Reconstruction of IncRNA-miRNA-mRNA network based on competitive endogenous RNA reveals functional IncRNAs in melanoma

CURRENT STATUS: Under Review

Junyou Zhu, Jin Deng, Lijun Zhang, Jingling Zhao, Fei Zhou, Ning Liu, Ruizhao Cai, Jun Wu, Bin Shu, Shaohai Qi

Junyou Zhu
Sun Yat-sen University First Affiliated Hospital

Jin Deng
Guangzhou Medical University Affiliated Cancer Hospital

Lijun Zhang
Sun Yat-sen University First Affiliated Hospital

Jingling Zhao
Sun Yat-sen University First Affiliated Hospital

Fei Zhou
Sun Yat-sen University First Affiliated Hospital

Ning Liu
Sun Yat-sen University First Affiliated Hospital

Ruizhao Cai
Sun Yat-sen University First Affiliated Hospital

Jun Wu
Sun Yat-sen University First Affiliated Hospital

Bin Shu
Sun Yat-sen University First Affiliated Hospital

Shaohai Qi
First affiliated hospital, Sun yat-sen University
qishh@mail.sysu.edu.cn Corresponding Author
Subject Areas

Oncology  Cancer Biology

Keywords

melanoma, IncRNA, competitive endogenous RNA, MALAT1, LINC00943, LINC00261, miRNA
Abstract

Background: Melanoma is the most common and dangerous skin tumor, and its pathogenesis is not fully understood.

Methods: To identify the key IncRNAs in melanoma, we reconstructed a global triple network based on the ceRNA theory. GO and KEGG pathway analysis were performed using DAVID. And, we verified our findings through qRT-PCR assay.

Results: According to ceRNA theory, 898 DEMs, 53 DELs and 16 DEMIs were selected to reconstruct the ceRNA network. MALAT1, LINCO00943, and LINCO00261 were selected as hub gene in the ceRNA network.

Conclusions: In this study, we found that MALAT1, LINCO00943 and LINCO00261 were closely associated with tumorigenesis and development of melanoma, and therefore, could be used as potential diagnostic biomarkers and therapeutic targets.

1. Background

Melanoma is the most common and dangerous skin cancer. Worldwide, cutaneous melanoma occurred in approximately 232,000 (1.7%) cases of all newly diagnosed primary malignant cancers, and resulted in about 55,500 cancer deaths (0.7% of all cancer deaths). The incidence of melanoma in Australia, New Zealand, Norway, Sweden, the UK, and USA from 1982 to 2011, had shown that the incidence increased about 3% annually, and will further increase at least until 2022. In 2015, there were 3.1 million people with melanoma, resulting in 59,800 deaths. Still, 95,710 cases of melanoma in situ will be newly diagnosed in 2020. The high incidence and high mortality of melanoma make it necessary for us to pay more attention on it. Although some achievements have been made in the genetic research of melanoma, more molecules related to diagnosis and treatment have been required to be found.

Tumorigenesis are often resulted from aberrant transcriptomes, including the aberrant levels of coding RNA and non-coding RNA. It had been proved that IncRNA have a variety of effects, including regulation of gene transcription, regulation of post-transcription and epigenetic regulation and more. In addition, dysregulation of IncRNA had been observed in various diseases. Unfortunately, the function of IncRNA are more difficult to identified in comparison with those of coding RNAs. Until now, only few IncRNA was identified as crucial factor in tumorigenesis and development of melanoma, including ZNNT1, THOR and SAMMSON. Thus, how to locate them and define its functions are the challenge of current research.

The effect of miRNA on malignancies had been well verified in many ways. Studies had suggested that IncRNAs can regulate miRNAs abundance by binding and sequestering them. This gave us a hint to study the function of IncRNAs, which is, by studying the interaction among IncRNAs, mRNAs and miRNAs. In 2011, the competitive endogenous RNA (ceRNA) hypothesis, a novel regulatory mechanism between non-coding RNA and coding RNA had been proposed. This theory indicated that any RNA transcript harboring miRNA-response elements (MREs) can sequester miRNAs from other targets sharing the same MREs, and thereby regulate their expressions. To be biologically predicted according to their common MREs. Evidences had been shown that ceRNAs existed in several species and contexts, and might play an important role in various biological processes, such as tumorigenesis. Systematic analysis of ceRNA network had been well performed in multiple tumors, such as gastric cancer, bladder cancer, and ovarian cancer, contributing to a better understanding of tumorigenesis and facilitate the development of IncRNA-directed diagnostics and therapeutics against this disease. Unfortunately, however, such functional interactions have not yet been elucidated in melanoma.
In this study, ceRNA networks had been reconstructed, in order to reveal the potential diagnosis and therapeutic molecules of melanoma.

2. Methods

2.1 Raw data

Human melanoma miRNA expression data were download from NCBI GEO database (GEO (http://www.ncbi.nlm.nih.gov/geo),

including GSE24996, GSE35579, GSE62372, which were array-based datasets. The GSE24996 dataset consists of 8 benign nevus tissue samples and 15 primary melanoma tissue samples. The GSE35579 dataset consists of 11 benign nevus tissue samples and 20 primary melanoma tissue samples. The GSE62372 dataset consists of 9 benign nevus tissue samples and 92 primary melanoma tissue samples. mRNA and IncRNA expression data were also download from NCBI GEO database (GSE112509), which is sequence-based dataset. The GSE112509 dataset consist of 23 benign nevus tissue samples and 57 primary melanoma tissue samples, respectively.

2.2 Identification of DEMis, DELs and DEMs

To identify the differential expressed miRNAs (DEMis) between primary melanoma and benign nevus samples, “R” (version 3.4.2, https://www.r-project.org/) was used with “limma” package after normalization. To identify the differential expressed IncRNAs (DELs) and mRNAs (DEMs) between primary melanoma and benign nevus samples, “R” (version 3.4.2, https://www.r-project.org/) was used with “DESeq2” package. The DEMis, DELs and DEMs were selected according to the \(|\log_2 FC| > 1\) and adjusted P-value < 0.05.

