Associated with Immune Function, miR-150-5p is a Favorable Biomarker for Head and Neck Cancer Patients

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Abstract

Background

Accumulating evidence has shown that dysregulated expression of microRNAs plays a key role in tumorigenesis. To explore the mechanisms of this we conducted this study.

Methods

Five Gene Expression Omnibus datasets (GEO) datasets, GSE32960, GSE36682, GSE43039, GSE70970 and GSE118613 and head and neck squamous cell carcinoma data of The Cancer Genome Atlas (TCGA) were analysis in this study.

Results

By analyzing the microRNA expression profile of nasopharyngeal carcinoma (NPC) in the five GEO datasets, we identified miR-150-5p as potential biomarker for patient survival. To explore the mechanisms of this, We examined the head and neck squamous cell carcinoma data of TCGA and found that miR-150-5p was correlated with high enrichment of tumor-infiltrating B cells, low enrichment of cancer-associated fibroblasts and down-regulated oncogenic pathways. miR-150-5p may also improve the immune response in the tumor microenvironment. These findings may explain how miR-150-5p improves outcome of head and neck squamous cell carcinoma patients including NPC. Additionally, the exosomal long non-coding RNA AC073130.1 was identified as a potential regulator of miR-150-5p. As miR-150-5p can also be released via exosomes, this study provides insight into the cross-talk of tumor cells and B cells in the tumor microenvironment via exosomal AC073130.1 and miR-150-5p.

Conclusion

MiR-150-5p improves outcome of head and neck squamous cell carcinoma patients by improving the immune response. There might be a cross-talk of tumor cells and B cells in the tumor microenvironment via exosomal AC073130.1 and miR-150-5p.

Background

Nasopharyngeal carcinoma (NPC), prevailing in the Southeast Asia, is an epithelial carcinoma arising from nasopharyngeal mucosa[1]. Although significant advances have been achieved in the treatment strategies for NPC, NPC is a major cause of death in epidemiological areas[1].

MicroRNAs (miRNAs) are a type of endogenous non-coding RNA approximately 21–23 nucleotides in length. miRNA can inhibit the translation of target mRNAs by forming a miRNA-induced silencing complex[2]. Studies have shown that miRNA can function as tumor suppressors or oncogenes in many types of cancer including NPC by affecting cell cycle, proliferation, apoptosis, migration, invasion and radiotherapy sensitivity[3, 4].
To gain more insights into the mechanism underlying NPC, we investigated the miRNA expression profile in NPC by analyzing five Gene Expression Omnibus (GEO) datasets. miR-150-5p was found as tumor suppressor and was down-regulated in NPC tissues compared with non-NPC tissues. However the function of miR-150-5p is controversial. For example, miR-150-5p was found to exhibit both tumor suppressor functions[5] as well as oncogenic functions in non-small cell lung cancer[6]. miR-150-5p was reported as an exosomal non-coding RNA[7] and can be released by components in tumor microenvironment (TME) [8]. Therefor we hypothesis that miR-150-5p can not only affect the biological behavior of tumor cells, but also affect TME around tumor cells. Then we analyzed samples in head and neck squamous cell carcinoma (HNSC) data of The Cancer Genome Atlas (TCGA) database to explore the potential anti-cancer mechanism of miR-150-5p.

**Methods**

**Datasets**

Five datasets, GSE32960, GSE36682, GSE43039, GSE70970 and GSE118613, including miRNA-seq data of NPC in GEO, were analyzed. GSE118613 data were from blood samples. Survival status, survival time, metastasis status, and time to metastasis in data from GSE70970 were used for analyses.

miRNA-seq data and mRNA-seq data of HNSC patients in TCGA who had miRNA-seq data available at the same time were collected. The clinical characteristics of the HNSC patients were also collected.

**Bioinformatics analysis**

Analyses were performed using R software 3.6.3. DESeq2 was used for differentially expressed gene (DEG) analysis. miRNAs with LogFC > 1 and adjusted p < 0.1 were selected as miRNAs with significant expression[9]. For mRNAs, LogFC > 1 and adjust p < 0.05 were considered to indicate mRNAs with significant expression. Volcano plot and heatmap of differentially expressed genes were obtained by ggplot2.

