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Research Article

CHARACTERIZATION OF OBESITY-ASSOCIATED LONG NONCODING RNAs IN LUNG

Xiaoying Fu[1, 2, 3*], Qinmiao Huang[4, 5], Yancheng Lin[1, 2, 3], Lin Ling[1, 2], Kuoting Ho[1, 2, 3, 6]

[1]Center for Precision Medicine, Yi He Hospital, China

[2]Hi. Q Biomedical Laboratory, Takyun Industrial Park, China

[3]R&D Incubation Center, Collaboration Unit of Quanzhou Medical College-Hi. Q Biomedical Laboratory, China

[4]Department of Pulmonary and Critical Care Medicine, Third Affiliated LuoHu Hospital of Shenzhen University, China

[5]Department of Pulmonary and Critical Care Medicine, Second Affiliated Hospital of Fujian Medical University, China

[6]School of Biomedical Science, Huaqiao University, China

Email: hkt@cnhiqc.com

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ABSTRACT

Obesity often increases the risk of cancer and worsens the prognosis. Unlike most types of cancer, several studies concluded that obesity had an inverse influence on LUAD survival. Increasing evidence showed that obesity-related and adipocyte-derived lncRNAs were associated with cancer initiation, progression, drug resistance, and the tumour microenvironment in lung cancer. In the present study, we identified two BMI-associated lncRNAs (LINC01500 and lnc-MAFB-1) that could potentially regulate tumour progression in LUAD. Both lncRNAs downregulated significantly in the obese lung. The increased expression level of LINC01500 could be observed in LUAD tumors and predict poorer survival.In addition, through estimation from bulk RNA-seq and profiling in single-cell sequencing, we found that the expressions of both BMI-associated lncRNAs were associated with macrophages. The downregulation of BMI-associated lncRNAs could create a less immunosuppressive microenvironment, leading to a more efficient response toward immunotherapy. To our knowledge, this is the first study to investigate BMI-associated lncRNAs in lung. We believe our findings can expand the understanding of obesity and the immune microenvironment in lung cancer.

Keywords: lncRNA; Obesity; Lung cancer; Immune microenvironment; Macrophage

INTRODUCTION

Whilst lung cancer remains the leading cause of cancer mortality worldwide, more and more researchers are engaged in the debate of the relationship between obesity and lung cancer. Extensive investigations on this topic have been conducted in large cohorts after adjusting potential confounders such as smoking and race. Several studies also included additional details to categorize obesity, for example, using BMI trajectories and metabolic obesity as phenotype. Although the effect of obesity on small cell lung cancer and lung squamous cell carcinoma (LUSC) remains inconclusive, a consistent inverse effect of obesity on lung adenocarcinoma (LUAD) were observed across studies, especially for those patients receiving immune checkpoint inhibitor treatments. However, the mechanism for this “obesity paradox” is still unclear. Some investigators suggested that the immunologic derangements led by obesity could upregulate PD-1 on immune cells [1-9].

Unlike most obesity-induced tumors, lung is not directly exposed to adipose tissue. Thus, the association between lung cancer and obesity may be more directly driven by the emic microenvironment. Increasing evidence suggested that lncRNAs, which often act as modulators in the tumor microenvironment, are associated with cancer initiation, progression, and drug resistance. Recent studies showed that obesity-related and adipocyte-derived lncRNAs, such as MALAT1, H19, and MEG3 could deregulate the cancer-associated pathways and affect the survival of lung cancer. Based on these current findings, we hypothesized that lncRNAs may serve as the missing link connecting obesity and lung cancer [10-24].

In this present study, we reported our preliminary findings of BMI-associated lncRNAs. We identified and examined the influence of these lncRNAs on lung cancer progression and prognosis. In addition, we explored the relationship between BMI, BMI-associated lncRNAs, and the immune microenvironment, as well as the potential immunotherapy response. Lastly, we were able to validate the presence of such lncRNAs and their potential immunosuppressive function from a single-cell study. To our knowledge, this is the first study to investigate BMI-associated lncRNAs in lung. We believe that our findings can expand the understanding of obesity and the immune microenvironment in lung cancer.