2.3 Prediction of target IncRNAs and mRNAs

To predict the target IncRNAs and mRNAs through DEMis, starbase (starbase.sysu.edu.cn) was used in our study. Multiple IncRNA/mRNA-predicting programs (PITA, RNA22, miRmap, DIANA-microT, miranda, PicTar and TargetScan) were used in starbase. In order to make our results more accurate, only when the target mRNA was predicted in at least four predicted programs on starbase, it would be chosen as the predicted target mRNA. Then, these predicted target IncRNAs and mRNAs were merged with DEMs and DELs, respectively.

2.4 Reconstruction of ceRNA network

The ceRNA network have been reconstructed based on ceRNA theory, and as follows: (1) Expression correlation between DELs and DEMs was evaluated using the Pearson correlation coefficient (PCC). The DELs-DEMs pairs with PCC > 0.4 and P-value < 0.01 were considered as co-expressed IncRNA-mRNA pairs. (2) Both IncRNAs and mRNAs in the pairs were negatively correlated with their common miRNAs. (3) The ceRNA network was reconstructed and visualized using Cytoscape (version 3.7.1, https://cytoscape.org/).

2.5 Functional enrichment analysis

In order to assess functional enrichment, Gene Ontology (GO) Biological Process (BP), Cell Component (CC), Molecular Function (MF) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of mRNAs in the ceRNA network were performed using DAVID (version 6.8, https://david.ncifcrf.gov/).

2.6 Hub gene selection and reconstruction of key ceRNA sub-network

To reconstruct our key ceRNA sub-network, we first select hub gene according to the node degrees of ceRNA network we reconstructed above, by calculating the number of IncRNA-miRNA and miRNA-mRNA pairs. For these key IncRNAs, GO-BP, GO-CC, GO-MF and KEGG pathway annotation were performed according to their first mRNA neighbors, by using DAVID (version 6.8, https://david.ncifcrf.gov/).

2.7 Selection of melanoma sample
The study protocol was approved by the Ethics Committee of The First Affiliated Hospital, Sun yat-sen University. All patients provided written informed consent in compliance with the code of ethics of World Medical Association (Declaration of Helsinki). Eligible patients were from The First Affiliated Hospital, Sun yat-sen University (Guangzhou, Guangdong, China) or Cancer Center of Guangzhou Medical University (Guangzhou, Guangdong, China). The eligible patients for study had to meet the following criteria: (1) histologically confirmed as melanoma; (2) received no radiotherapy, chemotherapy or biotherapy before surgery. Exclusion criteria include: (1) with previous malignancies; (2) with concomitant malignancies; (3) with serious active infection; (4) experiencing pregnancy or lactation. Melanoma tissues and its adjacent normal tissues, were collected from each patient. The adjacent normal tissue was taken more than 2cm away from the edge of the tumor, which was confirmed as normal skin tissue by at least three pathologists.

2.8 Selection of healthy sample

The study protocol was approved by the Ethics Committee of The First Affiliated Hospital, Sun yat-sen University. All patients provided written informed consent in compliance with the code of ethics of World Medical Association (Declaration of Helsinki). Eligible patients were from The First Affiliated Hospital, Sun yat-sen University (Guangzhou, Guangdong, China). Healthy patients between 18 and 50 years of age who were scheduled to undergo split-thickness skin grafting were included in the study. Exclusion criteria include: (1) with previous malignancies; (2) with concomitant malignancies; (3) with serious active infection; (4) experiencing pregnancy or lactation; (5) with diabetes or other skin diseases.

2.9 RNA isolation and qRT-PCR

The total RNA was extracted from all samples by the use of Trizol reagent (Invitrogen, USA). The OD value (260/280) of all RNA extracted samples was greater than 1.8. For each replicate, complementary DNA (cDNA) was synthesized from 2μg RNA using GoScript Reverse Transcription System (Promega, USA). The qRT-PCR reaction comprised 10μl of GoTaq qPCR Master Mix (2×) (Promega, USA), 2μl of diluted cDNA template (1:10) and 10μM of each primer contributing to a total volume of 20μl. Reactions were run in ABI 7500 real-time PCR system (Applied Biosystems, USA) under the following conditions: 95°C for 10 mins, and 40 cycles of 95°C for 15s and 60°C for 60s. Melting curves were derived for every reaction to insure a single product. Relative gene expression was evaluated according to ddCT method, using human GAPDH gene as endogenous controls for RNA load and gene expression in analysis. All experiments were performed in triplicate. The GraphPad Prism 8 (GraphPad Software, USA) was used to output figures.

The primers were as follows: MALAT1 Fw.: GACGAGTTGTGCTGCGAT; MALAT1 Rev.: TTCTGTGTTATGCCTGGTTA; LINC00943 Fw.: GGATTGGATTGTGGATTGC; LINC00943 Rev.: CAGGTCTCAGTTCAGTGTT; LINC00261 Fw.: CTTCTTGGACCACATCTTACAC; LINC00261 Rev.: GGACCATTGCCTCTTGATT; GAPDH Fw: GAGAGGGAAATCGTGCGTGAC; GAPDH Rev: CATCTGCTGGAAGGTGGACA.