Using the median expression of miR-150-5p, HNSC patients were divided into the high miR-150-5p expression group and low miR-150-5p expression group. Survival package and survminer package were used for Kaplan–Meier survival plots and log-rank test was used to compare the survival curves. P < 0.05 was considered to indicate statistical significance. Cox regression analysis was performed using the survival package.

Gene Ontology (GO) enrichment analysis, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis and Gene Set Enrichment Analysis (GSEA) were performed using clusterProfiler package.

Correlation analysis was tested by Spearman correlation.

**Tumor-infiltrating cells**
The tumor-infiltrating cells in HNSC were calculated by Timer[10], quanTIseq, MCP-Counter, EPIC, xCell[11] and CIBERSORT[12] respectively. The enrichment of different cells was compared by Wilcox test. The infiltrating cells that were significantly up-regulated or down-regulated were selected. GSVA package[13] was used for the correlation of AC073130.1 and B cells.

Network of ceRNAs

The lncRNAs that regulate miR-150-5p were identified by first determining the predicted miR-150-5p-regulating lncRNAs using LncBase[14] and the highly expressed lncRNAs in patients with low miR-150-5p expression and then identifying the overlapping lncRNAs. The potential mRNAs regulated by miR-150-5p were identified by first determining the predicted mRNA targets of miR-150-5p by TargetScan[15] and the highly expressed mRNA in patients with low miR-150-5p expression and then identifying the overlapping mRNAs.

Results

miR-150-5p showed decreased expression in NPC patients and was associated with poor prognosis

To examine the miRNA expression profile in NPC, we performed analysis of five NPC datasets in GEO, GSE32960, GSE36682, GSE43039, GSE70970 and GSE118613. Two microRNAs, miR-150-5p and miR-342-3p, were consistently down-regulated in the NPC group compared with the non-NPC group in all five GEO datasets (Fig. 1A–B, Fig. S1A). Decreased expression of miR-150-5p and miR-342-3p was associated with poor overall survival (OS) in NPC patients (Fig. 1C, Fig. S1B). Decreased miR-150-5p expression was also associated with poor metastasis-free survival in NPC patients (Fig. 1D). miR-342-3p had no effect on the metastasis-free survival in NPC patients (Fig. S1C).

Gene expression profile associated with miR-150-5p

We next explored the potential mechanism by which miR-150-5p affects NPC patient survival using TCGA HNSC database. miR-150-5p was decreased in HNSC tissues compared with paired non-tumor tissues (Fig. 2A). Decreased expression of miR-150-5p associated with poor OS and progression-free survival (PFS) in HNSC patients (Fig. 2B–C). miR-150-5p was also an independent prognostic factor of OS and PFS in HNSC (Fig. 2D).

We next compared the gene expression profiles of HNSC with high miR-150-5p expression and those with low miR-150-5p expression (Fig. 2E–G). GO enrichment analysis of the differentially expressed genes were mainly associated with immune-related terms, such as lymphocyte differentiation, T cell activation, B cell activation, lymphocyte-mediated immunity, humoral immune response, antigen-receptor mediated signal pathway, B cell–mediated immunity, B cell proliferation, and B cell receptor signal pathway (Fig. 2H and Supplementary table 1). KEGG pathway analysis revealed that the differentially expressed genes were also mainly involved in immune-related pathways, such as natural killer cell–mediated cytotoxicity, primary immunodeficiency, T cell receptor pathway, antigen processing and presentation, Th17 cell
differentiation, B cell receptor signal pathway, graft versus host disease, intestinal immune network for IgA production, and allograft rejection (Fig. 2I and Supplementary table 1).

GSEA analysis showed increased activated pathways in B cell activation, B cell–mediated immunity, leukocyte-mediated immunity, activation of immunity, immune response to tumor cell, allograft rejection, graft versus host disease, B cell receptor signal, antigen processing and presentation, T cell receptor signal, complement cascade and adaptive immune system in HNSC with high expression of miR-150. While the activation of pathways in MET signal, cell junction organization, non-integrin membrane–ECM interactions, hypoxia, glycolysis, angiogenesis and epithelial mesenchymal transition were decreased in HNSC with high expression of miR-150. (Fig. 2J–N and Supplementary table 2). These results suggested that the expression of miR-150-5p was associated with immune-related cells and the immune reaction in HNSC patients.

