers, including microRNA, in the diagnosis of melanoma[8]. MicroRNAs (miRs) are a class of small non-coding RNAs that measure 21-23 nucleotides in length[9]. MicroRNAs act to negatively regulate gene expression at the post-transcriptional level, blocking other RNA transcripts from being processed into their respective proteins[5,8]. The precursor to the microRNA is the pre-microRNA, which is transcribed by RNA Polymerase II. The pre-microRNA goes through normal post-transcriptional processing, including adenylation and capping. Before exiting the nucleus, it is cut to approximately 70 nucleotides in length by the RNAse III enzyme Drosha[5]. This precursor is exported out of the nucleus by the Ran-GTP-dependent nuclear export factor, exportin-5. It is then processed and cut by the RNase III endonuclease, Dicer, releasing a double-stranded mature microRNA strand. The microRNA is then incorporated into the RNA-induced silencing complex (RISC). However, only one strand of the microRNA remains stable enough to partially base complement with the 3' untranslated region (UTR) of the target

mRNA. The strand that is not functional, called the passenger strand, plays a role comparable to the guide strand functioning in the RISC complex[10]. The microRNA-RISC-mRNA association blocks the translation machinery from processing the mRNA into a protein and/or causes mRNA degradation, therefore silencing the mRNA[5]. This association ultimately results in the downregulation of the overall expression of microRNA target genes.

There are 721 identified microRNAs in the human genome that regulate approximately 30% of human genes[12]. MicroRNAs regulate mRNA targets that function in cellular differentiation, cell-cycle control, apoptosis, cell proliferation, and cell adhesion[5,7,10,13]. These processes are often altered in cancer development and progression, thereby implicating microRNA dysregulation in cancer pathogenesis[5,7,8,10,12,13,14,15,16,17,18,19,20, 21,22,23,24]. Fifty percent of microRNA genes have been located in cancer-associated genomic regions[15]. Consequently, microRNAs have received attention as potential biomarkers to be used as diagnostic and prognostic markers in cancer[8,10,12,25,26,27]. One method of applying this clincopathological potential is to develop a microRNA signature, a unique pattern of dysregulated microRNAs, specific to a type of cancer. The microRNA signature could then be compared to the microRNA pattern of a patient's lesion in order to determine an accurate classification of the tumor and its possible outcome[10].

MicroRNA signatures specific to some cancers have previously been investigated. These cancers include breast[28,29,30], hepatocellular[32], papillary thyroid[33], gastric and colon[35,36,37,38] carcinomas, as well as primary glioblastoma[31], endocrine tumors[10,33,39], lung[30,34], esophageal[40], pancreatic[41], and kidney[42] cancers. Several studies have applied microRNA signatures to determine the diagnosis and prognosis of patient tumors. MicroRNA signatures have been used

to determine prognoses in lung[43] and breast cancers[44] and diagnoses in kidney[45] and uterine carcinomas[46].

Several studies have also investigated the correlation between microRNA dysregulation and melanoma. Using real time polymerase chain reaction (qPCR), Satzger *et al.* identified miR-34a, miR-210, and miR-15b to be significantly dysregulated in primary melanoma tumors as compared to melanocytic nevi[99]. Philippidou *et al.* performed a microRNA microarray and found miR-23b, miR-125b, miR-148b, miR-150, miR-155, miR-200c, let-7a, and 23 other microRNAs to be significantly dysregulated in primary melanoma tumors as compared to benign nevi. These results were verified by qPCR[12]. Mueller *et al.* performed a microarray to find miR-17-5p, miR-148b, and 3 other microRNAs, to be significantly dysregulated in primary melanoma, which were also confirmed by qPCR[5]. Using microRNA microarray with qPCR verification, Segura *et al.* found miR-150 and miR-155 to be significantly dysregulated in 20 primary melanomas[8]. As a result, there is a great deal of interest in the potential diagnostic and prognostic uses of applying microRNA signatures to patient tissue samples by comparative means[,8,10,12,25,26,27]. These and several other publications were used in this study for the purpose of assembling a list of prominently dysregulated microRNAs in melanoma that was then studied in various melanocytic lesions.

Primary melanocytic lesions can be classified into several categories, including benign nevi, benign Spitz tumors, atypical Spitz tumors, and spitzoid melanomas. There are a number of gross and microscopic features (architectural and cytologic features) specific to each pathological type that contribute to the classification of a lesion. However, many patient lesions have a combination of features from more than one category or have a feature that exists outside the delineated categories. Therefore, the practical application of assigning these features in

spitzoid melanocytic lesions allows for subjectivity and error within a histopathological gray area[6,7]. Since the diagnosis of the lesion determines the aggressiveness of treatment necessary and the prognosis, it is imperative that the most accurate classification is made. A false-negative diagnosis could result in less treatment than necessary and could negatively impact the survival of the patient. Conversely, a false-positive diagnosis could result in unnecessary morbidity from surgery or adjuvant therapy for the patient[7].

This study involved the investigation of the microRNA signatures of four types of melanocytic lesions: benign nevi, benign Spitz tumors, atypical Spitz tumors, and spitzoid melanomas. Benign nevi are hyper-pigmented epithelial lesions that do not demonstrate any signs of malignancy at its current state. Benign Spitz tumors are benign tissue lesions that are composed of large spindled or epithelioid melanocytes that commonly develop in the first two decades of life. Spitzoid melanomas are malignant melanocytic lesions that have various architectural and cytologic attributes overlapping those of a benign Spitz tumor. Atypical Spitz tumors, which are sometimes categorized as borderline melanocytic lesions, belong to a diagnostic category that is less defined. Generally this tissue type includes lesions that have architectural and cytologic characteristics intermediate between benign Spitz tumors and spitzoid melanomas. Characteristics that indicate malignancy include displaying architectural atypicality, such as a lack of circumscription or observable maturation defects, and/or cytologic atypicality, such as a high number of cells in mitosis[6]. In this study, the candidate microRNAs were selected from a literature review and measured in each tissue type by qPCR to determine a microRNA signature for these spitzoid melanocytic lesions. Attaining differential microRNA expression between the tissue types would allow for comparative analysis and provide possible diagnostic tools for these spitzoid melanocytic lesions.

Materials and Methods

A Literature Review was conducted to determine a list of prominently dysregulated microRNAs that are suspected to influence the development of primary malignant melanoma. Nine scientific publications that focused on the presence of dysregulated microRNA in primary melanoma tissue samples were used in the collation of the list. The authors of these nine publications include: Grignol *et al.*[7], Mueller *et al.*[5], Mazar *et al.*[100], Penna *et al.*[102], Philippidou *et al.*[12], Satzger *et al.*[99], Segura *et al.*[8], Schultz *et al.*[103], and Jukic *et al.*[101]. Within these publications, the dysregulation of microRNAs in primary melanoma tissue samples was verified by qPCR. From these experiments, microRNAs were considered to be significantly dysregulated if they demonstrated a greater than 2-fold change. If the microRNA was verified to be dysregulated in more than one publication, it was considered for the list of potential microRNAs to investigate. Final discernment of which microRNA was to be included was indicated by the number of times the microRNA was confirmed by the other articles. The list was completed with twelve microRNAs that were suspected to influence the development of primary malignant melanoma. The twelve microRNAs (miRs) include: let-7a, miR-17-5p, miR-21, miR-22, miR-23b, miR-34a, miR-125b, miR-148b, miR-150, miR-155, miR-200c, and miR-211.