3. Results

3.1 Identification of DEMs, DELs and DEMis

After standardization of the GEO datasets, 56, 70 and 34 DEMis between benign nevus tissues and primary melanoma tissues, were identified respectively in GSE24996, GSE35579 and GSE62372 (Tab. 1, Fig. 1A-1F). The candidates 18 miRNAs were shared in at least two datasets (Fig. 2A), including hsa-miRNA-378a-3p, hsa-miRNA-23b-3p, hsa-miRNA-140-3p, hsa-miRNA-99a-5p, hsa-miRNA-100-5p, hsa-miRNA-204-5p, hsa-miRNA-211-5p, hsa-miRNA-205-5p, hsa-miRNA-224-5p, hsa-miRNA-200b-3p, hsa-miRNA-200c-3p, hsa-miRNA-125b-5p, hsa-miRNA-149-5p, hsa-miRNA-21-5p, hsa-miRNA-20b-5p, hsa-miRNA-424-5p, hsa-miRNA-203a-3p and hsa-miRNA-1826. According to method 2.3, 2,361 mRNAs and 277 IncRNAs were predicted using these miRNAs. And, we ruled out two of these 18 DEMis, hsa-miRNA-203a-3p and hsa-miRNA-1826, because no predicted gene was found in starbase according to method 2.3. In addition, 5,953 DEMs and 665 DELs between benign nevus tissues and primary melanoma tissues, were identified in GSE112509 (Fig. 1G and 1H). As a result, a total of 898 DEMs and 53 DELs were selected for further analysis according to method 2.3 (Fig. 2B and 2C). Finally, 898 DEMs,53 DELs
and 16 DEMs were selected for further reconstruct the lncRNA-miRNA-mRNA (ceRNA) network.

3.2 Reconstruction of lncRNA-miRNA-mRNA (ceRNA) network

The lncRNA-miRNA-mRNA (ceRNA) network, consisting of 53 lncRNA nodes, 16 miRNA nodes, 898 mRNA nodes and 609 edges was reconstructed and visualized by using Cytoscape (Fig. 3A).

3.3 KEGG pathway and GO enrichment analysis of lncRNAs based on ceRNA network

We used DAVID to analysis the biological classification of DEMs according to method 2.5. The results of top 15 significant GO terms and KEGG pathways are shown in Table 2 and Fig. 3B-3E. 60 pathways are significantly enriched through KEGG pathway analysis, including PI3K-Akt signaling pathway, focal adhesion, proteoglycans in cancer, pathway in cancer and most importantly in melanomagenesis. The results of GO-BP analysis reveal that 172 enriched terms, particularly in various regulation of transcription such as positive regulation of transcription from RNA polymerase II promoter, positive regulation of transcription (DNA-templated), transcription from RNA polymerase II promoter and etc.

3.4 Hub gene selection

According to the node degree in ceRNA network, we found that three lncRNAs, MALAT1, LINC00943, LINC00261, had the highest number of lncRNA-miRNA and miRNA-mRNA pairs, suggesting that these three lncRNAs could be chosen as hub nodes, and the results are shown in Table 3. Therefore, these three lncRNAs might play an essential role in melanomagenesis, which might be considered as the key lncRNAs.

3.5 Reconstruction of MALAT1/LINC00943/LINC00261-miRNA-mRNA sub-networks

MALAT, LINC00943, and LINC00261 and their paired miRNA and mRNA were used to reconstruct key ceRNA sub-network. The MALAT1 ceRNA network consists of 1 lncRNA nodes, 9 miRNA nodes, 158 mRNA nodes and 209 edges as shown in Fig. 4A. The LINC00943 ceRNA network consists of 1 lncRNA nodes, 7 miRNA nodes, 182 mRNA nodes and 209 edges as shown in Fig. 5A. The LINC00261 ceRNA network consists of 1 lncRNA nodes, 5 miRNA nodes, 123 mRNA nodes and 163 edges as shown in Fig. 6A. The results of functional analysis revealed that 75 GO-BP, 21 GO-CC, 15 GO-MF and 20 pathways were enriched in the MALAT1-miRNA-mRNA sub-network, 67 GO-BP, 14 GO-CC, 17 GO-MF and 13 pathways were enriched in the LINC00943-miRNA-mRNA sub-network, 42 GO-BP, 7 GO-CC, 10 GO-MF and 7 pathways were enriched in the LINC00261-miRNA-mRNA sub-network. The results of top 10 significant GO terms and KEGG pathways of these three lncRNAs were shown in Fig. 4B-5E, Fig. 5B-5E, Fig. 6B-6E, and Table 4-6.

The MALAT1/LINC00943 and LINC00261 sub-network show that GO-BP analysis revealed 75, 69 and 42 enriched terms respectively, which are also significantly enriched in regulation of transcription such as positive regulation of transcription from RNA polymerase II promoter, positive regulation of transcription (DNA-templated), transcription from RNA polymerase II promoter and more. Pathways analysis reveal that 19, 13 and 7 pathways are significantly enriched in tumor-related pathways, including pathway in cancer, focal adhesion, PI3K-Akt signaling pathway and more.

3.6 Expression of MALAT1/LINC00943 and LINC00261 are higher in tumor tissues

To confirm the expression of MALAT1/LINC00943 and LINC00261 in melanoma tissues, we had evaluated MALAT1/LINC00943 and LINC00261 expression level in cancer tissues from 12 melanoma patients and 3 healthy samples via qRT-PCR, as shown in Fig. 7. The results show that the expression of MALAT1/LINC00943 and LINC00261 are significantly higher in tumor tissues compared with healthy samples (p = 0.0243, p = 0.0005, p < 0.0001, respectively). Also, the expression of MALAT1/LINC00943 and LINC00261 are significantly higher in tumor tissues compared with its adjacent normal tissue (p = 0.0002, p < 0.0001, p < 0.0001, respectively). However, no significant difference was observed between healthy samples and adjacent normal skin tissues in expression of MALAT1/LINC00943 and LINC00261 (p = 0.366, p = 0.379, p = 0.262, respectively). The results are consistent with that discussed above. Thus, the expression of MALAT1/LINC00943 and LINC00261 are increased in melanoma and may responsible for the tumorigenesis of melanoma.
4. Discussion

Melanoma is the most common skin cancer.\textsuperscript{1,2} The high incidence and high mortality of melanoma make it necessary for us to pay more attention on it.\textsuperscript{5,6} Over the past few years, great efforts have been made to explore the molecular mechanisms of it.\textsuperscript{5} Previous studies have been focused on the protein-coding genes and miRNAs, however, it rarely involves in IncRNAs. In this study, three IncRNAs, \textit{MALAT1}, \textit{LINC00943} and \textit{LINC00261}, were identified according to the reconstructed ceRNA network.