miR-150-5p was associated with tumor-infiltrated B lymphocytes

The Extracellular Vesicles miRNA Database (EVmiRNA) contains 462 small non-coding RNA sequencing datasets of extracellular vesicles from 17 tissues/diseases[8]. In the EVmiRNA database, miR-150-5p was found in exosomes or microvesicles secreted by some cell types in the TME, such as lymphocytes, fibroblasts, endothelial cells, mast cells and mesenchymal stem cells[16–19] (Fig. 3A). Exosomes function as messengers of cell-cell communication and are released into paracellular and body fluids, such as plasma, salvia, urine, and breast milk[20, 21]. The cross-talk between tumor cells and stromal cells via exosomes in the TME regulates TME remodeling, invasion, metastasis, angiogenesis, immune suppression and drug resistance[22, 23]. Therefore, we investigate the correlation of miR-150-5p and TME.

The proportions of TME-infiltrating cells in HNSC were calculated using Timer, quanTIseq, MCP-Counter, EPIC, xCell and CIBERSORT. B cells, CD4+T cells, CD8+T cells, neutrophils, macrophages, myeloid-dendritic cells, NK cells, monocytes, mast cells and endothelial cells were upregulated while cancer-associated fibroblasts (CAFs) were decreased in the high miR-150-5p expression group (Fig. 3B–G). Correlation analysis revealed that B lymphocytes were correlated with the expression of miR-150-5p throughout all five predicting algorithms (Fig. 3H–L and Supplementary table 3). This suggested that decrease of miR-150-5p may result from the decrease of B lymphocytes. Survival analysis showed that a higher abundance of B lymphocytes indicated better survival in HNSC patients (Fig. 3M and Supplementary table 4).

CIBERSORT and xCell were used to calculate the abundance of the subtypes of B lymphocytes. Naïve B cells, memory B cells and plasma B cells were all positively correlated with miR-150-5p expression (Supplementary table 3). Higher level of plasma B cells was also correlated with longer survival of HNSC patients (Fig. 3N and Supplementary table 4). These results suggested that the impaired survival of HNSC patients may be related to the decrease of B lymphocyte in the TME.

miR-150-5p was regulated by AC073103.1.
As miR-150-5p was found down-regulated in NPC and TCGA HNSC compared with non-tumor tissues, we next investigate the potential cause of decreased miR-150-5p. To explore potential ceRNAs that may regulate miR-150-5p, we examined IncRNA expressions in HNSC with high miR-150-5p expression and those with low miR-150-5p expression (Fig. 4A–C). Subsequent analysis identified 89 potential IncRNAs that regulate miR-150-5p and 6 mRNAs that may be regulated by miR-150-5p (Fig. 4D, E). The ceRNA network associated with miR-150-5p is shown in Fig. 4F.

The exoRBase database is a repository of circular RNA (circRNA), IncRNA and mRNA derived from RNA-seq data analyses of human blood exosomes[24]. The IncRNA AC073103.1 was one of the candidate regulators of miR-150-5p identified above and was found in blood exosomes in the exoRBase database (Fig. 4G). AC073103.1 was increased in cancer patients compared with normal person (Fig. 4G). In TCGA HNSC, the expression of AC073103.1 was increased in tumor tissues compared with paired non-tumor tissues (Fig. 4H). High expression of AC073103.1 was associated with poor survival in HNSC. Furthermore, high expression of AC073103.1 was associated with low enrichment of B cells (Fig. 4J–L). These data indicated that tumor cells may down-regulated immune reaction pathways and decrease the enrichment of immune-related cells in the TME by increasing the expression of AC073103.1 (Fig. 4M).

**Discussion**

Dysregulation of miRNAs plays a key role in cancer progression in most cancers including NPC [25]. In addition to human miRNAs, miRNAs encoded by Epstein-Barr virus (EBV) also affect the proliferation, invasion, and immune evasion of tumor cells[25]. Only GSE43039[26] contained EBV-encoded miRNAs among all the five GEO datasets, and therefore no EBV-encoded miRNAs were analyzed in this study. miR-150-5p was down-regulated in GEO NPC and TCGA HNSC datasets. miR-150-5p was also associated with OS and DFS of NPC as well as OS and PFS of HNSC. Our findings suggest that a higher immune response, lower enrichment of CAF and down-regulation of oncogenic pathways may represent mechanisms by which high expression of miR-150-5p led to better patient prognosis.