Dermatological Tissue Samples of four different categories of melanocytic lesions were requested for this study. These tissue types consisted of benign nevi, benign Spitz tumors, atypical Spitz tumors, and spitzoid melanomas. These samples were identified as such by Dr. Sara Peters, a dermatopathologist at The Wexner Medical Center at the Ohio State University. The tissues were obtained from the Wexner Medical Center (IRB no. 2007 C0015). The tissues were requested and received from the Ohio State University Tissue Archive Service as four to ten formalin-fixed, paraffin-embedded tissue column strips of 10-20 microns each. Ten benign

nevi, eight benign Spitz tumors, twelve atypical Spitz tumors, and four spitzoid melanomas were used for this study.

RNA Extraction was performed on the 10-20 micron formalin-fixed, paraffin-embedded (FFPE) tissue samples. RecoverAll Total Nucleic Acid Isolation Kit for FFPE was used to harvest total RNA per the manufacturer's recommendations (Ambion, Foster City, CA, USA). One milliliter of 100% xylene was added to four 10-20 micron FFPE curls to dissolve the paraffin surrounding the tissue. The sample was then incubated at 50°C for 3 minutes, vortexed, and centrifuged. Since xylene is a carcinogenic substance, after xylene was decanted, 100% ethanol was used to dilute the xylene in a safe manner, and any remnant of either liquid was evaporated. Following this, Ambion's protease and digestion buffer was added to the sample and incubated at 50°C for 15 minutes, then subsequently at 80°C for 15 minutes to safely rid the sample of protein. Isolation additive mix, which included absolute ethanol, was added to the sample. This was followed by multiple washes, which was used to isolate the nucleic acids while being passed through a filter cartridge. Sixty microliters of DNase mix, consisting of DNase and 10X DNase buffer, was added to the sample to destroy any remaining DNA residues and then incubated for 30 minutes at room temperature. Again, multiple washes were used to purify and stabilize the RNA molecules in each sample. Thirty microliters of nuclease-free water was added to elute the RNA product through a filter cartridge. The RNA was then stored at -80°C.

Nano-Drop Quantification was performed to determine the relative concentration and quality of RNA using a BioTek Epoch microplate spectrophotometer. The computer program used to process the data was BioTek's Gen5 1.11. RNA quality was determined by the wavelength 260/280 ratio of approximately 2. This ratio indicates the relative RNA to protein content,

respectively, and allows for purity determination. The concentration was calculated in nanograms per microliter and a minimum of 10 ng/µl was needed to perform the qPCR reactions with each sample.

Quantitative PCR was performed to measure the amount of microRNA in each dermatological tissue sample. The microRNAs measured were chosen from the methods described in the literature review section[5,7,8,12,99,100,101,102,103]. MicroRNA-specific primer probes were used. All reagents and primer probes were obtained from Applied Biosystems (Foster City, CA, USA). Reverse transcription reactions and qPCR was performed by the Nucleic Acid Shared Resource (NASR) at the Wexner Medical Center James Comprehensive Cancer Center. The NASR utilizes the Fast TaqMan Chemistries as directed by Applied Biosystems. Applied Biosystems TaqMan Fast Advanced Master Mix is the key regulator in this experimental process which uses Optical Fast 96-Well Plates. Each microRNA quantity was normalized to a small nucleolar "housekeeping" RNA, RNU48, as a control. Gene expression levels were quantified using the ABI Prism 7900HT Sequence Detection System (Applied Biosystems). Comparative qPCR, using the delta CT method, was performed in triplicate. MicroRNA expression was calculated as $2^{(-\Delta CT)}$ relative to the internal control RNU48[104].

Statistical Analysis was performed using an unpaired two sample Student's t-test to compare the $2^{(-\Delta CT)}$ for each tissue type. A threshold of $p<0.05$ was utilized to determine statistically significant differences between the groups tested. MicroRNA fold change expression differences between tissue types were calculated by dividing the $2^{(-\Delta CT)}$ value of one type of tissue by the $2^{(-\Delta CT)}$ value of the comparing tissue. If statistically significant, fold change in expression is expected to be above 2.0-fold when using a two sample t-test.

Results

Literature review identified twelve candidate microRNAs to further investigate. A list of microRNA candidates was selected, following an extensive literature review, for the purpose of investigating a microRNA signature of spitzoid melanocytic lesions. Several criteria had to be met in order for the publication to be considered applicable to the literature review. Melanoma cell lines were determined not to be a reliable source of data due to their high variability and unclear clinical application to actual patients. The environment in which a cell line exists is chemically and biologically different than the environment of a cell within the epithelial layer of a patient tissue sample, and therefore, possible developmental differences could result. Data from studies that used patient tissue samples may translate more appropriately to clinical use. Therefore, only publications that studied expression levels of microRNA in primary melanoma tissues were considered for the literature review.

Another criterion for a paper to be considered applicable for the literature review was the use of qPCR in the attainment of data. Results from the qPCR analysis had to exhibit a 2.0-fold difference of microRNA expression levels in primary melanomas when compared to that of benign nevi. Another commonly used method to determine differential expression of microRNA is microarray. Although a microarray shows differential microRNA expression, it does not show the degree to which the microRNA is dysregulated, and the difference in expression may not be statistically significant. Therefore, it was determined that the use of microarray could not be a valid method of determining dysregulated microRNA expression without confirmation by qPCR. Furthermore, in order for a microRNA to be considered a candidate for this investigation, the microRNA needed to show consistent results in multiple publications.

The criteria narrowed the literature review to nine publications by Grignol *et al*[7], Mueller *et al.*[5], Mazar *et al.*[100], Penna *et al.*[102], Philippidou *et al.*[12], Satzger *et al.*[99], Segura *et al.*[8], Schultz *et al.*[103],and Jukic *et al.*[101]. From these nine publications, twelve microRNAs were shown to have consistently dysregulated expression in melanoma as compared to benign nevi. These five microRNAs were upregulated in melanoma as compared to benign nevi: miR-17-5p, miR-21, miR-22, miR-150, and miR-155[5,7,8,12,101]. These seven microRNAs were downregulated in melanoma as compared to benign nevi: miR-23b, miR-34a, miR-125b, miR-148b, miR-200c, miR-21, and let-7a[5,12,17,99,100,101,103]. Upregulation is defined by increased levels of expression seen in a tissue type as compared to the level of expression observed in benign nevi. Downregulation is defined by decreased levels of expression seen in a tissue type as compared to the level of expression observed in benign nevi.

Several microRNAs were found to be differently expressed in the four tissue types. Total RNA was extracted from formalin-fixed, paraffin-embedded patient samples, including ten benign nevi, eight benign Spitz tumors, twelve atypical Spitz tumors, and four spitzoid melanomas. qPCR was used to measure the expression of each microRNA in each tissue type (See Figure 1). A small nucleolar RNA, RNU48, was used as a control, and the measured candidate microRNAs were normalized to the expression of RNU48. The delta CT method was used, allowing for fold change to be evaluated by the formula, $2^{(-\Delta CT)}$ [104]. The expression levels were then compared between each tissue type by a two sample t-test comparing the values of $2^{(-\Delta CT)}$. To be determined statistically significant, the two sample t-test calculation had to represent $p<0.05$. This indicates a less than a 5% possibility that these results are due to chance alone.

MiR-22, miR-23b, miR-34a, miR-125b, and miR-150 were found to be dysregulated in benign Spitz tumors when compared to benign nevi. The comparison of benign Spitz tumors to benign nevi revealed five microRNAs to be significantly dysregulated: miR-22, miR-23b, miR-34a, miR-125b, and miR-150. The microRNAs that were found to be upregulated in benign Spitz tumors as compared to benign nevi were miR-22 by 5.9-fold (p=0.04), miR-23b by 4.4-fold (p=0.01), miR-34a by 4.8-fold (p=0.03), and mir-150 by 6.0-fold (p=0.03). The microRNA that was found to be downregulated in benign Spitz tumors as compared to benign nevi was miR-125b by 2.1-fold (p=0.05). (See Table 1). The differential expression of let-7a, miR-17-5p, miR-21, miR-148b, miR-155, miR-200c, and miR-211 were not found to be statistically significant, indicating similar expressions between the comparable tissue types. However, none of them were uniquely similar between only these benign samples.