With the increasing attention in the role of IncRNA, IncRNA have shown superior potential over protein-coding genes as a biomarker for diagnosis, prognosis and treatment.\textsuperscript{39–42} Among these key IncRNAs found in this study, \textit{MALAT1} has been demonstrated to be related to various malignant tumors.\textsuperscript{43–47} Studies had confirmed that \textit{MALAT1} is a valuable prognostic marker and a promising therapeutic target in lung cancer metastasis.\textsuperscript{43,44} A study had also suggested that \textit{MALAT1} play an important role in tumor progression and could be served as a promising therapeutic target.\textsuperscript{45} Through the study of whole-genome mutational landscape and characterization of noncoding and structural mutations in liver cancer, Fujimoto A. and colleges discovered \textit{MALAT1} closely related to liver carcinogenesis.\textsuperscript{46} In addition, a study revealed a novel mechanism of \textit{MALAT1}-regulated autophagy-related chemoresistance in gastric cancer.\textsuperscript{47} At present, it is believed that \textit{MALAT1} is mainly responsible for regulating the proliferation, migration and invasion of tumor cells. According to our findings, which indicated that \textit{MALAT1} might also be a crucial factor in the tumorigenesis and development of melanoma.

In this sub-network, we found that there are nine IncRNA-miRNA pairs, including miRNA-378a-3p, miRNA-23b-3p, miRNA-224-5p, miRNA-204-5p, miRNA-205-5p, miRNA-200c-3p, miRNA-200b-3p, miRNA-149-5p, miRNA-211-5p. Among them, study have demonstrated that \textit{MALAT1} can regulate chemoresistance via miRNA-23b-3p sequestration in gastric cancer.\textsuperscript{47} In ovarian cancer, a study suggested that \textit{MALAT1}-miRNA-211-5p may act as a key mediator in prevention of this disease.\textsuperscript{48} \textit{MALAT1} also involved in promoting renal cell carcinoma through interaction with miRNA-205-5p.\textsuperscript{49} Studies have confirmed that \textit{MALAT1} functions in liver and lung cancer through miRNA-204-5p.\textsuperscript{50,51} In addition, targeting \textit{MALAT1}/miRNA-200c-3p axis in xenograft endometrial carcinoma model can greatly inhibit tumor growth.\textsuperscript{52}

Moreover, studies had been illustrated that these miRNAs were closely related to melanoma in serval ways. miRNA-378a-3p can regulate oncogene PARVA expression in melanoma, preventing its progression.\textsuperscript{53} miRNA-23b-3p was shown as a tumor suppressor gene in melanoma.\textsuperscript{54} miRNA-224-5p can be regulated by E2F1 to drive EMT through TXNIP downregulation in melanoma, and it can inhibit uveal melanoma cell proliferation, migration, invasion by targeting PIK3R3/AKT3.\textsuperscript{55,56} miRNA-204-5p, known as tumor suppressor gene in melanoma, was associated with CDKN2A pathway and NRAS gene, and was contributed to BRAF inhibitor resistance.\textsuperscript{54,57,58} miRNA-205-5p suppresses proliferation and induces senescence via regulation of E2F1 in melanoma.\textsuperscript{54,59–61} miRNA-200b/c-3p act as potential diagnostic and prognostic markers for melanoma.\textsuperscript{62–64} Upregulation of miRNA-149-5p, directedly regulated by p53, results in increased expression of Mcl-1 and resistance to apoptosis in melanoma cells.\textsuperscript{65} Most importantly, studies have confirmed that miRNA-211-5p play a major role as tumor suppressor via various targets in melanoma.\textsuperscript{54,58,62,66,67} As these miRNAs we predicted were closely associated with melanoma, we believed that \textit{MALAT1} may play a crucial role in tumorigenesis and development of melanoma through competitive interactions with these miRNAs, and subsequently alter expression of downstream mRNAs.

Nothing was known about \textit{LINC00943}. According to \textit{LINC00943}-miRNA-mRNA sub-network, miRNA-99a-5p, miRNA-100-5p, miRNA-23b-3p, miRNA-204-5p, miRNA-224-5p, miRNA-149-5p and miRNA-125b-5p, were closely interacted with \textit{LINC00943}. No connection between \textit{LINC00943} and these miRNAs have been discovered yet, however, these miRNAs were also demonstrated to be associated with melanoma, except miRNA-99a-5p. The link between miRNA-204-5p, miRNA-224-5p, miRNA-149-5p and melanoma were discussed above. miRNA-23b was suggested as tumor suppressor gene.\textsuperscript{54} miRNA-100-5p and miRNA-125b-5p are associated with resistance.
to treatment with immune checkpoint inhibitors in melanoma patients. Therefore, understanding the relationship among LINC00943, miRNA and malignancies may provide a feasible way for the future research of melanoma and other malignancies.

LINC00261 is also significant based on ceRNA network, but its function remains controversial until now. We found that 43 GO-BP terms and 7 KEGG pathways were enriched, based on the sub-network. One of these pathways, PI3K/Akt signaling pathway, is proved to play a critical role in tumorigenesis, especially in melanoma. Also, study have demonstrated that LINC00261 promotes cancer cell proliferation and metastasis in human choriocarcinoma. However, LINC00261 have shown a great capacity in improving the chemotherapeutic response and survival of patients with esophageal cancer. And, in gastric cancer, LINC00261 can suppress the tumor metastasis by regulating epithelial-mesenchymal transition (EMT). Moreover, LINC00261 can block cellular proliferation by activating the DNA damage response. LINC00261 may affect the biological behavior of different tumors in different ways. Therefore, it is very essential to further explore the role of LINC00261 in different tumors. On the other hand, five miRNAs, including miRNA-23b-3p, miRNA-211-5p, miRNA-205-5p, miRNA-140-3p and miRNA-125b-5p were interacted with LINC00261 according to LINC00261-miRNA-mRNA sub-network. Similarly, no connection between LINC00261 and these miRNAs have been discovered yet, but these miRNAs were also demonstrated to be associated with melanoma. The role of miRNA-23b-3p, miRNA-211-5p, miRNA-205-5p, and miRNA-125b-5p in melanoma were discussed above. miRNA-140-3p was reported to be regulated by MALAT1 in uveal melanoma cell, while its role in cutaneous melanoma is still unknown.