In this study most of the differentially expressed genes between high and low miR-150-5p expression groups were enriched in immune-related GO terms and KEGG pathways. GSEA analysis also showed that the immune response was higher in patients with higher miR-150-5p expression. As immune suppression is one of the main factors that cause cancer immune evasion and tumor development[27], a suppressed immune response may be the most important potential cause of poor outcomes in patients with low miR-150-5p expression.

Recent studies has revealed that tumor-infiltrating B cells could promote the T cell response by forming a tertiary lymphoid structure[28] and improve immunotherapy response and survival[29]. In this study, many B cell-related pathways were activated in the miR-150-5p high expression group and B cells were correlated with the expression of miR-150-5p in all five predicting algorithms. B cells were also associated with the survival of HNSC patients. Furthermore, plasma B cells, the effector cell of B cells, also correlated
with the expression of miR-150-5p and survival of HNSC patients. This evidence suggested that tumor-infiltrating B cells in HNSC were responsible for patient outcomes.

Previous studies have indicated that miR-150 may function to maintain homeostasis of B cell development: as an exosomal miRNA, miR-150-5p is highly expressed in mature B cells, while it blocks maturation of premature B cells[30, 31]. Therefore, high level of miR-150-5p in TME could not increase the enrichment of mature B cells, but it could illustrate increased enrichment of mature B cells. However, some studies showed that high expression of miR-150 enhances the antigen presentation and B cell receptor signaling[32, 33]. Therefore high expression of miR-150-5p might up-regulate the immune response and improve patient outcome.

In this study, a lower abundance of CAFs was found in the high miR-150-5p expression group. CAFs originate from normal fibroblasts, and hypoxia-inducible factor (HIF)-1α transcription factor is one of the main factors that promote the conversion of normal fibroblasts to CAFs[34]. The down-regulated pathway of hypoxia in this study might be the reason underlying the low abundance of CAFs in the high miR-150-5p expression group. Recent studies have shown the tumor-promoting roles of CAFs, such as functions in promoting the proliferation and stemness of cancer, facilitating invasion and migration, controlling vascular and immune system and drug resistance[17, 35]. Therefore, a reduced abundance of CAFs might be another reason of better outcome in patients with high miR-150-5p expression.

The GSEA analysis also revealed that several oncogenic pathways were down-regulated in the case of high expression of miR-150. Although miR-150-5p acts as oncogene in some cancers[6, 36–38], miR-150-5p has mostly been show to play an anti-tumor role in cancer[5, 39–47]. The different roles of miR-150-5p in different studies and cancer types might depend on the target gene it regulates. The anti-cancer role of miR-150-5p in our study is consistent with others in HNSC[40, 42, 45].

Some lncRNAs contain binding sequences for specific target miRNAs and thus can act as ‘sponges’ or competitive endogenous RNAs to affect the subsequent activation of target mRNAs[48]. Studies have reported that a large number of RNA transcripts contain miRNA-binding sites, indicating that these RNA transcripts may regulate each other by competing for shared target miRNAs[49]. Additionally, dysregulation of non-coding RNAs is often involved with cancer[50]. Therefore, we explored the potential lncRNAs that may sponge miR-150-5p. Among the overlapping down-regulated lncRNAs in the miR-150-5p high expression group and lncRNAs predicted by LncBase, one lncRNA, AC073103.1, was correlated with patient survival and enrichment of B cells in the TME. Previous studies showed that AC073103.1 is released via exosomes and up-regulated in some types of cancers[24]. Therefore, tumor cells may reshape the TME by releasing exosomal AC073103.1. We also identified six potential target mRNAs of miR-150-5p and three, GHSR[51], IGF2BP1[52], and PEG10[53], were reported with oncogenic functions. Therefore, AC073103.1 might act as oncogene via competing for miR-150-5p.

Conclusions
Here we found the correlation of miR-150-5p and tumor infiltrating B cells and showed that miR-150-5p may improve the immune response in TME. This study provides insight into the cross-talk of HNSC cells and B cells in the TME through exosomal AC073130.1 and miR-150-5p.

