Journal of Positive School Psychology http://journalppw.com
2022, Vol. 6, No. 2, 1490 – 1496

MicroRNA 17-5p Overexpression Contributes to melanoma cancer metastasis

Esmaeil Abyar

Diploma in experimental science
Abyar.esmaeilhoo@gmail.com

ABSTRACT

Melanoma is a very aggressive form of skin cancer that spreads swiftly and creates distant metastases. Melanoma early identification and staging can benefit patient treatment. MicroRNA 17-5p is being investigated in this study to determine if it helps distinguish between metastatic and non-metastatic melanoma.. Forty individuals with melanoma diagnosed between 2010 and 2020 were enrolled in this cross-sectional study. Melanoma cell lines isolated from cancer tissue were cultured in the laboratory. The cell lines studied included WM115, BLM, K1735, WM793, and A375M. The concentration of microRNA 17-5p was quantified in real time by polymerase chain reaction. CT scans or chest radiographs were performed to detect metastases in both the control and case groups (n = 20). The levels of microRNA 17-5p were used to distinguish the incidence of metastases. Age and sex of metastatic and non-metastatic patients were similar (P > 0.05). 13 (70%) of metastatic patients had microRNA 17-5p compared to 20% of nonmetastatic patients (P = 0.004). Metastatic patients reported greater levels of microRNA 17-5p (-1.60±1.13)versus (−0.150.67; P = 0.001). MicroRNA 17-5p had an aUC of 0.923, with 100% accuracy and 94.44% specificity (P = 0.001; 95 percent confidence interval: 0.811-1). This study linked malignant melanoma to increased microRNA 17-5p levels. This marker's sensitivity and specificity for detecting metastatic melanoma are 100% and 94.4 percent, respectively.

Keywords:

INTRODUCTION

Melanoma is one of the most frequent kinds of skin cancer, accounting for around 5% of all cases. It is estimated that three persons per 100,000 globally are diagnosed with cutaneous melanoma each year. [1]. Tissue growth began with basal epidermis cells known as Melanocytes, which are responsible for producing melanin in the skin's basal layer. Depending on where and how far it has progressed, melanoma can be classified into a variety of phases. The presence of cancer cells in the epidermis is a hallmark of the early stages of melanoma (0 and 1). This is where the tumour is most commonly found. As the tumour grows in size, it has the potential to spread to other parts of your body. Melanoma is extremely metastatic in its mature stages. To reach other organs and tissues such as the lungs, bones, liver or the central nervous system it must first enter the lymphatic or blood systems. It is one of the worst forms of skin cancer, with a five-year survival rate of fewer than 20%. [2,3] Due to the common tissue fixation and paraffin embedding procedures, it is difficult for researchers to find useful biomarkers for melanoma. However, due to the unusually long lifespan of microRNAs (miRNAs), these molecules have deviated from their own attractiveness. [4] miRNAs are short, non-coding RNAs of 20-22 nucleotides that affect post-transcriptional gene expression; therefore, they are essential regulators of biological processes. [5] Recent publications in the literature indicate aberrant miRNA expression in human malignancies. It is possible that miRNAs play a role in oncogenesis or tumour suppression in carcinogenesis. [6,7] It has also been found that miRNAs can predict whether or not someone would get melanoma. [10,11] Several miRNAs have been found to be related with distinct melanoma subtypes. MiR-17-5p has recently been shown to be highly expressed in melanoma tumours, although its precise involvement in the course of the disease is yet unknown. MiR-17-5p was examined in

melanoma malignancy as part of our research[12].