METHODS

Data Collection and Processing

We acquired the count data and the fragments per kilobase of per million (FPKM) of RNA-sequencing data of the Clinical Proteomic Tumor Analysis Consortium (CPTAC) lung cancer study and TCGA lung study from the GDC Data Portal (https://portal.gdc.cancer.gov/). We further extracted body height, weight, gender, race, smoking status, and survival information for subsequent analysis if available. We also obtained normalized log2 transformed transcripts per million (TPM) of single-cell sequencing data, together with smoking status, from LUAD dataset GSE131907 to profile the expression of lncRNAs in the single-cell resolution.

Statistical Analysis

R version 4.1 was used in the following analysis. To identify the lncRNAs associated with BMI in lung, we divided subjects in the CPTAC study into

two groups, BMI>25 and BMI<25. We used DESeq2 package to identify the differential expressed lncRNAs between these two groups from adjacent normal lung samples in both LUAD and LUSC datasets. We then examined the relationship between lncRNA and lung cancer in the TCGA dataset with the one-sided t-test and validated them in the CPTAC dataset with the one-sided paired t-test. We used the R package "survminer" to decide the cutting point of lncRNA expression for survival analysis. The Kaplan-Meier curves and Cox proportional hazard regression were performed under the R package "survival" and visualized with "survminer". The online tool KM-plotter (https://kmplot.com/analysis/) was used to validate the finding. The average expression and the percentage of expression were calculated to profile the lncRNA expression in the single-cell dataset.

To investigate the relationship between lncRNAs and the immune microenvironment, we used 7 algorithms to estimate the immune cell compositions in the CPTAC dataset, including CIBERSORT-ABS, EPIC, MCP-counter, quanTIseq, xCELL, ImmuneCellAI and TIMER. In addition, we calculated the immunopheno score and TIDE (Tumor Immune Dysfunction and Exclusion) dysfunction score to predict immunotherapy response. We then calculated the correlation between the lncRNAs expression and these scores [25-33].

RESULTS

Identification of BMI-associated lncRNAs in Lung

We used the lncRNA expression from adjacent normal lung in LUAD and LUSC datasets to identify BMI-associated lncRNAs in lung. In the CPTAC study, there are 66 patients with BMI<=25 and 36 patients with BMI>25 in LUAD dataset. Meanwhile, there are 40 patients with BMI<=25 and 50 patients with BMI>25 in LUSC dataset. After quality control, differential expressed lncRNAs were defined as lncRNAs with log2 fold change>1 and adjusted p-value<0.05. A total of 14 lncRNAs were selected as differential expressed lncRNAs in LUAD and 13 in LUSC. We noticed that two BMI-associated lncRNAs (LINC01500 and lnc-MAFB-1) were downregulated in BMI>25 groups in both LUAD and LUSC datasets (seen in Table 1). We hypothesized that these two lncRNAs are BMI-associated lncRNAs in lung. SNPs in LINC01500 have been reported to link with childhood obesity and familial colorectal cancer. In the meantime, little is known for lnc-MAFB-1, which was once known as AL035665.1 and RP4-644L1.2 in previous human assembly annotations with ensemble id ENSG00000229771 [34, 35]. (Table 1)

Characterization of BMI-Associated lncRNAs in Lung Cancer

To further investigate these two BMI-associated lncRNAs, we examined their expression and their influence on survival in lung cancer. In the TCGA dataset, we found that the expression of LINC01500 was significantly increased in LUAD tumors, but not in LUSC. The upregulation of LINC01500 can also be observed in LUAD nonsmokers. We later validated this finding in the CPTAC dataset with paired samples. Similar patterns for LINC01500 were observed only in LUAD patients (Figure 1A). Nevertheless, the dynamic of lnc-MAFB-1 in lung cancer is nonconclusive.

Figure 1: A : The lncRNAs expression between normal adjacent lung tissues and tumors in LUAD.
B: The influence of lncRNA expressions on overall survival in CPTAC dataset.
C :The influence of lncRNA expressions on overall survival in KM plotter.