**Abbreviations**

GO: Gene Ontology; TCGA: The Cancer Genome Atlas; HNSC: head and neck squamous cell carcinoma; NPC: nasopharyngeal carcinoma; TME: tumor microenvironment; OS: overall survival; PFS: progression-free survival; KEGG: Kyoto Encyclopedia of Genes and Genomes; GSEA: Gene Set Enrichment Analysis; CAFs: cancer-associated fibroblasts; ceRNAs: competitive endogenous RNAs; EBV: Epstein-Barr virus.

**Declarations**

**Ethics approval and consent to participate**

The study was approved by the institutional ethics committee of Sun Yat-sen University (Guangzhou, China).

**Consent for publication**

Not applicable.

**Availability of data and materials**

The information of this study here is obtained from GEO (https://www.ncbi.nlm.nih.gov/geo/) and the TCGA (https://portal.gdc.cancer.gov/).

**Competing interests**

The authors declare that they have no conflicts of interest with the contents of this article.

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**Authors' contributions**

ZL participated in study design, performed the bioinformatics analysis and wrote the initial manuscript. LZ, LP, YM and CL participated in study design and edited the manuscript.
JW, LX, DM, XP, YL, MW, DX, LG, DS, LD and BH participated in data analysis and edited the manuscript. CQ conceived and designed the study and edited the manuscript.

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References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394–424.

2. Fabian MR, Sonenberg N. The mechanics of miRNA-mediated gene silencing: a look under the hood of miRISC. Nat Struct Mol Biol. 2012;19(6):586–93.

3. Lee KT, Tan JK, Lam AK, Gan SY. MicroRNAs serving as potential biomarkers and therapeutic targets in nasopharyngeal carcinoma: A critical review. Crit Rev Oncol Hematol. 2016;103:1–9.

4. Tian Y, Tang L, Yi P, Pan Q, Han Y, Shi Y, Rao S, Tan S, Xia L, Lin J, et al. MiRNAs in Radiotherapy Resistance of Nasopharyngeal Carcinoma. J Cancer. 2020;11(13):3976–85.

5. Dai FQ, Li CR, Fan XQ, Tan L, Wang RT, Jin H. miR-150-5p Inhibits Non-Small-Cell Lung Cancer Metastasis and Recurrence by Targeting HMGA2 and β-Catenin Signaling. Mol Ther Nucleic Acids. 2019;16:675–85.

6. Wu Z, Li W, Li J, Zhang Y, Zhang X, Xu Y, Hu Y, Li Q, Sun Q, Ma Z. Higher expression of miR-150-5p promotes tumorigenesis by suppressing LKB1 in non-small cell lung cancer. Pathol Res Pract. 2020;216(11):153145.

7. Zou SL, Chen YL, Ge ZZ, Qu YY, Cao Y, Kang ZX. Downregulation of serum exosomal miR-150-5p is associated with poor prognosis in patients with colorectal cancer. Cancer Biomark. 2019;26(1):69–77.

8. Liu T, Zhang Q, Zhang J, Li C, Miao YR, Lei Q, Li Q, Guo AY. EVmiRNA: a database of miRNA profiling in extracellular vesicles. Nucleic Acids Res. 2019;47(D1):D89–93.

9. Guo L, Lobenhofer EK, Wang C, Shippy R, Harris SC, Zhang L, Mei N, Chen T, Herman D, Goodsaid FM, et al. Rat toxicogenomic study reveals analytical consistency across microarray platforms. Nat Biotechnol. 2006;24(9):1162–9.

10. Li T, Fu J, Zeng Z, Cohen D, Li J, Chen Q, Li B, Liu XS. TIMER2.0 for analysis of tumor-infiltrating immune cells. Nucleic Acids Res. 2020;48(W1):W509–14.

11. Aran D, Hu Z, Butte AJ. xCell: digitally portraying the tissue cellular heterogeneity landscape. Genome Biol. 2017;18(1):220.

12. Newman AM, Steen CB, Liu CL, Gentles AJ, Chaudhuri AA, Scherer F, Khodadoust MS, Esfahani MS, Luca BA, Steiner D, et al. Determining cell type abundance and expression from bulk tissues with digital cytometry. Nat Biotechnol. 2019;37(7):773–82.
13. Bindea G, Mlecnik B, Tosolini M, Kirilovsky A, Waldner M, Obenauf AC, Angell H, Fredriksen T, Lafontaine L, Berger A, et al. Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. Immunity. 2013;39(4):782–95.

