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Causal Association Between Adipokines And Lung Cancers: A Two-Sample Mendelian Randomization Study

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1. Abstract

1.1. Background: The involvement of certain adipokines link to carcinogenesis, development, and prognosis. However, the roles of adipokines in lung cancer and its histological subtypes remains indistinct. Therefore, the aims of this study are to explore the causal relationship between adipokines and lung cancers.

1.2. Methods: Summary-level data for exposures (six adipokines) and outcomes (lung cancer and its histological subtypes) were collected from the IEU OpenGWAS, International Lung Cancer Consortium(ILCCO) and lectures. Two-sample mendelian randomization (MR) was conducted to estimate the causality by employing single nucleotide polymorphisms (SNPs) as instrument variables (IVs).

1.3. Results: LEPR was associated with risk of lung squamous cell carcinoma (LUSC, OR: 1.05, 95% CI: 1.01-1.08; $P < 0.0125$), and no other adipokines associated with lung cancer and its histological subtypes ($P > 0.05$).

1.4. Conclusion: This results provide the specific adipokines may act a vital role in risk of LUSC.

2. Keywords:
Adipokines, Lung caner, histological subtypes, Mendelian randomization, causal association

3. Introduction

Lung cancer remains the leading cause of cancer death, with anestimated 2.5 million new cases and over 1.8 million deaths globallyin 2022 [1]. In most countries, the five-year survival rate for lung cancer trends to below 20% [2] and is not be significantly influenced by levels of human development [3]. Tobacco remains the primary risk factor for lung cancer, other risk factors include air pollution [4, 5]. Small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) are the two main types of lung cancer, with over 80% of cases diagnosed as NSCLC. Furthermore, NSCLC can be divided into four histological subtypes: lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LSCC or LUSC), and large-cell carcinoma (LCC)[6].Most lung cancers are diagnosed at a advanced stage when curative treatment is no longer possible [1]. Therefore, identifyingmore risk factors related to lung cancer progression is crucial for the early detection and treatment of lung cancer.

Adipokines are generated and secreted by adipocytes or tissue-infiltrating immune cells and are involved in a range of biological functions and processes in cancer, including metabolism, inflammatory responses, and carcinogenesis [7, 8]. Adipocyte-secreted adipokines, such as leptin, adiponectin, Nesfatin-1, resistin, chemerin, and visfatin, have been found to drive lung cancer bone metastasis [9]. These adipokines play a crucial role in determining the effectiveness of anti-cancer immunotherapy in patients with extensive-stage small cell lung cancer (ES-SCLC) [10]. Additionally, visceral-secreted omentin serum levels are reduced in smokers with lung cancer and are associated with their prognosis [11]. Leptin serum levels also serve as a prognostic indicator for lung cancer [12], but another case-control study indicates that serum leptin levels have no prognostic implications in advanced lung cancer patients [13]. The current research results are inconsistent, and the consistency and quality of these studies

have not been well evaluated. They are often subject to confounding and reverse causation bias, which limits the objective assessment of the impact of adipokines on lung cancer. Mendelian randomization (MR) is a powerful statistical technique investigating causal relationships in epidemiological research by using genetic variants as instrumental variables [14]. Since genetic variants are randomly assigned and unaffected by reverse causation, MR effectively avoids the confounding and reverse causation biases that commonly impact traditional observational studies [15]. In this study, we conducted MR analysis to explore the potential relationships between adipokines and lung cancer and its three histological subtypes.

4. Methods

4.1 Study design

The study design is shown in Two-sample MR analysis was conducted to explore the causal relationship between adipokines and lung cancers by using genetic variables as the instruments. Genetic instrument variables (IVs) were selected by stratifying three assumptions. (a) IVs should have a strong association with the exposure (adipokines). (b) IVs should not be associatedwith confounding factors. (c) IVs should be linked to the outcome (lung cancers) only through their effect on the exposure [16]. Since the data used in this study were derived from publicly available GWAS summary-level data, no additional ethical approval was required. To avoid errors due to population stratification, all subjects involved in this study were of European ancestry. The STROBE-MR checklist has been checked [17].

4.2 Data source of exposure

The summary GWAS statistics for adipokines, including adiponectin, leptin, resistin, monocyte chemoattractant protein-1 (MCP-1), leptin receptor (LEPR), and plasminogen activator inhibitor (PAI-1), were obtained from the ADIPOGen consortium belongs to IEU OpenGWAS data project and recent research [18-20]. As shown in Table S1, these statisticsencompass data from39,883 individuals (ieu-a-1), 21,758 individuals (ebi-a-GCST90012076), 21,758 individuals (prot-c-5400_52_3), 21,758 individuals (ebi-a-GCST90012034), 997 individuals (ebi-a-GCST90012007), 34448 individuals (ebi-a-GCST90014291), respectively.