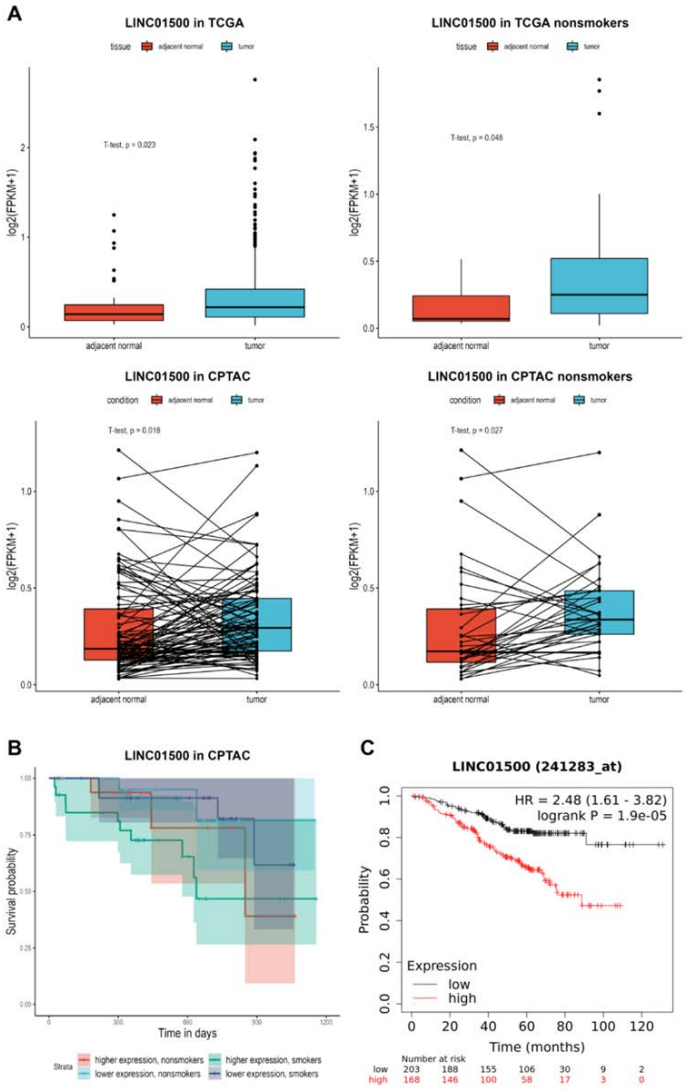

Table 1: The BMI-associated lncRNAs and their influence on LUAD survival

Ensembl gene id	Gene	LUAD (BMI>25 vs. BMI<=25)			LUSC (BMI>25 vs. BMI<=25)			Overall survival in LUAD+	
		log2 (Fold-Change)	p-value	p-value(adjusted)	log2 (Fold-Change)	p-value	p-value(-adjusted)	HR	p-value
ENSG00000229771	lnc-MAFB-1	-1.23	1.05E-09*	4.68E-06	-1	5.18E-05*	0.023	3.395 (1.264,9.119)	0.015*
ENSG00000258583	LINC01500	-1.31	2.97E-06*	1.29E-03	-1	1.36E-04*	0.044	2.963 (1.113,7.892)	0.030*
Noted: * denote statistically significant; + fitting Cox proportional hazards model with expression of lncRNAs from normal adjacent tissues, after adjusting for smoking									

Furthermore, from CPTAC dataset, after adjusting for smoking, we found that both LINC01500 and lnc-MAFB-1 in the normal adjacent lung can predict tumor survival in LUAD (LINC01500: hazard ratio=2.963, 95%CI 1.113-7.892, p=0.03; lnc-MAFB-1: hazard ratio=3.395, 95%CI 1.264-9.119, p=0.015) (seen in Table 1, Figure 1B). Higher expression of these two lncRNAs suggested a poorer overall survival. A similar influence on survival could also be observed from the lncRNAs expression in tumors, but not statistically significant. This is consistent with the finding that tumor-adjacent tissues could build a superior prognosis model. In addition, after adjusting for smoking, we validated the influence of LINC01500 on LUAD survival in a larger sample from the KM plotter (hazard ratio=2.48, 95%CI 1.61-3.82, p=1.9E-05) (Figure 1C). We could not validate the influence of lnc-MAFB-1 in the KM plotter because of the lack of lncRNA coverage in previous studies. Moreover, the influence of these lncRNAs on cancer survival was not able to be observed in LUSC [36, 37].

BMI-Associated lncRNAs and Immune Microenvironment in LUAD

First, we calculated the correlation between BMI-associated lncRNAs and immune cell compositions in CPTAC nonsmokers. We found that the expression of both LINC01500 and lnc-MAFB-1 were positively correlated with the composition of macrophages significantly in all 7 algorithms. In addition, the expression of LINC01500 was also significantly positively correlated with myeloid dendritic cells using CIBERSORT.ABS, MCP-counter, xCELL, and TIMER algorithms. Meanwhile, lnc-MAFB-1 was significantly negatively correlated with neutrophil cells, according to CIBERSORT.ABS, MCP-counter, quanTIseq, and TIMER. Macrophages, dendritic, and neutrophil cells are all considered major immunity mediators in lung.