14. Paraskevopoulou MD, Vlachos IS, Karagkouni D, Georgakilas G, Kanellos I, Vergoulis T, Zagganas K, Tsanakas P, Floros E, Dalamagas T, et al. DIANA-LncBase v2: indexing microRNA targets on non-coding transcripts. Nucleic Acids Res. 2016;44(D1):D231–8.

15. Agarwal V, Bell GW, Nam JW, Bartel DP. Predicting effective microRNA target sites in mammalian mRNAs. Elife 2015, 4.

16. Mao Y, Keller ET, Garfield DH, Shen K, Wang J. Stromal cells in tumor microenvironment and breast cancer. Cancer Metastasis Rev. 2013;32(1–2):303–15.

17. Bu L, Baba H, Yoshida N, Miyake K, Yasuda T, Uchihara T, Tan P, Ishimoto T. Biological heterogeneity and versatility of cancer-associated fibroblasts in the tumor microenvironment. Oncogene. 2019;38(25):4887–901.

18. Whiteside TL. Exosome and mesenchymal stem cell cross-talk in the tumor microenvironment. Semin Immunol. 2018;35:69–79.

19. Catalano V, Turdo A, Di Franco S, Dieli F, Todaro M, Stassi G. Tumor and its microenvironment: a synergistic interplay. Semin Cancer Biol. 2013;23(6 Pt B):522–32.

20. Principe S, Hui AB, Bruce J, Sinha A, Liu FF, Kislinger T. Tumor-derived exosomes and microvesicles in head and neck cancer: implications for tumor biology and biomarker discovery. Proteomics. 2013;13(10–11):1608–23.

21. Zhou Y, Xia L, Lin J, Wang H, Oyang L, Tan S, Tian Y, Su M, Wang H, Cao D, et al. Exosomes in Nasopharyngeal Carcinoma. J Cancer. 2018;9(5):767–77.

22. Taghikhani A, Farzaneh F, Sharifzad F, Mardpour S, Ebrahimi M, Hassan ZM. Engineered Tumor-Derived Extracellular Vesicles: Potentials in Cancer Immunotherapy. Front Immunol. 2020;11:221.

23. Mashouri L, Yousefi H, Aref AR, Ahadi AM, Molaei F, Alahari SK. Exosomes: composition, biogenesis, and mechanisms in cancer metastasis and drug resistance. Mol Cancer. 2019;18(1):75.

24. Li S, Li Y, Chen B, Zhao J, Yu S, Tang Y, Zheng Q, Li Y, Wang P, He X, et al. exoRBase: a database of circRNA, lncRNA and mRNA in human blood exosomes. Nucleic Acids Res. 2018;46(D1):D106–12.

25. Bruce JP, Yip K, Bratman SV, Ito E, Liu FF. Nasopharyngeal Cancer: Molecular Landscape. J Clin Oncol. 2015;33(29):3346–55.

26. Cai L, Ye Y, Jiang Q, Chen Y, Lyu X, Li J, Wang S, Liu T, Cai H, Yao K, et al. Epstein-Barr virus-encoded microRNA BART1 induces tumour metastasis by regulating PTEN-dependent pathways in nasopharyngeal carcinoma. Nat Commun. 2015;6:7353.

27. Shimizu K, Iyoda T, Okada M, Yamasaki S, Fujii SI. Immune suppression and reversal of the suppressive tumor microenvironment. Int Immunol. 2018;30(10):445–54.

28. Zhu W, Germain C, Liu Z, Sebastian Y, Devi P, Knockaert S, Brohawn P, Lehmann K, Damotte D, Validire P, et al. A high density of tertiary lymphoid structure B cells in lung tumors is associated with
increased CD4(+) T cell receptor repertoire clonality. Oncoimmunology. 2015;4(12):e1051922.

29. Helmkink BA, Reddy SM, Gao J, Zhang S, Basar R, Thakur R, Yizhak K, Sade-Feldman M, Blando J, Han G, et al. B cells and tertiary lymphoid structures promote immunotherapy response. Nature. 2020;577(7791):549–55.

30. Xiao C, Calado DP, Galler G, Thai TH, Patterson HC, Wang J, Rajewsky N, Bender TP, Rajewsky K. MiR-150 controls B cell differentiation by targeting the transcription factor c-Myb. Cell. 2007;131(1):146–59.