4.3 Data source of outcome

The GWAS summary statistics of lung cancers and three histologic subtypes of lung cancer were obtained from the International Lung Cancer Consortium (ILCCO, https://ilcco.iarc.fr/). The data includes, lung cancer (ieu-a-987): 29,863 cases and 55,586 controls, lung adenocarcinoma (ieu-a-984): 11,245cases and 54,619 controls, squamous cell carcinoma (ieu-a-989): 7,704cases and 54,763controls, and small cell lung cancer (ieu-a-988): 2,791casesand 20,580controls). The detailinformation was shown in.

4.4 Selection of instrument variables (IVs)

SNPs significantly associated with adipokines were identified based on the following criterion, genome-wide significance ($P < 5 \times 10\text{-}6$), clumping R2< 0.01and a distance of 5,000 kb to avoid the linkage disequilibrium (LD). an F-statistic > 10 was used to prevent bias from weak IVs. PhenoScannerwas utilized to provide phenotype information for the SNPs, ensuring they were not associated with confounding factors (such as smoking, body mass index, and type 2 diabetes) or the outcome (lung cancer). This was done by searching all screened SNPs on PhenoScanner V2 (http://www.phenoscanner.medschl.cam.ac.uk/) [21] with a threshold of $P > 1 \times 10\text{-}5$.

4.5 Mendelian randomization (MR)and sensitivity analyses

The inverse variance weighted (IVW) approach was used as the primary method for performing MR analysis. IVW evaluates overall causal effects through a meta-analysis of the Wald ratios of multiple SNPs [22]. Sensitivity analyses included MR-Egger regression, weighted median estimator (WME), MR robust adjusted profile score (MR-RAPS), and MR pleiotropy residual sum and outlier (MR-PRESSO). MR-Egger regressionis a method that tests causal effects by considering the existence of an intercept term to evaluate pleiotropy [23]. WMErobust estimation even if more than half of the SNPs are invalid [24]. MR-RAPS is a statistical inference method for two-sample summary data MR based on robust adjusted profile scores, providing robust estimates even with many weak instrumental variables and increasing statistical power [25]. MR-PRESSO method can outlier SNPs and estimates corrected results excluding horizontal pleiotropy. If no outliers are detected, the results remain consistent with IVW [26]. Statistical power was estimated using an online tool (https://sb452.shinyapps.io/power/), with a power greater than 80% indicating sufficient statistical power for MR. The MR-Egger intercept was used to assess horizontal pleiotropy, with $P < 0.05$ indicating significant pleiotropy. Cochran's Q test was performed to test for heterogeneity, with $P < 0.05$ indicating significant heterogeneity. A leave-one-out analysis was also conducted to assess sensitivity. The Steiger filtering method was used to test the direction of causality.

4.6 Statistical analysis

The IVW, MR-Egger regression, WME, and MR-RAPS methods were employed to evaluate the causal associations between adipokines and lung cancer and its subtypes using the "Twosample MR" package (version 0.5.7). If there was no heterogeneity, a fixed-effects model IVW was used; if heterogeneity was present, a multiple random-effects model IVW was applied. MR-PRESSO analysis was conducted using the "MRPRESSO' R package (version 3.3.2) to identify and remove outlier SNPs and to determine whether outliers influenced the MR results. Forest plots of the leave-one-out results were drawn using the "forestplot" R package (version 3.1.3). Due to multiple testing, a Bonferroni correction P value < 0.0125 (0.05/4) was considered significant. In other analyses, a P value < 0.05 is considered statistically significant.

5. Results

5.1 Selection of IVs

As shown in a total of 34 SNPs for adiponectin, 4 SNPs for Leptin, 44 SNPs for Resistin, 27 SNPs for MCP-1, 5 SNPs for LEPR, and 20 SNPs for PAI-

1 were identified as IV saccording to the genome-wide significance (P < 5 x 10-6), clumping R2< 0.01 and a distance of 5,000 kb, all F-statistic > 10.

5.2 Evaluating the casual effects of six adipokines on lung cancer and three histologic subtypes

No causal association between six adipokines and lung cancer was observed by IVW analysis (P > 0.0125). No horizontal pleiotropy was detected by MR-Egger intercept estimates (Pleiotropy p-value > 0.05), and no heterogeneity was detected by Cochran's Q test (Q p-value > 0.05). WME, MR-Egger, MR-RAPS, and MR-PRESSO provided consistent results. MR-Steiger filtering results indicated no invalid genetic instruments for these MR analyses. Due to the horizontal pleiotropy observed in the genetic instruments of MCP-1 (Q_pval< 0.05), an IVW-random effect model was used to estimate the causal association between MCP-1 and LUAD. No causal effects of the six adipokines on LUAD were found by IVW analysis (P > 0.05). Other analyses yielded results consistent with the IVW findings (P > 0.05). No horizontal pleiotropy was observed according to MR-Egger intercept estimates (Pleiotropy p-value > 0.05). MR-Steiger filtering results indicated no invalid genetic instruments for these MR analyses.