We then inspected the correlation between the BMI-associated lncRNAs and potential immunotherapy response. Immunopheno score and TIDE dysfunction score are two models to evaluate and predict immunotherapy response. A potential positive immunotherapy response is often correlated with a higher immunopheno score and lower TIDE dysfunction score. Although there's no correlation between BMI and immunopheno score, both LINC01500 and lnc-MAFB-1 were negatively correlated with immunopheno score (LINC01500, r=-0.36, p=2E-04; lnc-MAFB-1, r=-0.27, p=0.007). This finding suggested that the downregulation of these lncRNAs were related to higher immunopheno score and better immunotherapy response. On the other hand, BMI was negatively correlated with TIDE dysfunction score (r=-0.24, p=0.01) while LINC01500 showed a positive correlation (r=0.34, p=4E-04). This finding suggested that higher BMI and lower LINC01500 expression would generate a lower TIDE dysfunction score and hence predict better immunotherapy response. We noted that in both prediction systems, these two lncRNAs showed a significantly positive correlation with myeloid-derived suppressor cells (MDSCs) which are closely related to T cell exclusion signature.

BMI-Associated lncRNAs in Single-Cell LUAD Profile

We used dataset GSE131907 to profile the BMI-associated lncRNAs in single-cell resolution, with clusters annotated by the author. We found that LINC01500 was specifically expressed in monocyte-derived macrophages in LUAD and metastatic brain tumors. In the meantime, lnc-MAFB-1 not only showed expression in monocyte-derived macrophages in tumor cells but metastatic lymph node cells. Lnc-MAFB-1 was also sparsely expressed in exhausted CD8+ T cells and regulatory T cells in lung tumors. These findings agree with our previous finding that both BMI-associated lncRNAs are linked to macrophages and immunosuppressive microenvironments [38].

DISCUSSION

The As far as we know, this is the first study to identify BMI-associated lncRNAs in lung. Most lncRNAs express in a cell-, tissue- and situation-specific manner. Therefore, our finding could hint at the lung-specific mediators that link obesity and cancer. Our finding suggested that these BMI-associated lncRNAs could influence the progress and survival of LUAD, but not LUSC. This is consistent with the recent finding that, the inverse causal relation of obesity and cancer only exists in LUAD, but not in other types of lung tumors. In addition, we validated that the tumor-adjacent tissue could better predict cancer survival with fewer samples [3].

We also predicted the relationship between these BMI-associated lncRNAs and macrophages and validated it in a single-cell LUAD study. However, we found out that in peripheral blood samples, both these two lncRNAs were significantly upregulated in obese lung cancer patients, compared to lung cancer patients with normal BMI (samples not described in this manuscript). The opposite direction of the regulation pattern in peripheral blood indicated that the lncRNA-mediated network could appear in a tissue-specific manner. A recent study indicates that macrophage populations showed diverse regulation function in different tissues, and between tissue-resident and recruited macrophages in lung. For example, macrophages in lung are associated with lipid catabolic process, response to oxidative stress, and myeloid leukocyte migration. Hence, investigators should put more effort to inspect the obesity-induced metabolic reprogramming of myeloid cells in lung. Even though the mechanisms of efficiency of immunotherapy in obesity LUAD patients are largely unknown, some researchers suggested that obesity may enhance the sensitivity of MDSCs and macrophages towards immunotherapy. This hypothesis is agreed with our finding that BMI-associated lncRNAs were linked to macrophages and MDSCs, and thus could affect the response of immunotherapy [39, 40].

This is a preliminary study highlighting the BMI-associated lncRNAs and their potential regulator roles in lung cancer. Even though further validations are still in need, we hope that this study can provide a fresh perspective to investigate the relationship between obesity and the immune microenvironment in lung cancer.

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AUTHORS' CONTRIBUTIONS

KH conceived and supervised this project. QH provided clinical samples and advice. YL and LL performed experiments. XF analyzed the data and drafted

this manuscript.

AVAILABILITY OF DATA AND MATERIALS

The datasets used in the current study are available from the corresponding author on reasonable request.

COMPETING INTERESTS

The authors declare no conflicts of interest.

CONSENT FOR PUBLICATION

Not applicable

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