Let-7a, miR-22, miR-34a, miR-125b, miR-148b, miR-155 were found to be dysregulated in spitzoid melanomas when compared to benign nevi. When comparing the microRNA expressions of benign nevi and spitzoid melanomas, there were six microRNAs that were found to be significantly dysregulated in spitzoid melanoma: let-7a, miR-22, miR-34a, miR-125b, miR-148b, miR-155. The microRNAs that were found to be upregulated in spitzoid melanomas as compared to benign nevi were miR-22 by 20.9-fold (p=0.0001), miR-34a by 11.3-fold (p=0.0002), miR-148b by 76.2-fold (p=0.0002), and miR-155 by 13.1-fold (p=0.0001). The microRNAs that were found to be downregulated in spitzoid melanomas as compared to benign nevi were let-7a by 26.6-fold (p=0.01) and miR-125b by 310.0-fold (p=0.008). (See Table 2.)

Let-7a, miR-22, miR-125b, and miR-148b were found to be dysregulated in spitzoid melanomas when compared to benign Spitz tumors. The comparison of spitzoid melanomas to benign Spitz tumors revealed four microRNAs to be significantly dysregulated: let-7a,

miR-22, miR-125b, and miR-148b. The microRNAs that were found to be upregulated in spitzoid melanomas as compared to benign Spitz tumors were miR-22 by 3.5-fold (p=0.02) and mir-148b by 40.4-fold (p=0.001). The microRNAs that were found to be downregulated in spitzoid melanomas as compared to benign Spitz tumors were let-7a by 47.3-fold (p=0.05) and miR-125b by 147.0-fold (p=0.03). (See Table 3).

MiR-23b, miR-125b, and miR-155 were found to be dysregulated in atypical Spitz tumors when compared to benign nevi. The comparison of atypical Spitz tumors to benign nevi revealed three microRNAs to be significantly dysregulated: miR-23b, miR-125b, and miR-211. The microRNA that was found to be upregulated in atypical Spitz tumors as compared to benign nevi was miR-23b by 3.5-fold (p=0.008). The microRNAs that were found to be downregulated in atypical Spitz tumors as compared to benign nevi were miR-125b by 3.2-fold (p=0.002) and miR-211 by 6.0-fold (p=0.01). (See Table 4).

MiR-150 was found to be dysregulated in atypical Spitz tumors when compared to benign Spitz tumors. The comparison of benign Spitz tumors and atypical Spitz tumors demonstrated that miR-150 was under-expressed by 3.7-fold in atypical Spitz tumors (p=0.04). (See Table 5).

Let-7a, miR-22, miR-34a, miR-125b, miR-148b, and miR-155 were found to be dysregulated in spitzoid melanomas when compared to atypical Spitz tumors. The comparison of spitzoid melanomas to atypical Spitz tumors revealed seven microRNAs to be significantly dysregulated: let-7a, miR-22, miR-34a, miR-125b, and miR-148b, and miR-155. The microRNAs that were found to be upregulated in spitzoid melanomas as compared to atypical Spitz tumors were miR-22 by 12.9-fold (p=0.00004), miR-34a by 5.5-fold (p=0.0005), miR-148b by 92.8-fold (p=0.00005), and miR-155 by 3.2-fold (p=0.03). The microRNAs that

were found to be downregulated in spitzoid melanomas as compared to atypical Spitz tumors were let-7a by 28.0-fold (p=0.02) and miR-125b by 3.2-fold (p=0.02). (See Table 6). The expressions of miR-17-5p, miR-21, and miR-200c were not found to be statistically different in any tissue type comparison.

The expression levels of miR-125b, miR-148b, miR-211, and let-7a have exhibited general trends across the tissue types. There are general trends following the expression of particular microRNAs within the tissue type. MiR-125b is shown to be downregulated as the tissue samples have more architectural and cytologic characteristics of malignancy. The fold change as normalized to RNU48 is as follows: 3.2 ±1.9 in benign nevi, 1.5 ± 1.1 in benign Spitz tumors, 0.99 ±0.75 in atypical Spitz tumors, and 0.010±0.010 in spitzoid melanomas. Each expression comparison was found to be statistically significant except the fold change of expression in atypical Spitz tumors as compared to benign Spitz tumors. MiR-125b was found to be significantly under-expressed in spitzoid melanomas when compared to benign nevi (p=0.008), benign Spitz tumors (p=0.03), and atypical Spitz tumors (p=0.02). MiR-125b was also downregulated in benign Spitz tumors when compared to benign nevi (p=0.05), and in atypical Spitz tumors when compared to benign nevi (p=0.002). Also, every comparison of expression was found to be above 2.0-fold except for the comparison of the atypical Spitz tumors to benign Spitz tumors. (See Figure 2G).

MiR-148b was found to be upregulated in spitzoid melanomas with an expression of 0.71±0.456, normalized to RNU48. Conversely, miR-148b showed very small expression levels when normalized to RNU48 in benign nevi, benign Spitz tumors, and atypical Spitz tumors, (0.0093±0.0082, 0.018±0.016, 0.0077±0.0066, respectively). MiR-148b was significantly

over-expressed in spitzoid melanomas when compared to benign nevi (p=0.0002), benign Spitz tumors (p=0.002), and atypical Spitz tumors (p=0.00005). (See Figure 2H).

MiR-211 was observed to be upregulated in benign nevi tissue in comparison to other tissue types with the expression of 3.6±3.7, normalized to RNU48. Benign Spitz tumors, atypical Spitz tumors, and spitzoid melanomas have exhibited expression levels lower than benign nevi at 1.2±1.4, 0.6±0.6, and 1.1±0.80, respectively. However, the only statistically significant upregulation of miR-211 was found in benign nevi when compared to atypical Spitz tumors (p=0.01). (See Figure 2L.)

Let-7a was found to be downregulated as the architectural and cytologic characteristics of malignancy increase within the tissue spectrum of benign Spitz tumors, atypical Spitz tumors, and spitzoid melanomas. Let-7a expression in benign Spitz tumors, atypical Spitz tumors, and spitzoid melanomas were found to be 2.1±1.8, 1.2±0.89, 0.044±0.051, respectively. All fold changes were found to be statistically significant when let-7a expression in benign nevi (p=0.01), benign Spitz tumors (p=0.5), atypical Spitz tumors (p=0.02), was compared to spitzoid melanomas. (See Figure 2A).

Discussion

This study was conducted to determine a microRNA signature of spitzoid melanocytic lesions. A list of candidate microRNAs was collected following a literature review, and the microRNA expression levels were measured by qPCR from formalin-fixed, paraffin-embedded tissue patient samples. A number of microRNAs were found to have significantly different expression patterns among the following types of skin lesions: benign nevi, benign Spitz tumors, atypical Spitz tumors, and spitzoid melanomas. Analysis was conducted according to the expression pattern of each tissue type comparison.