METHODS

Study subject population

From 2010, researchers from Shahid Beheshti University of Medical Sciences (SBUMS) collaborated with patients at Imam Ali Hospital to conduct a cross-sectional study including forty participants who were newly diagnosed with melanomas. The Ethics Committee of Shahid Beheshti University of Medical Sciences approved the study's protocol (IR.MUI. MED . REC .1397.105) .the first time. The study protocol was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences (IR.MUI. MED . REC .1397.105) (IR.MUI. MED . REC .1397.105). Patients were then informed about the confidentiality of their personal data and signed informed consent was obtained. Those whose melanoma diagnosis was confirmed and whose records were available were considered. In addition, patients were selected if they had melanoma deeper than 1.5 mm based on Breslow thickness, which indicates a high risk of metastasis, and Clark stage 2. [18] Both incomplete or faulty medical records and faulty tissue samples met the exclusion criteria. In this study, participants were randomly selected from a random sample with a block size of 1:1 from the general population. As a result, data were collected from twenty patients with distant metastases as the case group and twenty patients without metastases as the control group. Patients' medical records were reviewed for evidence of metastasis based on reports that included history, physical examination, complete blood count, serum biochemical analysis, chest radiography and computed tomography (CT), cranial CT or magnetic resonance imaging, whole body examination, and abdominal CT or ultrasound examination.

Procedure

Dulbecco's modified Eagle's medium with streptomycin/penicillin and 10% foetal bovine serum was used to cultivate melanoma cells at 37 °C in a humidified environment containing 5% CO2.

Real-time polymerase chain reaction (PCR)

Deparaffinization and microdissection were used to examine miR-17-5p expression levels in the samples. As a result, we used a technique known as manual microdissection. In 500 1 of lysis solution (0.05 M Tris, pH 7.65, 0.1% SDS, and 100 g/ml proteinase K), deparaffinized sections were digested overnight at 37°C. It was kept at 20°C for the duration of the experiment. RNA was isolated from digested tissues by following the manufacturer's instructions for tissue or plasma and using Tri reagent (Sigma Aldrich, Gillingham, UK). CA The QIAGEN RNeasy kit was used to precipitate total RNA using 1.25 times the quantity of absolute ethanol recommended by the manufacturer (QIAGEN, Valencia, CA, USA). After extraction, the RNA was kept at 20°C in a 25-litre RNase-free buffer. In order to synthesise miRNAs, cDNA was created from whole RNA. TaqMan Megaplex Preamplification Primers (Human Pool A v2.0) and the TaqMan Preamplification Master Mix were used to pre-amplify this reaction in order to improve its sensitivity and detectability of miRNAs in a 25-litre reaction. To get the pre-amplified cDNA down to 100 litres, we utilised 0.1xTE (pH 8.0). It was our goal to find out why it is so difficult to analyse clinical specimens. Because of this, we used a semi-quantitative scoring method to quantify miRNA expression levels. The miR-17-5p in situ hybridization was done using 6 m thick formalin-fixed, paraffin-embedded slices in accordance with a previously reported technique [19]. 7.5 g/ml proteinase K was used to digest them after deparaffinization and rehydration in 50 mM sodium bicarbonate. An Exiqon hybridization buffer (Exiqon, Vedbaek, Denmark) for 15 minutes at 56 °C, followed with 80 nM double DIG labelled mi for 2 hours was used for prehybridization. When using the full-length mi R-17-5p CA, CTA, ATT, and CCG, the R-17-5p probe has 30% blocked nucleic acid alterations complementary to full-length mi R-17-5p CA, TAC, TAC, ACG, and GCA (base to plus is blocked) strictly with 5 SSC, 1 SSC, and 0.2 SSC, respectively, Vector Laboratories in Burlingame, California, USA, provided us with a nuclear rapid red counterstain, which we warmed to 25°C for two minutes. In order to mount the slides, we utilised VWR's Eukitt mounting medium (Herlev, Denmark). Use of an unlabeled probe (Exiqon, cat. no. 99004 15) or a scrambled probe (80 nM) were used as negative controls, as was not using a probe at all during hybridization. RNA retention was tested using a double DIG labelled