31. Zhou B, Wang S, Mayr C, Bartel DP, Lodish HF. miR-150, a microRNA expressed in mature B and T cells, blocks early B cell development when expressed prematurely. Proc Natl Acad Sci U S A. 2007;104(17):7080–5.

32. Mi QS, Xu YP, Qi RQ, Shi YL, Zhou L: Lack of microRNA miR-150 reduces the capacity of epidermal Langerhans cell cross-presentation. Exp Dermatol 2012, 21(1):876–877.

33. Mraz M, Chen L, Rassenti LZ, Ghia EM, Li H, Jepsen K, Smith EN, Messer K, Frazer KA, Kipps TJ. miR-150 influences B-cell receptor signaling in chronic lymphocytic leukemia by regulating expression of GAB1 and FOXP1. Blood. 2014;124(1):84–95.

34. Kojima Y, Acar A, Eaton EN, Mellody KT, Scheel C, Ben-Porath I, Onder TT, Wang ZC, Richardson AL, Weinberg RA, et al. Autocrine TGF-beta and stromal cell-derived factor-1 (SDF-1) signaling drives the evolution of tumor-promoting mammary stromal myofibroblasts. Proc Natl Acad Sci U S A. 2010;107(46):20009–14.

35. Kalluri R. The biology and function of fibroblasts in cancer. Nat Rev Cancer. 2016;16(9):582–98.

36. Tung CH, Kuo LW, Huang MF, Wu YY, Tsai YT, Wu JE, Hsu KF, Chen YL, Hong TM. MicroRNA-150-5p promotes cell motility by inhibiting c-Myb-mediated Slug suppression and is a prognostic biomarker for recurrent ovarian cancer. Oncogene. 2020;39(4):862–76.

37. Oboshi W, Hayashi K, Takeuchi H, Ikeda K, Yamaguchi Y, Kimura A, Nakamura T, Yukimasa N. MicroRNA-150 suppresses p27(Kip1) expression and promotes cell proliferation in HeLa human cervical cancer cells. Oncol Lett. 2020;20(5):210.

38. Liu F, Di Wang X. miR-150-5p represses TP53 tumor suppressor gene to promote proliferation of colon adenocarcinoma. Sci Rep. 2019;9(1):6740.

39. Misono S, Seki N, Mizuno K, Yamada Y, Uchida A, Sanada H, Moriya S, Kikkawa N, Kumamoto T, Suetsugu T, et al: Molecular Pathogenesis of Gene Regulation by the miR-150 Duplex: miR-150-3p Regulates TNS4 in Lung Adenocarcinoma. Cancers (Basel) 2019, 11(5).

40. Kolenda T, Guglas K, Kopczyńska M, Teresiak A, Bliźniak R, Mackiewicz A, Lamperska K, Mackiewicz J. Oncogenic Role of ZFAS1 IncRNA in Head and Neck Squamous Cell Carcinomas. Cells 2019, 8(4).

41. Suetsugu T, Koshizuka K, Seki N, Mizuno K, Okato A, Arai T, Misono S, Uchida A, Kumamoto T, Inoue H. Downregulation of matrix metalloproteinase 14 by the antitumor miRNA, miR-150-5p, inhibits the aggressiveness of lung squamous cell carcinoma cells. Int J Oncol. 2018;52(3):913–24.

42. Koshizuka K, Hanazawa T, Kikkawa N, Katada K, Okato A, Arai T, Idichi T, Osako Y, Okamoto Y, Seki N. Antitumor miR-150-5p and miR-150-3p inhibit cancer cell aggressiveness by targeting SPOCK1 in
head and neck squamous cell carcinoma. Auris Nasus Larynx. 2018;45(4):854–65.

43. Osako Y, Seki N, Koshizuka K, Okato A, Idichi T, Arai T, Omoto I, Sasaki K, Uchikado Y, Kita Y, et al. Regulation of SPOCK1 by dual strands of pre-miR-150 inhibit cancer cell migration and invasion in esophageal squamous cell carcinoma. J Hum Genet. 2017;62(11):935–44.