One standard deviation (SD) increased in LEPR was associated with the risk of LUSC by IVW analysis (OR: 1.05, 95% CI: 1.01-1.08; P: 0.05 < 0.0125). The analysis had sufficient statistical power (power > 80%). WEM and MR-RAPS results were consistent with IVW findings. No causal effects of adiponectin, leptin, resistin, MCP-1, and PAI-1 on LUSC were found (P > 0.05). No horizontal pleiotropy was detected by MR-Egger intercept estimates (Pleiotropy p-value > 0.05), and no heterogeneity was detected by Cochran's Q test (Q p-value > 0.05). MR-Steiger filtering results indicated no invalid genetic instruments for these MR analyses. We also found no causal association between the six adipokines and SCLC by IVW analysis (P > 0.05). No horizontal pleiotropy was detected by MR-Egger intercept estimates (Pleiotropy p-value > 0.05), and no heterogeneity was detected by Cochran's Q test (Q p-value > 0.05). MR-Steiger filtering results indicated no invalid genetic instruments for these MR analyses.

5.3 Sensitivity analyses

A series of sensitivity analyses were employed to assess potential horizontal pleiotropy. No horizontal pleiotropy was detected by MR-Egger intercept estimates (Pleiotropy p-value > 0.05). Moreover, the association between the six adipokines and lung cancer and its subtypes was not driven by any single SNP.

6. Discussion

Increasing evidence have indicated that adipokines are involved in lung cancer development, progression, and prognosis [27, 28]. In the present study, we discovered that the certain effects of adipokines on lung cancer and histological subtypes of lung cancer. We found thatincrease of LEPRwas associated withrisk of LUSC, and no other adipokines associated with lung cancer and histological subtypes of lung cancer.

Leptin (LEP) is a polypeptide hormone composed of 167 amino acids, known for its role in regulating neural, immune, and endocrine functions [29-31]. It binds to the leptin receptor (LEPR) to activate various intracellular signaling pathways, including TGF-β [32], JAK/AKT/STAT [33], PI3K/ATK [34], HIF [35, 36], and MAPK signaling pathways [34]. The roles of LEP and LEPR in carcinogenesis, development, and progression are controversial.. In breast cancer, patients with high expression of LEPR have worse recurrence-free survival (RFS) compared to those with low LEPR expression [37]. However, a case-control study suggests that neither LEP nor the LEPR gene has a strong association with premenopausal breast cancer risk, although there is a suggestive association between the LEPR gene and breast cancer grade [38]. Polymorphic variations in the LEP and LEPR genes are associated with disease-free survival (DFS) and colorectal cancer (CRC)-specific survival in the Newfoundland Familial Colorectal Cancer Study [39]. Another clinical study supports that LEP and LEPR are linked to CRC risk [40]. However, no significant differences in serum LEP levels and tissue LEPR expression were observed in CRC patients in Northern Iran [41]. These discrepancies may be due to population stratification caused by factors such as different races, regions, diets, and lifestyles.

LEPR has been recognized as a metabolic checkpoint for pulmonary inflammation, sustaining AMPK signaling in alveolar macrophages (AMs) to suppress necroptosis and subsequently attenuate pulmonary inflammation [42]. LEP and LEPR are highly expressed in NSCLCand accelerate its progression [43], and they can serve as independent prognostic factors for NSCLC [44]. Polymorphisms in the LEPR gene have been identified as being associated with the occurrence and lymph node metastases in NSCLC patients [45]. Thus, LEPR may play a vital role in the etiology and development of NSCLC. Our findings support that high LEPR expression is linked to an increased risk of LUSC. There are several advantages to our Mendelian randomization (MR) analysis. Firstly, the GWAS summary statistics used in this study are derived from individuals of European ancestry, which helps to avoid bias caused by population stratification. Secondly, we conducted rigorous checks for multiplicity and heterogeneity of our instrumental variables, and performed sensitivity analyses and Q-tests using multiple methods to ensure the stability of our analytical results. However, some limitations should also be considered. First, the focus on a single European ancestry limits the generalizability of our findings to other populations. Secondly, relaxing the IV significance threshold to P < 5×10^-6 introduces the possibility of false positives and bias, although the consistent F-statistics >10 suggest that weak instrument bias is less likely. Therefore, we need to validate our findings in larger populations in future studies and employ additional bioinformatic and experimental techniques to confirm the results.

7. Conclusion

Taken together, our MR study highlights the causal effects of LEPR on LUSC. These findings may offer potential diagnostic and treatment targets for LUSC, and support the use of dietary improvements in conjunction with treatment to combat tumors.

8. Availability of data and material:

All datasets used in this study have been described in Methods section and supplied in the supplementary tables.

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