probe for U6 snRNA (0.1 nM). [4] For the last step, the delta Ct technique was employed to quantify the concentration of miR-17-5p in the samples. A miR-17-5p reference standard (Horizon Diagnostics) was utilised in each experiment to calibrate the delta Ct technique to the projected cancer cell count. [17] Analyses of statistical data are conducted.IBM NY Data were imported into the Statistical Package for the Social Sciences (SPSS) (version 22, IBM Corporation, Armonk, NY, USA). For continuous variables with normal distribution, the mean (standard deviation) was reported; for non-normal distributions, the median (interquartile range) was published; and for categorical variables, the frequency (percent of patients) was recorded. The mean difference in scores between metastatic and non-metastatic tumours was determined using the independent samples t-test (Mann-Whitney for data with non-normal distribution). In addition, the performance of miR-17-5p was evaluated by the area under the curve (AUC) of its receiver operating parameters and diagnostic accuracy metrics such as sensitivity, specificity and accuracy value. The Youden index, which is equal to (sensitivity + specificity 1), was used to find the optimal cutoff value for the model. The statistical significance level was set at P = 0.05.

RESULTS

Twenty individuals with metastatic melanoma and twenty patients without it were studied in this study. Table 1 demonstrates that the two groups tested were similar in age (P = 0.59) and sex distribution (P = 0.34). Table 2 and Figure 1 demonstrate the comparison of miR-17-5p quantitative and qualitative scores in the two groups. Metastatic and non-metastatic melanoma patients differed statistically (P 0.05) in both qualitative and quantitative measures, as demonstrated in Table 2.

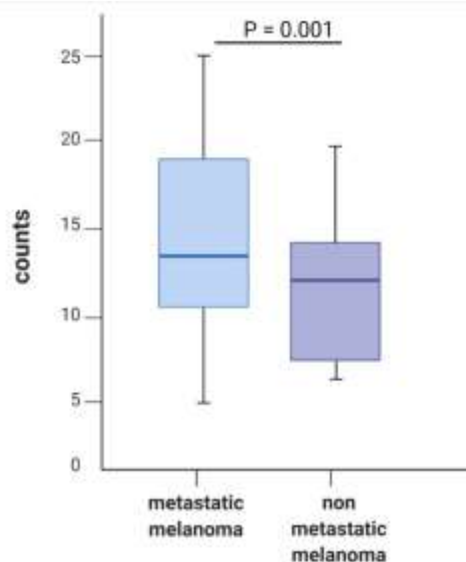

Figure 1: Demonstrate the comparison of miR-17-5p quantitative and qualitative scores in the two groups

Table 1: Demographic information of the study population			
Variables Metastatic	Metastatic melanoma	NonMetastatic melanoma	P
Age	43.50±12.32	43.50±13.65	0.59*
Gender (%)			
Male	8(22.5)	13(30)	0.34**
Female	20 (27.5)	7 (20)	

Table1: the table reveals Two groups of patients, those with metastatic melanoma and those without it, had comparable age (P = 0.59) and gender distribution (P = 0.34) characteristics.

Table 2: Quantitative and qualitative assessments of miR-17-5p in the study population			
Variables Metastatic	melanoma	Nonmetastatic	P
Quantitative miR-17-5p levels	-1.60±1.13	−0.150.67	0.001*
Qualitative miR-17-5p assessments (%)			
Positive	13 (70)	5(20)	0.004**
Negative	7 (30)	15 (80)	

Table2: the table reveals the quantitative and qualitative evaluations of miR-17-5p in the two groups.

DISCUSSION

In this article, distant metastases from melanoma and miR-17-5p are examined. Since both quantitative and qualitative studies found significantly higher levels and higher positivity in metastatic cases compared to non-metastatic cases, our results justify the use of miR-17-5p in pathology samples to predict distant metastases in patients with melanoma. MiRNAs are among the most novel indicators currently used for the diagnosis and prognosis of malignancies in humans; therefore, many studies are being conducted to explore these markers. Medical professionals still use tissue samples to detect cancers, but they are useless when it comes to tracking patients receiving radiation therapy. Tumour cells can leak miRNAs into the bloodstream, leading to increased expression in future cancers that have deregulated miRNAs. Because miRNAs are relatively stable, constant, and reproducible in blood, higher levels of specific miRNAs may be an excellent marker for disease diagnosis or prognosis prediction. (20) The relevance of this biomarker can be demonstrated in the prognosis of patients with malignant melanoma of different ages. [21-23] This study supports our findings on the miR-17 role of 5p in predicting melanoma prognosis, although the research designs are different. Although this study used a novel approach to examine miR-17 5p expression in tissue samples rather than blood, measurements in serum are less invasive, less expensive, and more accessible. The small sample population of the study is a major drawback. In addition, several complicating variables, such as melanoma duration, metastatic site, and primary lesion site, may affect miR-17-5p expression. In addition, other suspected unmeasured factors affecting miR-17-5p expression may have been neglected. Further investigation is essential. In the current study, we found that miR-17-5p has exceptional values for predicting melanoma metastasis, with a sensitivity of 100 percent and a specificity of 94.4 percent. Although this is the second study to address the use of miR-17-5p in melanoma,