From analysis of the qPCR data, it was observed that several microRNAs exhibited trends of differential expression. For instance, miR-125b gradually decreased in expression as the tissue type increased in architectural and cytological characteristics of malignancy. MiR-125b expression was found to be significantly different in every tissue type comparison, with the exception of atypical Spitz tumors compared to benign Spitz tumors. Philippidou *et al.*[12] found miR-125b to be downregulated in 17 primary malignant melanomas. MiR-125b is also found to be significantly dysregulated in endometrial[47,48], cervical[49,50], breast[28,51,52], prostate[53,54], thyroid[57], ovarian[58,59], colorectal[60], and bladder[61,62] cancers as well as leukemia[55,56]. Amongst the many targets of miR-125b, a prominent and well-studied target is the *BCL-2*, whose respective protein, Bcl-2, functions in apoptosis through the mitochondrial pathway[59,63,64,65,66]. Another target includes *MAZ*, whose respective protein is a Myc-associated Zinc finger transcription factor for a gene that promotes tumor-associated angiogenesis, *VEGF*[67]. One other target is *BMF*, whose respective protein is a bcl-2 modifying factor[68]. In addition, miR-125b was also found to regulate *ARID3B*, whose protein functions in the motility of breast cancer cells[69]. The downregulation of miR-125b in spitzoid melanomas would, therefore, likely result in

increased translation of *BCL-2, MAZ, BMF*, and *ARID3B.* This suggests that the effects of dysregulated miR-125b are not specific to a particular type of cancer, but are a part of the general processes that lead to cancer.

This investigation also showed that miR-148b was significantly upregulated in spitzoid melanomas when compared to the expression levels of benign nevi, benign Spitz tumors, and atypical Spitz tumors. However, Philippidou *et al.* [12] and Mueller *et al.* [5] found miR-148b to be downregulated in primary melanoma tumors. MiR-148b has also been found to be dysregulated in other cancers, including ovarian[70], colorectal[71], oral[72], gastric[73], and esophageal[74] cancers. In gastric and colorectal cancers, miR-148b is known to target a mRNA of a cholecystokinin-B receptor, which is active during digestion[71,73]. The upregulation of miR-148b in spitzoid melanomas would likely result in the downregulated translation of the mRNA of a cholecystokinin-B receptor. MiR-148b is also known to cause aberrant glycosylation in IgA nephropathy via *C1GALT1*[74]. MiR-148b may play different roles depending on the type of cell it functions in. This study's results of miR-148b upregulation in spitzoid melanomas differed from previous studies' results that showed downregulation of miR-148b in primary melanomas. This disagreement may be because this study looked at only the spitzoid type of melanoma and not all types of melanoma.

Another microRNA that exhibited differential expression was miR-211, which was upregulated in benign nevi. However, only the expression comparison of benign nevi to atypical Spitz tumors was found to be statistically significant. Jukic *et al.* [101], Mazar *et al.* [100], and Grignol *et al.* [7] found miR-211 to be downregulated in primary melanoma as compared to benign nevi. MiR-211 is found to be upregulated in colorectal cancer, and it acts by suppressing translation of *CHD5* to its respective protein, a chromodomain-helicase DNA binding protein

and an identified tumor-suppressor. When the expression of miR-211 was increased in a colorectal cancer cell line, the p53 pathway, which involves apoptosis, was also altered. This indicates that miR-211 may have an influence on the regulation of apoptosis[75]. Although this may imply a role in overall process of cancer progression, further investigation is needed to determine if miR-211 alterations are required for tumor progression.

In addition, let-7a was found to be downregulated in spitzoid melanomas when compared to benign nevi, benign Spitz tumors, and atypical Spitz tumors. Let-7a was also found to be dysregulated in melanoma in publications by Philippidou *et al.* [12] and Schultz *et al.* [103]. The dysregulation of let-7a has been found to be involved in many different types of cancer, including breast[77,78,79], lung[80], gastric[81,82,83,84], prostate[85], renal[86], colorectal [87,88], and ovarian[89] cancers. Let-7a is suspected to regulate many different targets, including the *RAS* [90,91] and *MYC*[86,92] mRNAs. The respective proteins of these target genes are both involved in the regulation of apoptosis[93,94]. The downregulation of let-7a would allow for increased translation of its targets, *RAS* and *MYC*. It is thought that involvement of let-7a in cancer is not specific to a particular tissue type, but that it is involved in the overall processes of cell proliferation, apoptosis, and the cell cycle.

A factor that may alter microRNA expression in the tissues types, benign nevi, benign Spitz tumors, and spitzoid melanomas is the V600E BRAF mutation. The protein of the BRAF gene functions to phosphorylate and activate the MAP Kinase pathway, which influences cell proliferation, cell differentiation, and transcriptional regulation. The V600E BRAF mutation causes a missense mutation in the translational code of the protein, substituting a glutamic acid for a valine. The BRAF mutation is found to be in several cancers, including non-small cell lung, colorectal, papillary thyroid, and ovarian cancers[105]. Fullen et al. found the V600E BRAF

mutation to be present in 82% of the benign tissues and in 33% of primary cutaneous melanomas studied. In addition, Fullen *et al.* found the mutation in 21% of the 28 benign Spitz tumors and 15% of the 12 spitzoid melanomas studied. However, the mutation has not been found in atypical Spitz tumors[106]. Evidence by Caramuta *et al.* suggests that the BRAF mutation may have an effect on the transcriptional regulation of microRNAs[107]. Therefore, the BRAF mutation may alter the transcriptional regulation of microRNAs in hyper-pigmented lesions. As a result, the presence of this mutation may be a confounding factor in the correlation of microRNA expression and malignancy.

In this investigation, there were some discrepancies in the results of microRNA expression as compared to previous studies. For instance, miR-23b was found to be upregulated in benign Spitz tumors and in atypical Spitz tumors when compared to benign nevi. Conversely, miR-23b was found to be downregulated in publications by Philippidou *et al.* [12] and Jukic et al[101], when comparing melanoma samples to benign nevi. In this study, miR-34a was observed to be upregulated in benign Spitz tumors as compared to benign nevi, in spitzoid melanomas as compared to benign nevi, and in spitzoid melanomas as compared to atypical Spitz tumors. Conversely, miR-34a was found to be downregulated by Satzger *et al.* when comparing melanoma to benign nevi[99]. In this study, miR-148b was found to be upregulated in spitzoid melanomas as compared to benign nevi, benign Spitz tumors, and atypical Spitz tumors. Conversely, miR-148b was found to be downregulated in publications by Philippidou *et al.* [12] and Mueller *et al.* [5] when comparing melanoma to benign nevi. Although the results disagree with the direction of dysregulation that was previously established, the expression of the microRNAs seems to be consistent across the spectrum of tissue types within this study. In addition, only melanoma and benign nevi were compared in the previous studies reviewed.

These studies exclude melanoma, specific to spitzoid type, benign Spitz tumors, and the atypical Spitz tumors, which are the main focus of this study. Other reasons that could account for the discrepancy are the differing techniques, methods, or statistical analysis used, variation upon interpretation of results, or error on the part of the investigator.

Another discrepancy is in the lack of significance found in the expression comparisons of miR-21, miR-17-5p, and miR-200c in the melanoma types studied. MiR-21 was found to be significantly upregulated in malignant melanoma as compared to benign nevi by Grignol *et al.*[7] and Jiang *et al.* [98]. MiR-17-5p was found to be significantly upregulated in malignant melanoma as compared to benign nevi by Mueller *et al.* [5] and Greenberg *et al.* [97]. MiR-200c was found to be downregulated in malignant melanoma as compared to benign nevi by Philippidou *et al.* [12], Xu *et al.* [96], and Elson Schwab *et al.* [95]. The lack of consistency between this investigation and other studies may be accounted for by the limited number of samples available for this study, specifically spitzoid melanomas. Also, this investigation looked specifically at melanoma of the spitzoid type, which may have different microRNA expression compared to that of all melanoma types combined.

This study found a number of microRNAs to be significantly dysregulated in comparisons between benign nevi, benign Spitz tumors, atypical Spitz tumors, and spitzoid melanomas. These results may develop a signature that could distinguish one tissue type from another tissue type in a patient sample. However, as there were a limited number of tissue samples available for use, further investigation measuring the microRNAs in more samples of these melanocytic lesions is necessary to establish a definitive, indisputable signature for these spitzoid melanocytic lesions. Specifically, spitzoid melanomas should be further investigated in greater number than what was attained in this study. Future studies should also include applying

the signature to patient samples, verifying it with previously established histopathological methods, and following the patient outcomes. The development of a microRNA signature in these spitzoid melanocytic lesions may lead to improved accuracy in providing a diagnosis, prognosis, and treatment plan for patients with these spitzoid melanocytic lesions.