Three of the 16 predicted miRNAs were not associated with MALAT1, LINC00943 and LINC00261, including miRNA-21-5p, miRNA-20b-5p and miRNA-424-5p. They closely related to SGMS1.AS1, EPB41L4A.AS1 and SNHG1, on the other hand. Little was known about miRNA-424-5p in melanoma, while studies have suggested that miRNA-20b-5p may inhibit tumor metastasis via regulation of PAR-1 receptor in melanoma cells, and miRNA-21 may regulate melanoma cell proliferation, migration, apoptosis through ERK/NF-κB signaling pathway by targeting SPRY1, PDCD4 and PTEN.

All in all, we reconstructed a ceRNA network, which for the first time enables an overall view and analysis of the IncRNA-associated ceRNA mediated genes in the tumorigenesis and development of melanoma at a system-wide level based on ceRNA theory. Our study discovered that serval IncRNAs, including MALAT1, LINC00943 and LINC00261, might play an essential role in skin cutaneous melanoma. Finally, we verified our findings through q(RT)-PCR assay. This study will advance our understanding of the tumorigenesis and development of melanoma from the perspective of IncRNAs and provide some novel IncRNAs as candidate diagnostic biomarkers or potential therapeutic targets for melanoma. Further studies are required to explore the biological functions and molecular mechanisms of MALAT1, LINC00943 and LINC00261 in melanoma.

5. Conclusions

This study advances our understanding of tumorigenesis and development in melanoma from the perspective of IncRNAs, and provides three novel IncRNAs as candidate diagnostic biomarkers or potential therapeutic targets for melanoma. Further studies are required to verify the role of MALAT1, LINC00943 and LINC00261 in melanoma.

Declarations

Ethics approval and consent to participate: The study protocol was approved by the Ethics Committee of The First Affiliated Hospital, Sun yat-sen University. All patients provided written informed consent in compliance with the code of ethics of World Medical Association (Declaration of Helsinki).

Consent for publication: This manuscript is approved by all authors for publication.
Availability of data and material: The data that support results of the present study are available from GEO datasets (including GSE24996(https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE24996), GSE35579(https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE35579), GSE62372(https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE62372), and GSE112509(https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE112509)), DAVID(https://david.ncifcrf.gov/), and starbase miRNA-mRNA Interactions (http://starbase.sysu.edu.cn/agoClipRNA.php?source=mRNA), and starbase miRNA-IncRNA Interactions http://starbase.sysu.edu.cn/agoClipRNA.php?source=IncRNA) database.

Competing interests: No potential conflict of interest was declared by the authors.

Funding: This article is funded by the Science and Technology Program of Guangzhou (201704020165) and Natural Science Foundation of Guangdong Province (2017A030313619).

Author contributions: JZ, NL and RC collected the data from GEO database; JZ and JZ analyzed the data; JW, BS and SQ provided project administration, and resources; and JZ, JD and LZ wrote the paper. All authors have read and approved this manuscript.

Acknowledgements: This manuscript is approved by all authors for publication. And we sincerely thank those who help finishing this article.

Abbreviations

cRNA- competitive endogenous RNA
IncRNA- long non-coding RNAs
NCBI GEO-National Center for Biotechnology Information Gene Expression Omnibus
GO- Gene Ontology
KEGG- Kyoto Encyclopedia of Genes and Genomes
DAVID- Database for Annotation, Visualization, and Integration Discovery
MREs- miRNA-response elements
DEMis- differential expressed miRNAs
DELs- differential expressed IncRNAs
DEMs- differential expressed mRNAs