efforts to establish a stand-alone component for melanoma prognostic evaluation are ongoing. On the other hand, while most previous publications advocate a panel of markers, the cost-effectiveness and cost-effectiveness of markers should be examined. [24] Bai et al. were the first to investigate whether miR-17-5p could be used to diagnose melanoma and predict its stage of development. They were able to discriminate between healthy and cancerous volunteers and identify metastatic melanoma with a sensitivity and specificity of 76 and 74%, respectively. [The high expression of miR-17-5p in a variety of malignancies, including squamous cell carcinomaof the oesophagus [25], gastric cancer diagnosis [26], bone metastasis in breast cancer [27], and hepatocellular carcinoma in chronic liver disease [28], indicates that the use of this biomarker for melanoma prognosis or follow-up needs further exploration. Overexpression of miR-17-5p has also been found in a number of malignancies other than melanoma. For example, higher serum miR-17-5p expression was found in patients with metastatic breast cancer. Zhao et al. found that individuals with metastatic breast cancer had significantly higher biomarker levels than healthy individuals or patients with non-metastatic breast cancer. [27] In another study, miR-17-5p was found to be useful in both identifying and distinguishing hepatocellular carcinoma from other chronic liver diseases. [28] Overexpression of miR-17-5p is observed in a variety of cancers, including non-melanoma skin cancer, and further studies are needed to find specific biomarkers that can be used for diagnosis and prognosis of these cancers[29-31]. Perhaps using a panel instead of miR-17-5p will allow us to find the most specific markers; however, we found a specificity of 94.4%.

CONCLUSION

MiR-17-5p levels have been shown to be significantly associated with metastatic melanoma. The sensitivity and specificity of this marker in distinguishing metastatic melanoma from non-metastatic melanoma were 100% and 94.4%, respectively. Consequently, this biomarker can be used to monitor patients suspected of having metastatic melanoma. It is imperative that further investigations be performed.

Conflicts of interest

There are no conflicts of interest.

References

[1] H. Ahrend, G. Daeschlein, E. Grove, M. Paditz, A. Mustea, M. Burchardt, M.B. Stope, M. Stope, H. Ahrend, others, MicroRNA-20a-3p and microRNA-20a-5p exhibit anti-proliferative activities in a melanoma in vitro model, (n.d.).

[2] M.-A. Beleaua, I. Jung, C. Braicu, D. Milutin, S. Gurzu, Relevance of BRAF Subcellular Localization and Its Interaction with KRAS and KIT Mutations in Skin Melanoma, International Journal of Molecular Sciences. 22 (2021) 11918.

[3] Y. Chen, J. Cui, Y. Gong, S. Wei, Y. Wei, L. Yi, MicroRNA: a novel implication for damage and protection against ionizing radiation, Environmental Science and Pollution Research. (2021) 1–13.

[4] M. Dragomir, B. Chen, X. Fu, G.A. Calin, Key questions about the checkpoint blockade-are microRNAs an answer?, Cancer Biology & Medicine. 15 (2018) 103.

[5] F. Duan, Y. Yang, W. Liu, J. Zhao, X. Song, L. Li, F. Li, Quantifying the prognostic significance of microRNA-17/17-5P in cancers: a meta-analysis based on published studies, Cancer Management and Research. 10 (2018) 2055.