Acknowledgements

I would like to thank Dr. Carson, Dr. Martin Del Campo, Dr. Fisk, Cristina Jaime-Ramirez, and Elizabeth Foust for their invested time and effort in providing me with exceptional mentorship while pursuing this thesis. I have learned a great deal concerning molecular processes and melanoma. I have also learned about the logistics of how different departments of molecular laboratories and derm-pathological laboratories work together. I have enjoyed my time at the Carson laboratory greatly and cannot wait to further pursue research in the medical field. I would like to give a special thanks to Dr. Peters for confirming the pathological classification of the tissue samples and providing this project with means to obtain the tissue samples. I would also like to give a special thanks to Dr. Alder, director of the NASR, who provided this project with means to use qPCR.

References

1. Parkin DM, Bray F, Ferlay J, Pisani P. Estimating the world cancer burden: Globocan 2000. Int J Cancer. 2001 Oct 15; 94(2):153-6.
2. Miller AJ, Mihm MC Jr. Melanoma. N Engl J Med. 2006 Jul 6;355(1):51-65.
3. Gray-Schopfer V, Wellbrock C, Marais R. Melanoma biology and new targeted therapy. Nature. 2007 Feb 22;445(7130):851-7.
4. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin. 2005 Mar-Apr;55(2):74-108.
5. Mueller DW, Bosserhoff AK. Role of miRNAs in the progression of malignant melanoma. Br J Cancer. 2009 Aug 18;101(4):551-6.
6. Zedek DC, McCalmont TH. Spitz nevi, atypical spitzoid neoplasms, and spitzoid melanoma. Clin Lab Med. 2011 Jun;31(2):311-20.
7. Grignol V, Fairchild ET, Zimmerer JM, Lesinski GB, Walker MJ, Magro CM, Kacher JE, Karpa VI, Clark J, Nuovo G, Lehman A, Volinia S, Agnese DM, Croce CM, Carson WE 3rd. miR-21 and miR-155 are associated with mitotic activity and lesion depth of borderline melanocytic lesions. Br J Cancer. 2011 Sep 27;105(7):1023-9. doi: 10.1038/bjc.2011.288.
8. Segura MF, Belitskaya-Lévy I, Rose AE, Zakrzewski J, Gaziel A, Hanniford D, Darvishian F, Berman RS, Shapiro RL, Pavlick AC, Osman I, Hernando E. Melanoma MicroRNA signature predicts post-recurrence survival. Clin Cancer Res. 2010 Mar 1;16(5):1577-86.
9. Zeng Y, Cullen BR. Recognition and cleavage of primary microRNA transcripts. Methods Mol Biol. 2006;342:49-56.
10. Molnár V, Tamási V, Bakos B, Wiener Z, Falus A. Changes in miRNA expression in solid tumors: an miRNA profiling in melanomas. Semin Cancer Biol. 2008 Apr;18(2):111-22.
11. Engels BM, Hutvagner G. Principles and effects of microRNA-mediated post-transcriptional gene regulation. Oncogene. 2006 Oct 9;25(46):6163-9.
12. Philippidou D, Schmitt M, Moser D, Margue C, Nazarov PV, Muller A, Vallar L, Nashan D, Behrmann I, Kreis S. Signatures of microRNAs and selected microRNA target genes in human melanoma. Cancer Res. 2010 May 15;70(10):4163-73.
13. Levati L, Alvino E, Pagani E, Arcelli D, Caporaso P, Bondanza S, Di Leva G, Ferracin M, Volinia S, Bonmassar E, Croce CM, D'Atri S. Altered expression of selected microRNAs in melanoma: antiproliferative and proapoptotic activity of miRNA-155. Int J Oncol. 2009 Aug;35(2):393-400.
14. Bargaje R, Hariharan M, Scaria V, *et al.* Consensus miRNA expression profiles derived from interplatform normalization of microarray data. RNA 2010;16:16–25.
15. Calin GA, Croce CM. MicroRNA signatures in human cancers. Nat Rev Cancer 2006;6:857–66.
16. Saunders MA, Lim LP. (micro)Genomic medicine: microRNAs as therapeutics and biomarkers. *RNA Biol* 2009;6:324–8.

17. Esquela-Kerscher A and Slack FJ: Oncomirs - microRNAs with a role in cancer. Nat Rev Cancer 6: 259-269, 2006.
18. Barbarotto E, Schmittgen TD and Calin GA: MicroRNAs and cancer: profile, profile, profile. Int J Cancer 122: 969-977,2008.
19. Gartel AL and Kandel ES: miRNAs: Little known mediators of oncogenesis. Semin Cancer Biol 18: 103-110, 2008.
20. Ma L, Teruya-Feldstein J, Weinberg RA. Tumour invasion and metastas initiated by microRNA-10b in breast cancer. Nature 2007;449:682–8.
21. Segura MF, Hanniford D, Menendez S, *et al.* Aberrant miR-182 expression promotes melanoma metastasis by repressing FOXO3 and microphthalmia-associated transcription factor. Proc Natl Acad Sci U S A 2009;106:1814–9.
22. Tavazoie SF, Alarcon C, Oskarsson T, *et al.* Endogenous human microRNAs that suppress breast cancer metastasis. Nature 2008;451: 147–52.
23. Visone R, Croce CM. MiRNAs and cancer. Am J Pathol. 2009 Apr;174(4):1131-8.
24. Mirnezami AH, Pickard K, Zhang L, Primrose JN, Packham G. MicroRNAs: key players in carcinogenesis and novel therapeutic targets. Eur J Surg Oncol. 2009 Apr;35(4):339-47.
25. Yi R, Fuchs E. MicroRNA-mediated control in the skin. Cell Death Differ 2010;17:229–35.
26. CL, Tsongalis GJ. MicroRNAs: novel biomarkers for human cancer. Clin Chem 2009;55:623–31.
27. Sand M, Gambichler T, Sand D, *et al.* MicroRNAs and the skin: tiny players in the body's largest organ. J Dermatol Sci 2009;53:169–75.
28. M.V. Iorio, M. Ferracin, C.G. Liu, A. Veronese, R. Spizzo, S. Sabbioni *et al.* MicroRNA gene expression deregulation in human breast cancer. Cancer Res, 65 (2005), pp. 7065–7070.
29. Alshatwi AA, Shafi G, Hasan TN, Syed NA, Al-Hazzani AA, Alsaif MA, Alsaif AA. Differential expression profile and genetic variants of microRNAs sequences in breast cancer patients. PLoS One. 2012;7(2):e30049. Epub 2012 Feb 20.
30. Yanaihara N, Caplen N, Bowman E, Seike M, Kumamoto K, Yi M, Stephens RM, Okamoto A, Yokota J, Tanaka T, Calin GA, Liu CG, Croce CM, Harris CC. Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. Cancer Cell. 2006 Mar;9(3):189-98.
31. S.A. Ciafre, S. Galardi, A. Mangiola, M. Ferracin, C.G. Liu, G. Sabatino *et al.* Extensive modulation of a set of microRNAs in primary glioblastoma. Biochem Biophys Res Commun, 334 (2005), pp. 1351–1358.
32. Y. Murakami, T. Yasuda, K. Saigo, T. Urashima, H. Toyoda, T. Okanoue *et al.* Comprehensive analysis of microRNA expression patterns in hepatocellular carcinoma and non-tumorous tissues. Oncogene, 25 (2006), pp. 2537–2545.
33. He, K. Jazdzewski, W. Li, S. Liyanarachchi, R. Nagy, S. Volinia *et al.* The role of microRNA genes in papillary thyroid carcinoma. Proc Natl Acad Sci USA, 102 (2005), pp. 19075–19080.
34. J. Takamizawa, H. Konishi, K. Yanagisawa, S. Tomida, H. Osada, H. Endoh *et al.* Reduced expression of the let-7 microRNAs in human lung cancers in association with shortened postoperative survival. Cancer Res, 64 (2004), pp. 3753–3756