References

1. McGuire, S. World Cancer Report 2014. Geneva, Switzerland: World Health Organization, International Agency for Research on Cancer, WHO Press, 2015. Adv Nutr, 418-419, doi:10.3945/an.116.012211 (2016).
2. Berwick, M., Erdei, E. & Hay, J. Melanoma Epidemiology and Public Health. Dermatologic Clinics (2009). doi:10.1016/j.det.2008.12.002
3. Schadendorf, D. et al. Melanoma. Lancet392, 971-984, doi:10.1016/S0140-6736(18)31559-9 (2018).
4. Vos, T. et al. Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet (2016). doi:10.1016/S0140-6736(16)31678-6
5. Siegel, R. L., Miller, K. D. & Jemal, A. Cancer Statistics, 2017. CA Cancer J Clin67, 7-30, doi:10.3322/caac.21387 (2017).
6. Siegel, R. L., Miller, K. D. & Jemal, A. Cancer statistics, 2020. CA Cancer J Clin70, 7-30, doi:10.3322/caac.21590 (2020).
7. Burrell, R. A., McGranahan, N., Bartek, J. & Swanton, C. The causes and consequences of genetic heterogeneity in cancer evolution. Nature (2013). doi:10.1038/nature12625
8. Lander, E. S. et al. Initial sequencing and analysis of the human genome. Nature409, 860-921, doi:10.1038/35057062 (2001).
9. Yost, S. E. et al. Identification of high-confidence somatic mutations in whole genome sequence of formalin-fixed breast cancer specimens. Nucleic Acids Res. (2012). doi:10.1093/nar/gks299
10. Goodrich, J. A. & Kugel, J. F. Non-coding-RNA regulators of RNA polymerase II transcription. Nature Reviews Molecular Cell Biology (2006). doi:10.1038/nrm1946
11. Yoon, J. H., Abdelmohsen, K. & Gorospe, M. Posttranscriptional gene regulation by long noncoding RNA. Journal of Molecular Biology (2013). doi:10.1038/nature06008
12. Kiefer, J. C. Epigenetics in development. Developmental Dynamics (2007). doi:10.1002/dvdy.21094
13. Mikkelsen, T. S. et al. Genome-wide maps of chromatin state in pluripotent and lineage-committed cells. Nature (2007). doi:10.1038/nature06008
14. Joung, J. et al. Genome-scale activation screen identifies a lncRNA locus regulating a gene neighbourhood. Nature (2017). doi:10.1038/nature23451
15. Leucci, E. et al. Melanoma addiction to the long non-coding RNA SAMMSON. Nature (2016). doi:10.1038/nature17161
16. Hosono, Y. et al. Oncogenic Role of THOR, a Conserved Cancer/Testis Long Non-coding RNA. Cell (2017). doi:10.1016/j.cell.2017.11.040
17. Schmidt, K. et al. The lncRNA SLNCR1 Mediates Melanoma Invasion through a Conserved SRA1-like Region. Cell Rep. (2016). doi:10.1016/j.celrep.2016.04.018
18. Montes, M. et al. The lncRNA MIR31HG regulates p16 INK4A expression to modulate senescence. Nat. Commun. (2015). doi:10.1038/ncomms7967
19. Li, P. et al. ZNNT1 long noncoding RNA induces autophagy to inhibit tumorigenesis of uveal melanoma by regulating key autophagy gene expression. Autophagy (2019). doi:10.1080/15548627.2019.1659614
20. Hosono, Y. et al. Oncogenic Role of THOR, a Conserved Cancer/Testis Long Non-coding RNA. Cell (2017). doi:10.1016/j.cell.2017.11.040
21. Leucci, E. et al. Melanoma addiction to the long non-coding RNA SAMMSON. Nature (2016). doi:10.1038/nature17161
22. Jalali, S., Bhartiya, D., Lalwani, M. K., Sivasubbu, S. & Scaria, V. Systematic Transcriptome Wide Analysis of lncRNA-miRNA Interactions. PLoS One (2013). doi:10.1371/journal.pone.0053823
23. Ala, U. et al. Integrated transcriptional and competitive endogenous RNA networks are cross-regulated in permissive molecular environments. Proc. Natl. Acad. Sci. (2013). doi:10.1073/pnas.1222509110
24. Salmena, L., Poliseno, L., Tay, Y., Kats, L. & Pandolfi, P. P. A ceRNA hypothesis: The rosetta stone of a hidden RNA language? Cell (2011). doi:10.1016/j.cell.2011.07.014
25. Tay, Y., Rinn, J. & Pandolfi, P. P. The multilayered complexity of ceRNA crosstalk and competition. Nature (2014). doi:10.1038/nature12986
26. Rinn, J. L. & Chang, H. Y. Genome Regulation by Long Noncoding RNAs. Annu. Rev. Biochem. (2012). doi:10.1146/annurev-biochem-051410-092902
27. Liu, X. hua et al. Lnc RNA HOTAIR functions as a competing endogenous RNA to regulate HER2 expression by sponging miR-331-3p in gastric cancer. Mol. Cancer (2014). doi:10.1186/1476-4598-13-92
28. Zhong, Z. et al. Circular RNA MYLK as a competing endogenous RNA promotes bladder cancer progression through modulating VEGFA/VEGFR2 signaling pathway. Cancer Lett. (2017). doi:10.1016/j.canlet.2017.06.027
29. Chang, L., Guo, R., Yuan, Z., Shi, H. & Zhang, D. LncRNA HOTAIR Regulates CCND1 and CCND2 Expression by Sponging miR-206 in Ovarian Cancer. Cell. Physiol. Biochem. (2018). doi:10.1159/000493408
30. Huang, D. W., Sherman, B. T. & Lempicki, R. A. Bioinformatics enrichment tools: Paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res. (2009). doi:10.1093/nar/gkn923
31. Huang, D. W., Sherman, B. T. & Lempicki, R. A. Systematic and integrative analysis of large gene lists
using DAVID bioinformatics resources. Nat. Protoc. (2009). doi:10.1038/nprot.2008.211
32. Edgar, R. Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. Nucleic Acids Res. (2002). doi:10.1093/nar/30.1.207
33. Smyth G.K. (2005) limma: Linear Models for Microarray Data. In: Gentleman R., Carey V.J., Huber W., Irizarry R.A., Dudoit S. (eds) Bioinformatics and Computational Biology Solutions Using R and Bioconductor. Statistics for Biology and Health. Springer, New York, NY
34. M. I. Love, W. Huber, S. Anders: Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology 2014, 15:550.
35. Breuer, J. (2017). R (Software). In The International Encyclopedia of Communication Research Methods (eds J. Matthes, C.S. Davis and R.F. Potter). doi:10.1002/9781118901731.iecrm0201
36. Li, J. H., Liu, S., Zhou, H., Qu, L. H. & Yang, J. H. StarBase v2.0: Decoding miRNA-ceRNA, miRNA-ncRNA and protein-RNA interaction networks from large-scale CLIP-Seq data. Nucleic Acids Res. (2014). doi:10.1093/nar/gkt1248
37. Liao, Q. et al. Large-scale prediction of long non-coding RNA functions in a coding-non-coding gene expression network. Nucleic Acids Res. (2011). doi:10.1093/nar/gkq1348
38. Shannon, P. et al. Cytoscape: A software Environment for integrated models of biomolecular interaction networks. Genome Res. (2003). doi:10.1101/gr.1239303
39. Malik, B. & Feng, F. Y. Long noncoding RNAs in prostate cancer: overview and clinical implications. Asian J Andro/18, 568-574, doi:10.4103/1008-682X.177123 (2016).
40. Zhou, M. et al. Identification and validation of potential prognostic LncRNA biomarkers for predicting survival in patients with multiple myeloma. J. Exp. Clin. Cancer Res. (2015). doi:10.1186/s13046-015-0219-5
41. Scully, C. & Bagan, J. Oral squamous cell carcinoma overview. Oral Oncol. (2009). doi:10.1016/j.oraloncology.2009.01.004
42. Jiao, F. et al. Elevated expression level of long noncoding RNA MALAT-1 facilitates cell growth, migration and invasion in pancreatic cancer. Oncol. Rep. (2014). doi:10.3892/or.2014.3518
43. Gutschner, T. et al. The noncoding RNA MALAT1 is a critical regulator of the metastasis phenotype of lung cancer cells. Cancer Res. (2013). doi:10.1158/0008-5472.CAN-12-2850
44. Gutschner, T., Hämmerle, M. & Diederichs, S. MALAT1 - A paradigm for long noncoding RNA function in cancer. Journal of Molecular Medicine (2013). doi:10.1007/s00109-013-1028-y
45. Lai, M. C. et al. Long non-coding RNA MALAT-1 overexpression predicts tumor recurrence of hepatocellular carcinoma after liver transplantation. Med. Oncol. (2012). doi:10.1007/s12032-011-0004-z
46. Fujimoto, A. et al. Whole-genome mutational landscape and characterization of noncoding and structural mutations in liver cancer. Nat. Genet. (2016). doi:10.1038/ng.3547
47. YiRen, H. et al. Long noncoding RNA MALAT1 regulates autophagy associated chemoresistance via miR-23b-3p sequestration in gastric cancer. Mol Cancer16, 174, doi:10.1186/s12943-017-0743-3 (2017).
48. Tao, F., Tian, X., Ruan, S., Shen, M. & Zhang, Z. miR-211 sponges IncRNA MALAT1 to suppress tumor growth and progression through inhibiting PHF19 in ovarian carcinoma. FASEB. J. (2018). doi:10.1096/fj.201800495RR
49. Hirata, H. et al. Long Noncoding RNA MALAT1 Promotes Aggressive Renal Cell Carcinoma through Ezh2 and interacts with miR-205. Cancer Res. (2015). doi:10.1158/0008-5472.CAN-14-2931
50. Li, J. et al. LncRNA MALAT1 exerts oncogenic functions in lung adenocarcinoma by targeting miR-204. Am J Cancer Res6, 1099-1107 (2016).
51. Tan, X., Huang, Z. & Li, X. Long Non-Coding RNA MALAT1 Interacts With miR-204 to Modulate Human Hilar Cholangiocarcinoma Proliferation, Migration, and Invasion by Targeting CXCR4. J. Cell Biochem. (2017). doi:10.1002/jcb.25862
52. Li, Q. et al. Disrupting MALAT1/miR-200c sponge decreases invasion and migration in endometrioid endometrial carcinoma. Cancer Lett. (2016). doi:10.1016/j.canlet.2016.09.019
53. Velazquez-Torres, G. et al. A-to-I miR-378a-3p editing can prevent melanoma progression via regulation of PARVA expression. Nat. Commun. (2018). doi:10.1038/s41467-018-02851-7
54. Kozubek, J. et al. In-depth characterization of microRNA transcriptome in melanoma. PLoS One (2013). doi:10.1371/journal.pone.0072699
55. Knoll, S. et al. E2F1 induces miR-224/452 expression to drive EMT through TXNIP downregulation. EMBO Rep. (2014). doi:10.15252/embr.201439392
56. Li, J., Liu, X., Li, C. & Wang, W. miR-224-5p inhibits proliferation, migration, and invasion by targeting PIK3R3/AKT3 in uveal melanoma. J. Cell Biochem. (2019). doi:10.1002/jcb.28507