[6] Ç.Ö. Ecevit, S. Aktaş, H. Tosun Yildirim, B. Demirağ, A. Erbay, I. Karaca, A. Celik, A.B. Demir, A.P. Erçetin, N. Olgun, MicroRNA-17, microRNA-19b, microRNA-146a, microRNA-302d expressions in hepatoblastoma and clinical importance, Journal of Pediatric Hematology/Oncology. 41 (2019) 7–12.

[7] K. Han, F.-W. Wang, C.-H. Cao, H. Ling, J.-W. Chen, R.-X. Chen, Z.-H. Feng, J. Luo, X.-H. Jin, J.-L. Duan, others, CircLONP2 enhances colorectal carcinoma invasion and metastasis through modulating the maturation and exosomal dissemination of microRNA-17, Molecular Cancer. 19 (2020) 1–18.

[8] M. Hasanzadeh, M. Movahedi, M. Rejali, F. Maleki, M. Moetamani-Ahmadi, S. Seifi, Z. Hosseini, M. Khazaei, F. Amerizadeh, G.A. Ferns, others, The potential prognostic and therapeutic application of tissue and

circulating microRNAs in cervical cancer, Journal of Cellular Physiology. 234 (2019) 1289–1294.
[9] X. Jiang, Y. Yuan, L. Tang, J. Wang, Q. Liu, X. Zou, L. Duan, Comprehensive Pan-Cancer Analysis of the Prognostic and Immunological Roles of the METTL3/lncRNA-SNHG1/miRNA-140-3p/UBE2C Axis, Frontiers in Cell and Developmental Biology. 9 (2021).
[10] X. Jiao, X. Qian, L. Wu, B. Li, Y. Wang, X. Kong, L. Xiong, microRNA: the impact on cancer stemness and therapeutic resistance, Cells. 9 (2020) 8.
[11] C. Just, J. Knief, P. Lazar-Karsten, E. Petrova, R. Hummel, C. Röcken, U. Wellner, C. Thorns, MicroRNAs as potential biomarkers for chemoresistance in adenocarcinomas of the esophagogastric junction, Journal of Oncology. 2019 (2019).
[12] J. Liu, L. Dang, X. Wu, D. Li, Q. Ren, A. Lu, G. Zhang, microRNA-mediated regulation of bone remodeling: a brief review, JBMR Plus. 3 (2019) e10213.
[13] S.N. Lone, A.A. Bhat, N.A. Wani, T. Karedath, S. Hashem, S. Nisar, M. Singh, P. Bagga, B.C. Das, D. Bedognetti, others, miRNAs as novel immunoregulators in cancer, in: Seminars in Cell & Developmental Biology, Elsevier, 2021.
[14] A. Malhotra, U. Sharma, S. Puhan, N.C. Bandari, A. Kharb, P. Arifa, L. Thakur, H. Prakash, K.M. Vasquez, A. Jain, Stabilization of miRNAs in esophageal cancer contributes to radioresistance and limits efficacy of therapy, Biochimie. 156 (2019) 148–157.
[15] R.R. Malla, S. Kumari, M.M. Gavara, A.K. Badana, S. Gugalavath, D.K.G. Kumar, P. Rokkam, A perspective on the diagnostics, prognostics, and therapeutics of microRNAs of triple-negative breast cancer, Biophysical Reviews. 11 (2019) 227–234.
[16] M. Mowla, A. Hashemi, Functional roles of exosomal miRNAs in multi-drug resistance in cancer chemotherapeutics, Experimental and Molecular Pathology. 118 (2021) 104592.
[17] S. Murlistyarini, L.P. Aninda, U.A. Afridafaz, S. Widyarti, A.T. Endharti, T.W. Sardjono, The Identification of HSA-MIR-17-5P Existence in the Exosome of Adipose-Derived Stem Cells and Adipocytes, in: Journal of Biomimetics, Biomaterials and Biomedical Engineering, Trans Tech Publ, 2021: pp. 66–75.
[18] M.-H.T. Nguyen, Y.-H. Luo, A.-L. Li, J.-C. Tsai, K.-L. Wu, P.-J. Chung, N. Ma, miRNA as a Modulator of Immunotherapy and Immune Response in Melanoma, Biomolecules. 11 (2021) 1648.
[19] M. Riefolo, E. Porcellini, E. Dika, E. Broseghini, M. Ferracin, Interplay between small and long non-coding RNA s in cutaneous melanoma: a complex jigsaw puzzle with missing pieces, Molecular Oncology. 13 (2019) 74–98.
[20] M.A. Saral, S.B. Tuncer, D.A. Odemis, O.S. Erdogan, S.K. Erciyas, P. Saip, S. Ozel, H. Yazici, New biomarkers in peripheral blood of patients with ovarian cancer: high expression levels of miR-16-5p, miR-17-5p, and miR-638, Archives of Gynecology and Obstetrics. (2021) 1–9.
[21] M. Shelton, C. Anene, J. Nsengimana, W. Roberts, J. Newton-Bishop, J. Boyne, The role of CAF derived exosomal microRNAs in the tumour microenvironment of melanoma, Biochimica et Biophysica Acta (BBA)-Reviews on Cancer. 1875 (2021) 188456.
[22] K.N. Smit, J. Chang, K. Derks, J. Vaarwater, T. Brands, R.M. Verdijk, E.A. Wiemer, H.W. Mensink, J. Pothof, A. de Klein, others, Aberrant MicroRNA expression and its implications for uveal melanoma metastasis, Cancers. 11 (2019) 815.
[23] M. Wang, M. Zhao, Q. Guo, J. Lou, L. Wang, Non-small cell lung cancer cell–derived exosomal miR-17-5p promotes osteoclast differentiation by targeting PTEN, Experimental Cell Research. 408 (2021) 112834.
[24] R. Wen, C. Chen, X. Zhong, C. Hu, PAX6 upstream antisense RNA (PAUPAR) inhibits colorectal cancer progression through modulation of the microRNA (miR)-17-5p/zinc finger protein 750 (ZNF750) axis, Bioengineered. 12 (2021) 3886–3899.
[25] S. Wu, H. Chen, N. Han, C. Zhang, H. Yan, Long Noncoding RNA PVT1 Silencing Prevents the Development of Uveal Melanoma by Impairing MicroRNA-17-3p–Dependent MDM2 Upregulation, Investigative Ophthalmology & Visual Science. 60 (2019) 4904–4914.