35. Michael MZ, SM OC, van Holst Pellekaan NG, Young GP, James RJ. Reduced accumulation of specific microRNAs in colorectal neoplasia. Mol Cancer Res 2003; 1:882–91.
36. S. Volinia, G.A. Calin, C.G. Liu, S. Ambs, A. Cimmino, F. Petrocca *et al.* A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci USA, 103 (2006), pp. 2257–2261
37. Liu M, Chen H. The role of microRNAs in colorectal cancer. J Genet Genomics. 2010 Jun;37(6):347-58. Epub 2010 Jul 1.
38. Kim CH, Kim HK, Rettig RL, Kim J, Lee ET, Aprelikova O, Choi IJ, Munroe DJ, Green JE. miRNA signature associated with outcome of gastric cancer patients following chemotherapy. BMC Med Genomics. 2011 Nov 23;4:79
39. A. Bottoni, D. Piccin, F. Tagliati, A. Luchin, M.C. Zatelli, E.C. degli Uberti. miR-15a and miR-16-1 down-regulation in pituitary adenomas. J Cell Physiol, 204 (2005), pp. 280–285
40. Zhang C, Wang C, Chen X, Yang C, Li K, Wang J, Dai J, Hu Z, Zhou X, Chen L, Zhang Y, Li Y, Qiu H, Xing J, Liang Z, Ren B, Yang C, Zen K, Zhang CY. Expression profile of microRNAs in serum: a fingerprint for esophageal squamous cell carcinoma. Clin Chem. 2010 Dec;56(12):1871-9. Epub 2010 Oct 13.
41. Liu R, Chen X, Du Y, Yao W, Shen L, Wang C, Hu Z, Zhuang R, Ning G, Zhang C, Yuan Y, Li Z, Zen K, Ba Y, Zhang CY. Serum microRNA expression profile as a biomarker in the diagnosis and prognosis of pancreatic cancer. Clin Chem. 2012 Mar;58(3):610-8. Epub 2011 Dec 22
42. Petillo D, Kort EJ, Anema J, Furge KA, Yang XJ, Teh BT. MicroRNA profiling of human kidney cancer subtypes. Int J Oncol. 2009 Jul;35(1):109-14.
43. Yu SL, Chen HY, Chang GC, Chen CY, Chen HW, Singh S, Cheng CL, Yu CJ, Lee YC, Chen HS, Su TJ, Chiang CC, Li HN, Hong QS, Su HY, Chen CC, Chen WJ, Liu CC, Chan WK, Chen WJ, Li KC, Chen JJ, Yang PC. MicroRNA signature predicts survival and relapse in lung cancer. Cancer Cell. 2008 Jan;13(1):48-57.
44. Volinia S, Galasso M, Sana ME, Wise TF, Palatini J, Huebner K, Croce CM. Breast cancer signatures for invasiveness and prognosis defined by deep sequencing of microRNA. Proc Natl Acad Sci U S A. 2012 Feb 21;109(8):3024-9. Epub 2012 Feb 6.
45. Youssef YM, White NM, Grigull J, Krizova A, Samy C, Mejia-Guerrero S, Evans A, Yousef GM. Accurate molecular classification of kidney cancer subtypes using microRNA signature. Eur Urol. 2011 May;59(5):721-30. Epub 2011 Jan 13.
46. Ratner ES, Tuck D, Richter C, Nallur S, Patel RM, Schultz V, Hui P, Schwartz PE, Rutherford TJ, Weidhaas JB. MicroRNA signatures differentiate uterine cancer tumor subtypes. Gynecol Oncol. 2010 Sep;118(3):251-7. Epub 2010 Jun 9.
47. Jiang F, Liu T, He Y, Yan Q, Chen X, Wang H, Wan X. MiR-125b promotes proliferation and migration of type II endometrial carcinoma cells through targeting TP53INP1 tumor suppressor in vitro and in vivo. BMC Cancer. 2011 Oct 5;11:425.
48. Shang C, Lu YM, Meng LR. MicroRNA-125b down-regulation mediates endometrial cancer invasion by targeting ERBB2. Med Sci Monit. 2012 Apr 1;18(4):BR149-155.
49. Wilting SM, Snijders PJ, Verlaat W, Jaspers A, van de Wiel MA, van Wieringen WN, Meijer GA, Kenter GG, Yi Y, le Sage C, Agami R, Meijer CJ, Steenbergen RD. Altered microRNA expression associated with chromosomal changes contributes to cervical carcinogenesis. Oncogene. 2012 Feb 13. doi: 10.1038/onc.2012.20.

50. Huang L, Lin JX, Yu YH, Zhang MY, Wang HY, Zheng M. Downregulation of Six MicroRNAs Is Associated with Advanced Stage, Lymph Node Metastasis and Poor Prognosis in Small Cell Carcinoma of the Cervix. PLoS One. 2012;7(3):e33762. Epub 2012 Mar 16.
51. Hui AB, Shi W, Boutros PC, Miller N, Pintilie M, Fyles T, McCready D, Wong D, Gerster K, Waldron L, Jurisica I, Penn LZ, Liu FF. Robust global micro-RNA profiling with formalin-fixed paraffin-embedded breast cancer tissues. Lab Invest. 2009 May;89(5):597-606. Epub 2009 Mar 16. Erratum in: Lab Invest. 2009 Jun;89(6):726. Waldron, Levi [added].
52. Hannafon BN, Sebastiani P, de las Morenas A, Lu J, Rosenberg CL. Expression of microRNA and their gene targets are dysregulated in preinvasive breast cancer. Breast Cancer Res. 2011 Mar 4;13(2):R24. PubMed PMID: 21375733; PubMed Central PMCID: PMC3219184.
53. Ozen M, Creighton CJ, Ozdemir M, Ittmann M. Widespread deregulation of microRNA expression in human prostate cancer. Oncogene. 2008 Mar 13;27(12):1788-93.
54. Schaefer A, Jung M, Mollenkopf HJ, Wagner I, Stephan C, Jentzmik F, Miller K, Lein M, Kristiansen G, Jung K. Diagnostic and prognostic implications of microRNA profiling in prostate carcinoma. Int J Cancer. 2010 Mar 1;126(5):1166-76.
55. Klusmann JH, Li Z, Böhmer K, Maroz A, Koch ML, Emmrich S, Godinho FJ, Orkin SH, Reinhardt D. miR-125b-2 is a potential oncomiR on human chromosome 21 in megakaryoblastic leukemia. Genes Dev. 2010 Mar 1;24(5):478-90.
56. Bousquet M, Harris MH, Zhou B, Lodish HF. MicroRNA miR-125b causes leukemia. Proc Natl Acad Sci U S A. 2010 Dec 14;107(50):21558-63.
57. Vriens MR, Weng J, Suh I, Huynh N, Guerrero MA, Shen WT, Duh QY, Clark OH, Kebebew E. MicroRNA expression profiling is a potential diagnostic tool for thyroid cancer. Cancer. 2011 Oct 17. doi: 10.1002/cncr.26587.
58. Sorrentino A, Liu CG, Addario A, Peschle C, Scambia G, Ferlini C. Role of microRNAs in drug-resistant ovarian cancer cells. Gynecol Oncol. 2008 Dec;111(3):478-86.
59. Guan Y, Yao H, Zheng Z, Qiu G, Sun K. MiR-125b targets BCL3 and suppresses ovarian cancer proliferation. Int J Cancer. 2011 May 15;128(10):2274-83. doi: 10.1002/ijc.25575.
60. Nishida N, Yokobori T, Mimori K, Sudo T, Tanaka F, Shibata K, Ishii H, Doki Y, Kuwano H, Mori M. MicroRNA miR-125b is a prognostic marker in human colorectal cancer. Int J Oncol. 2011 May;38(5):1437-43. doi: 10.3892/ijo.2011.969.
61. Ichimi T, Enokida H, Okuno Y, Kunimoto R, Chiyomaru T, Kawamoto K, Kawahara K, Toki K, Kawakami K, Nishiyama K, Tsujimoto G, Nakagawa M, Seki N. Identification of novel microRNA targets based on microRNA signatures in bladder cancer. Int J Cancer. 2009 Jul 15;125(2):345-52.
62. Huang L, Luo J, Cai Q, Pan Q, Zeng H, Guo Z, Dong W, Huang J, Lin T. MicroRNA-125b suppresses the development of bladder cancer by targeting E2F3. Int J Cancer. 2011 Apr 15;128(8):1758-69. doi: 10.1002/ijc.25509.
63. Kong F, Sun C, Wang Z, Han L, Weng D, Lu Y, Chen G. miR-125b confers resistance of ovarian cancer cells to cisplatin by targeting pro-apoptotic Bcl-2 antagonist killer 1. J Huazhong Univ Sci Technolog Med Sci. 2011 Aug;31(4):543-9.