44. Lu W, Zhang H, Niu Y, Wu Y, Sun W, Li H, Kong J, Ding K, Shen HM, Wu H, et al. Long non-coding RNA linc00673 regulated non-small cell lung cancer proliferation, migration, invasion and epithelial mesenchymal transition by sponging miR-150-5p. Mol Cancer. 2017;16(1):118.

45. Koshizuka K, Nohata N, Hanazawa T, Kikkawa N, Arai T, Okato A, Fukumoto I, Katada K, Okamoto Y, Seki N. Deep sequencing-based microRNA expression signatures in head and neck squamous cell carcinoma: dual strands of pre-miR-150 as antitumor miRNAs. Oncotarget. 2017;8(18):30288–304.

46. Wu X, Xia M, Chen D, Wu F, Lv Z, Zhan Q, Jiao Y, Wang W, Chen G, An F. Profiling of downregulated blood-circulating miR-150-5p as a novel tumor marker for cholangiocarcinoma. Tumour Biol. 2016;37(11):15019–29.

47. Sakr M, Takino T, Sabit H, Nakada M, Li Z, Sato H. miR-150-5p and miR-133a suppress glioma cell proliferation and migration through targeting membrane-type-1 matrix metalloproteinase. Gene. 2016;587(2):155–62.

48. Statello L, Guo CJ, Chen LL, Huarte M. Gene regulation by long non-coding RNAs and its biological functions. Nat Rev Mol Cell Biol. 2021;22(2):96–118.

49. Salmena L, Poliseno L, Tay Y, Kats L, Pandol PP: A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? Cell 2011, 146(3):353–358.

50. Gutschner T, Diederichs S. The hallmarks of cancer: a long non-coding RNA point of view. RNA Biol. 2012;9(6):703–19.

51. Liu A, Huang C, Xu J, Cai X. Lentivirus-mediated shRNA interference of ghrelin receptor blocks proliferation in the colorectal cancer cells. Cancer Med. 2016;5(9):2417–26.

52. Kato T, Hayama S, Yamabuki T, Ishikawa N, Miyamoto M, Ito T, Tsuchiya E, Kondo S, Nakamura Y, Daigo Y. Increased expression of insulin-like growth factor-II messenger RNA-binding protein 1 is associated with tumor progression in patients with lung cancer. Clin Cancer Res. 2007;13(2 Pt 1):434–42.

53. Peng YP, Zhu Y, Yin LD, Zhang JJ, Wei JS, Liu X, Liu XC, Gao WT, Jiang KR, Miao Y. PEG10 overexpression induced by E2F-1 promotes cell proliferation, migration, and invasion in pancreatic cancer. J Exp Clin Cancer Res. 2017;36(1):30.

Figures
miR-150-5p was down-regulated in the five GEO datasets. A, Venn plot of five GEO datasets. B miR-150-5p was down-regulated in GSE32960, GSE36682, GSE43039, GSE70970 and GSE118613. C, Overall survival and metastasis-free survival of miR-150-5p in GSE70970.
Figure 2

Gene expression profile associated with miR-150-5p in HNSC. A, miR-150-5p was down-regulated in HNSC. B–D, miR-150-5p expression was associated with overall survival and progress-free survival. E–G, Gene expression profile associated with miR-150-5p. H–I, GO and KEGG analysis of different genes between miR-150-5p high expression group and low expression group in HNSC. J–N, GSEA analysis of different genes between miR-150-5p high expression group and low expression group in HNSC.

Figure 3

miR-150-5p was associated with tumor-infiltrated B lymphocytes. A, miR-150-5p was released via extracellular vesicles. B–G, Radar plot of tumor-infiltrating cells predicted by different methods. H–L, correlation of miR-150-5p and B cells. M–N, survival analysis of different enrichment of B cells.
miR-150-5p was regulated by AC073103.1. A-C, lncRNAs expression profile associated with miR-150-5p. D-E Potential lncRNAs and mRNAs were identified. F, ceRNA network about miR-150-5p; G, AC071130.1 was found expressing in blood (NP, Normal patient; CHD, Coronary heart disease; CRC, Colorectal cancer; HCC, Hepatocellular carcinoma; PADD, Pancreatic adenocarcinoma, WhB, Whole blood). H, AC071130.1 decreased in HNSC. I, AC071130.1 was associated with patient survival. J-L, AC071130.1 was associated with B cell. M, Mechanism diagram of the cross-talk of tumor cells and B cells in TME.

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