57. Galasso, M. et al. Loss of miR-204 expression is a key event in melanoma. Mol. Cancer (2018). doi:10.1186/s12943-018-0819-8

58. Diaz-Martinez, M. et al. miR-204-5p and miR-211-5p Contribute to BRAF Inhibitor Resistance in Melanoma. Cancer Res. (2018). doi:10.1158/0008-5472.CAN-17-1318

59. Xu, Y., Brenn, T., Brown, E. R., Doherty, V. & Melton, D. W. 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). doi:10.1038/bjc.2011.568

60. Dar, A. A. et al. miRNA-205 suppresses melanoma cell proliferation and induces senescence via regulation of E2F1 protein. J. Biol. Chem. (2011). doi:10.1074/jbc.M111.227611

61. Sanchez-Sendra, B. et al. Downregulation of intratumoral expression of miR-205, miR-200c and miR-125b in primary human cutaneous melanomas predicts shorter survival. Sci. Rep. (2018). doi:10.1038/s41598-018-35317-3

62. Mirzaei, H. et al. MicroRNAs as potential diagnostic and prognostic biomarkers in melanoma. Eur. J. Cancer (2016). doi:10.1016/j.ejca.2015.10.009

63. Elson-Schwab, I., Lorentzen, A. & Marshall, C. J. MicroRNA-200 family members differentially regulate morphological plasticity and mode of melanoma cell invasion. PLoS One (2010). doi:10.1371/journal.pone.0013176

64. Zhao, H. et al. Long noncoding RNA HEIH promotes melanoma cell proliferation, migration and invasion via inhibition of miR-200b/a/429. Biosci. Rep. (2017). doi:10.1042/BSR20170682

65. Jin, L. et al. MicroRNA-149*, a p53-responsive microRNA, functions as an oncogenic regulator in human melanoma. Proc. Natl. Acad. Sci. U. S. A. (2011). doi:10.1073/pnas.1019312108

66. Bell, R. E. et al. Transcription factor/microRNA axis blocks melanoma invasion program by miR-211 targeting NUAK1. J. Invest. Dermatol. (2014). doi:10.1038/jid.2013.340

67. Levy, C. et al. Intronic miR-211 assumes the tumor suppressive function of its host gene in melanoma. Mol. Cell (2010). doi:10.1016/j.molcel.2010.11.020

68. Huber, V. et al. Tumor-derived microRNAs induce myeloid suppressor cells and predict immunotherapy resistance in melanoma. J. Clin. Invest. (2018). doi:10.1172/JCI98060

69. Fruman, D. A. & Rommel, C. PI3K and cancer: Lessons, challenges and opportunities. Nat. Rev. Drug Discov. (2014). doi:10.1038/nrd4204

70. Davies, M. A. The role of the PI3K-AKT pathway in melanoma. Cancer Journal. (2012). doi:10.1097/PPO.0b013e31824d448c

71. Wang, Y. et al. Long Noncoding RNA LINC00261 Suppresses Cell Proliferation and Invasion and Promotes Cell Apoptosis in Human Choriocarcinoma. Oncol Res25, 733-742, doi:10.3727/096504016X14772362173376 (2017).