64. Zhao A, Zeng Q, Xie X, Zhou J, Yue W, Li Y, Pei X. MicroRNA-125b induces cancer cell apoptosis through suppression of Bcl-2 expression. J Genet Genomics. 2012 Jan;39(1):29-35. doi: 10.1016/j.jgg.2011.12.003.
65. Zeng CW, Zhang XJ, Lin KY, Ye H, Feng SY, Zhang H, Chen YQ. Camptothecin Induces Apoptosis in Cancer Cells via MicroRNA-125b-Mediated Mitochondrial Pathways. Mol Pharmacol. 2012 Apr;81(4):578-86.
66. Zhou M, Liu Z, Zhao Y, Ding Y, Liu H, Xi Y, Xiong W, Li G, Lu J, Fodstad O, Riker AI, Tan M. MicroRNA-125b confers the resistance of breast cancer cells to paclitaxel through suppression of pro-apoptotic Bcl-2 antagonist killer 1 (Bak1) expression. J Biol Chem. 2010 Jul 9;285(28):21496-507.
67. Smits M, Wurdinger T, van Het Hof B, Drexhage JA, Geerts D, Wesseling P, Noske DP, Vandertop WP, de Vries HE, Reijerkerk A. Myc-associated zinc finger protein (MAZ) is regulated by miR-125b and mediates VEGF-induced angiogenesis in glioblastoma. FASEB J. 2012 Mar 13.
68. Xia HF, He TZ, Liu CM, Cui Y, Song PP, Jin XH, Ma X. MiR-125b expression affects the proliferation and apoptosis of human glioma cells by targeting Bmf. Cell Physiol Biochem. 2009;23(4-6):347-58.
69. Akhavantabasi S, Sapmaz A, Tuna S, Erson-Bensan AE. miR-125b targets ARID3B in breast cancer cells. Cell Struct Funct. 2012;37(1):27-38.
70. Chang H, Zhou X, Wang ZN, Song YX, Zhao F, Gao P, Chiang Y, Xu HM. Increased expression of miR-148b in ovarian carcinoma and its clinical significance. Mol Med Report. 2012 May;5(5):1277-80. doi: 10.3892/mmr.2012.794.
71. Song Y, Xu Y, Wang Z, Chen Y, Yue Z, Gao P, Xing C, Xu H. MicroRNA-148b suppresses cell growth by targeting cholecystokinin-2 receptor in colorectal cancer. Int J Cancer. 2011 Oct 23. doi: 10.1002/ijc.26485.
72. Lu YC, Chen YJ, Wang HM, Tsai CY, Chen WH, Huang YC, Fan KH, Tsai CN, Huang, SF, Kang CJ, Chang JT, Cheng AJ. Oncogenic Function and Early Detection Potential of miRNA-10b in Oral Cancer as Identified by microRNA Profiling. Cancer Prev Res (Phila). 2012 Apr;5(4):665-74.
73. Song YX, Yue ZY, Wang ZN, Xu YY, Luo Y, Xu HM, Zhang X, Jiang L, Xing CZ, Zhang Y. MicroRNA-148b is frequently down-regulated in gastric cancer and acts as a tumor suppressor by inhibiting cell proliferation. Mol Cancer. 2011 Jan 4;10:1.
74. Zhang C, Wang C, Chen X, Yang C, Li K, Wang J, Dai J, Hu Z, Zhou X, Chen L, Zhang Y, Li Y, Qiu H, Xing J, Liang Z, Ren B, Yang C, Zen K, Zhang CY. Expression profile of microRNAs in serum: a fingerprint for esophageal squamous cell carcinoma. Clin Chem. 2010 Dec;56(12):1871-9.
75. Serino G, Sallustio F, Cox SN, Pesce F, Schena FP. Abnormal miR-148b Expression Promotes Aberrant Glycosylation of IgA1 in IgA Nephropathy. J Am Soc Nephrol. 2012 Feb 23.
76. Cai C, Ashktorab H, Pang X, Zhao Y, Sha W, Liu Y, Gu X. MicroRNA-211 expression promotes colorectal cancer cell growth in vitro and in vivo by targeting tumor suppressor CHD5. PLoS One. 2012;7(1):e29750. Epub 2012 Jan 3.
77. Kim SJ, Shin JY, Lee KD, Bae YK, Sung KW, Nam SJ, Chun KH. MicroRNA let-7a suppresses breast cancer cell migration and invasion through downregulation of C-C chemokine receptor type 7. Breast Cancer Res. 2012 Jan 18;14(1):R14.
78. Lu L, Katsaros D, Zhu Y, Hoffman A, Luca S, Marion CE, Mu L, Risch H, Yu H. Let-