[26] Y. Xing, Z. Wang, Z. Lu, J. Xia, Z. Xie, M. Jiao, R. Liu, Y. Chu, MicroRNAs: immune modulators in cancer immunotherapy, Immunotherapy Advances. 1 (2021) ltab006.

[27] K. Xu, P. Zhang, J. Zhang, H. Quan, J. Wang, Y. Liang, Identification of potential micro-messenger RNAs (miRNA–mRNA) interaction network of osteosarcoma, Bioengineered. 12 (2021) 3275–3293.

[28] Y. Xu, Q. Zhang, F. Lin, L. Zhu, F. Huang, L. Zhao, R. Ou, Casiopeina II-gly acts on lncRNA MALAT1 by miR-17-5p to inhibit FZD2 expression via the Wnt signaling pathway during the treatment of cervical carcinoma, Oncology Reports. 42 (2019) 1365–1379.

[29] C. Yang, Y. Wang, P. Hardy, Emerging roles of microRNAs and their implications in uveal melanoma, Cellular and Molecular Life Sciences. 78 (2021) 545–559.

[30] M.T. Yildiz, L. Tutar, N.I. Giritlioğlu, B. Bayram, Y. Tutar, MicroRNAs and heat shock proteins in breast cancer biology, in: MiRNomics, Springer, 2022: pp. 293–310.

[31] A. Zebardast, S.S. Tehrani, T. Latifi, F. Sadeghi, Critical review of Epstein–Barr virus microRNAs relation with EBV-associated gastric cancer, Journal of Cellular Physiology. (2021).