72. Lin, K. et al. Long noncoding RNA LINC00261 induces chemosensitization to 5-fluorouracil by mediating methylation-dependent repression of DPYD in human esophageal cancer. FASEB. J. (2019). doi:10.1096/fj.201800759R

73. Yu, Y. et al. Long non-coding RNA linc00261 suppresses gastric cancer progression via promoting Slug degradation. J. Cell. Mol. Med. (2017). doi:10.1111/jcmm.13035

74. Shahabi, S. et al. LINC00261 Is an Epigenetically Regulated Tumor Suppressor Essential for Activation of the DNA Damage Response. Cancer Res. (2019). doi:10.1158/0008-5472.CAN-18-2034

75. Sun, L., Sun, P., Zhou, Q. Y., Gao, X. & Han, Q. Long noncoding RNA MALAT1 promotes uveal melanoma cell growth and invasion by silencing of miR-140. Am J Transl Res8, 3939-3946 (2016).

76. Saleibani, A., Faxalv, L., Claesson, K., Jonsson, J. I. & Osman, A. miR-20b regulates expression of proteinase-activated receptor-1 (PAR-1) thrombin receptor in melanoma cells. Pigment Cell Melanoma Res. (2014). doi:10.1111/jpcr.12217

77. Mao, X. H. et al. MicroRNA-21 regulates the ERK/NF-kappaB signaling pathway to affect the proliferation, migration, and apoptosis of human melanoma A375 cells by targeting SPRY1, PDCD4, and PTEN. Mol. Carcinog. (2017). doi:10.1002/mc.22542

78. Yang, C. H., Yue, J., Pfeffer, S. R., Handorf, C. R. & Pfeffer, L. M. MicroRNA miR-21 regulates the metastatic behavior of B16 melanoma cells. J. Biol. Chem. (2011). doi:10.1074/jbc.M111.285098
Due to technical limitations, all tables are available as downloads in the Supplementary Files section.
Figure 1

(A) Heatmap analysis of miRNA differential expressed profiles in GSE24996; (B) Volcano analysis of miRNA expressed profiles in GSE24996; (C) Heatmap analysis of miRNA differential expressed profiles in GSE35579; (D) Volcano analysis of miRNA expressed profiles in GSE35579; (E) Heatmap analysis of miRNA differential expressed profiles in GSE62372; (F) Volcano analysis of miRNA expressed profiles in GSE62372; (G) Heatmap analysis of RNA differential expressed profiles in GSE112509; (H) Volcano analysis of RNA expressed profiles in
Figure 2

Venn diagram: (A) DEMs were selected with $|\log_{2}FC| > 1$ and adjusted P-value < 0.05 among the non-coding RNA profiling sets, GSE24996, GSE35579 and GSE62372. The candidates 18 miRNAs were shared in at least two datasets. (B) DEMs were selected by intersecting mRNAs predicted by DEMis through starbase and differential expressed mRNAs in GSE112509. (C) DELs were selected by intersecting IncRNAs predicted by DEMis through starbase and differential expressed IncRNAs in GSE112509.
Figure 3

(A) ceRNA network. The round rectangle represents IncRNAs, the diamond represents miRNAs, and the ellipse represents mRNAs. There are 53 IncRNA nodes, 16 miRNA nodes, 898 mRNA nodes and 609 edges in the network. (B-E) Biological function and pathway analysis of differentially expressed mRNAs. (B) The top 15 significant changes in GO-BP. (C) The top 15 significant changes in the GO-CC. (D) The top 15 significant changes in the GO-MF. (E) The top 15 significant changes in the KEGG pathway. Note: more details are shown in Table 1.
(A) The ceRNA sub-network of MALAT1. The round rectangle represents lncRNAs, the diamond represents miRNAs, and the ellipse represents mRNAs. There are 1 lncRNA nodes, 9 miRNA nodes, 158 mRNA nodes and 209 edges in the network. (B-E) Biological function and pathway analysis of MALAT1 paired mRNAs. (B) The top 10 significant changes in the GO-BP. (C) The top 10 significant changes in the GO-CC. (D) The top 10 significant changes in the GO-MF. (E) The top 10 significant changes in the KEGG pathway.
Figure 5

(A) The ceRNA sub-network of LINC00943. The round rectangle represents IncRNAs, the diamond represents miRNAs, and the ellipse represents mRNAs. There are 1 IncRNA node, 7 miRNA nodes, 182 mRNA nodes and 209 edges in the network. (B-E) Biological function and pathway analysis of LINC00943 paired mRNAs. (B) The top 10 significant changes in the GO-BP. (C) The top 10 significant changes in the GO-CC. (D) The top 10 significant changes in the GO-MF. (E) The top 10 significant changes in the KEGG pathway.
Figure 6

(A) The ceRNA sub-network of LINC00261. The round rectangle represents IncRNAs, the diamond represents miRNAs, and the ellipse represents mRNAs. There are 1 IncRNA nodes, 5 miRNA nodes, 123 mRNA nodes and 163 edges in the network. (B-E) Biological function and pathway analysis of LINC00261 paired mRNAs. (B) The top 10 significant changes in the GO-BP. (C) The changes in the GO-CC. (D) The top 10 significant changes in the GO-MF. (E) The changes in the KEGG pathway.
Figure 7

The expression level of MALAT1 (A), LINC00943 (B) and LINC00261 (C) in normal skin, adjacent normal skin and melanoma tissues.

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- [Table6.jpg](#)
- [Table2.jpg](#)
- [Table3.jpg](#)
- [Table4.jpg](#)
- [Table5.jpg](#)
- [Table1.jpg](#)