7a regulation of insulin-like growth factors in breast cancer. Breast Cancer Res Treat. 2011 Apr;126(3):687-94.
79. Oliveras-Ferraros C, Cufí S, Vazquez-Martin A, Torres-Garcia VZ, Del Barco S, Martin-Castillo B, Menendez JA. Micro(mi)RNA expression profile of breast cancer epithelial cells treated with the anti-diabetic drug metformin: induction of the tumor suppressor miRNA let-7a and suppression of the TGFβ-induced oncomiR miRNA-181a. Cell Cycle. 2011 Apr 1;10(7):1144-51. Epub 2011 Apr 1. PubMed PMID: 21368581.
80. Heegaard NH, Schetter AJ, Welsh JA, Yoneda M, Bowman ED, Harris CC. Circulating micro-RNA expression profiles in early stage nonsmall cell lung cancer. Int J Cancer. 2012 Mar 15;130(6):1378-86. doi: 10.1002/ijc.26153. Epub 2011 Aug 26
81. Song J, Bai Z, Han W, Zhang J, Meng H, Bi J, Ma X, Han S, Zhang Z. Identification of Suitable Reference Genes for qPCR Analysis of Serum microRNA in Gastric Cancer Patients. Dig Dis Sci. 2012 Apr;57(4):897-904. Epub 2011 Dec 25.
82. Ma YY, Tao HQ. Microribonucleic acids and gastric cancer. Cancer Sci. 2012 Apr;103(4):620-5. doi: 10.1111/j.1349-7006.2011.02185.x. Epub 2012 Jan 13.
83. Yang Q, Jie Z, Cao H, Greenlee AR, Yang C, Zou F, Jiang Y. Low-level expression of let-7a in gastric cancer and its involvement in tumorigenesis by targeting RAB40C. Carcinogenesis. 2011 May;32(5):713-22. Epub 2011 Feb 24.
84. Zhu Y, Zhong Z, Liu Z. Lentiviral vector-mediated upregulation of let-7a inhibits gastric carcinoma cell growth in vitro and in vivo. Scand J Gastroenterol. 2011 Jan;46(1):53-9. Epub 2010 Sep 1.
85. Dong Q, Meng P, Wang T, Qin W, Qin W, Wang F, Yuan J, Chen Z, Yang A, Wang H. MicroRNA let-7a inhibits proliferation of human prostate cancer cells in vitro and in vivo by targeting E2F2 and CCND2. PLoS One. 2010 Apr 14;5(4):e10147.
86. Liu Y, Yin B, Zhang C, Zhou L, Fan J. Hsa-let-7a functions as a tumor suppressor in renal cell carcinoma cell lines by targeting c-myc. Biochem Biophys Res Commun. 2012 Jan 6;417(1):371-5. Epub 2011 Dec 1. PubMed PMID: 22155254.
87. Vickers MM, Bar J, Gorn-Hondermann I, Yarom N, Daneshmand M, Hanson JE, Addison CL, Asmis TR, Jonker DJ, Maroun J, Lorimer IA, Goss GD, Dimitroulakos J. Stage-dependent differential expression of microRNAs in colorectal cancer: potential role as markers of metastatic disease. Clin Exp Metastasis. 2012 Feb;29(2):123-32. doi: 10.1007/s10585-011-9435-3.
88. Wang F, Zhang P, Ma Y, Yang J, Moyer MP, Shi C, Peng J, Qin H. NIRF is frequently upregulated in colorectal cancer and its oncogenicity can be suppressed by let-7a microRNA. Cancer Lett. 2012 Jan 28;314(2):223-31.
89. Lu L, Katsaros D, Zhu Y, Hoffman A, Luca S, Marion CE, Mu L, Risch H, Yu H. Let-7a regulation of insulin-like growth factors in breast cancer. Breast Cancer Res Treat. 2011 Apr;126(3):687-94.
90. Khodayari N, Mohammed KA, Goldberg EP, Nasreen N. EphrinA1 inhibits malignant mesothelioma tumor growth via let-7 microRNA-mediated repression of the RAS oncogene. Cancer Gene Ther. 2011 Nov;18(11):806-16. doi: 10.1038/cgt.2011.50. Epub 2011 Aug 26.
91. Johnson SM, Grosshans H, Shingara J, Byrom M, Jarvis R, Cheng A, Labourier E, Reinert KL, Brown D, Slack FJ. RAS is regulated by the let-7 microRNA family. Cell. 2005 Mar 11;120(5):635-47.
92. Sampson VB, Rong NH, Han J, Yang Q, Aris V, Soteropoulos P, Petrelli NJ, Dunn SP,

Krueger LJ. MicroRNA let-7a down-regulates MYC and reverts MYC-induced growth in Burkitt lymphoma cells. Cancer Res. 2007 Oct 15;67(20):9762-70.
93. Downward J. Ras signalling and apoptosis. Curr Opin Genet Dev. 1998 Feb;8(1):49-54. Review.
94. Prendergast GC. Mechanisms of apoptosis by c-Myc. Oncogene. 1999 May 13;18(19):2967-87. Review.
95. Elson-Schwab I, Lorentzen A, Marshall CJ. MicroRNA-200 family members differentially regulate morphological plasticity and mode of melanoma cell invasion. PLoS One. 2010 Oct 4;5(10). pii: e13176.
96. Xu Y, Brenn T, Brown ER, Doherty V, Melton DW. Differential expression of microRNAs during melanoma progression: miR-200c, miR-205 and miR-211 are downregulated in melanoma and act as tumour suppressors. Br J Cancer. 2012 Jan 31;106(3):553-61. doi: 10.1038/bjc.2011.568. Epub 2012 Jan 5.
97. Greenberg E, Hershkovitz L, Itzhaki O, Hajdu S, Nemlich Y, Ortenberg R, Gefen N, Edry L, Modai S, Keisari Y, Besser MJ, Schachter J, Shomron N, Markel G. Regulation of cancer aggressive features in melanoma cells by microRNAs. PLoS One. 2011 Apr 25;6(4):e18936.
98. Jiang L, Lv X, Li J, Li J, Li X, Li W, Li Y. The status of microRNA-21 expression and its clinical significance in human cutaneous malignant melanoma. Acta Histochem. 2011 Nov 28.
99. Satzger I, Mattern A, Kuettler U, Weinspach D, Voelker B, Kapp A, Gutzmer R. MicroRNA-15b represents an independent prognostic parameter and is correlated with tumor cell proliferation and apoptosis in malignant melanoma. Int J Cancer. 2010 Jun 1;126(11):2553-62.
100. Mazar J, DeYoung K, Khaitan D, Meister E, Almodovar A, Goydos J, Ray A, Perera RJ. The regulation of miRNA-211 expression and its role in melanoma cell invasiveness. PLoS One. 2010 Nov 1;5(11):e13779.
101. Jukic DM, Rao UN, Kelly L, Skaf JS, Drogowski LM, Kirkwood JM, Panelli MC. Microrna profiling analysis of differences between the melanoma of young adults and older adults. J Transl Med. 2010 Mar 19;8:27.
102. Penna E, Orso F, Cimino D, Tenaglia E, Lembo A, Quaglino E, Poliseno L, Haimovic A, Osella-Abate S, De Pittà C, Pinatel E, Stadler MB, Provero P, Bernengo MG, Osman I, Taverna D. microRNA-214 contributes to melanoma tumour progression through suppression of TFAP2C. EMBO J. 2011 May 18;30(10):1990-2007
103. Schultz N, Marenstein DR, De Angelis DA, Wang WQ, Nelander S, Jacobsen A, Marks DS, Massagué J, Sander C. Off-target effects dominate a large-scale RNAi screen for modulators of the TGF-β pathway and reveal microRNA regulation of TGFBR2. Silence. 2011 Mar 14;2:3.
104. Schmittgen TD, Lee EJ, Jiang J, Sarkar A, Yang L, Elton TS, Chen C. Real-time PCR quantification of precursor and mature microRNA. Methods. 2008 Jan;44(1):31-8. Review.
105. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W, Davis N, Dicks E, Ewing R, Floyd Y, Gray K, Hall S, Hawes R, Hughes J, Kosmidou V, Menzies A, Mould C, Parker A, Stevens C, Watt S, Hooper S, Wilson R, Jayatilake H, Gusterson BA, Cooper C, Shipley J, Hargrave D, Pritchard-Jones K, Maitland N, Chenevix-Trench G, Riggins GJ, Bigner DD, Palmieri G, Cossu

A, Flanagan A, Nicholson A, Ho JW, Leung SY, Yuen ST, Weber BL, Seigler HF, Darrow TL, Paterson H, Marais R, Marshall CJ, Wooster R, Stratton MR, Futreal PA. Mutations of the BRAF gene in human cancer. Nature. 2002 Jun 27;417(6892):949-54.

106. Fullen DR, Poynter JN, Lowe L, Su LD, Elder JT, Nair RP, Johnson TM, Gruber SB. BRAF and NRAS mutations in spitzoid melanocytic lesions. Mod Pathol. 2006 Oct;19(10):1324-32.
107. Caramuta S, Egyházi S, Rodolfo M, Witten D, Hansson J, Larsson C, Lui WO. MicroRNA expression profiles associated with mutational status and survival in malignant melanoma. J Invest Dermatol. 2010 Aug;130(8):2062-70.