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Evaluating the Level of Homeobox B8 and its association with cell lung cancer proliferation downregulation: A systematic review and meta-analysis

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ABSTRACT

Background and aim: Homeobox family genes have been identified as cancer growth regulators. There is no meta-analysis or comprehensive study on the activity of Homeobox B8 (HOXB8) in lung cancer. The aim of the present study was to Evaluate the level of Homeobox B8 and its association with cell lung cancer proliferation downregulation.

Material and methods: For this systematic review and meta-analysis study, international databases such as MEDLINE (PubMed and Ovid), Web of Science, and Scopus were searched until August 2024 using keywords relevant to the study objectives. STATA/MP. v17 software was used to analyze data.

Results: Five studies were reviewed. high HOXB8 level was a risk factor for unfavorable lung cancer patient overall survival (HR = 3.32; 95% CI 2.78, 3.86; p<0.001). The results suggested a shorter survival time for patients with high Homeobox B8 (HOXB8) expression.

Conclusions: Homeobox B8 (HOXB8) is highly expressed in lung cancer and associated with overall survival.

1. Introduction

According to available statistics, the prevalence of lung cancer is high worldwide, but early diagnosis of this disease can reduce the high mortality rate.[1, 2] According to epidemiological studies, less than 15% of lung cancer patients achieve 5-year survival.[3] According to the American Cancer Society (ACS), the number of patients will exceed 35 million by 2050.[4] Currently, lung cancer is the highest cancer death rate in men and the second leading cause of death in women.[1] When lung cancer is divided into two categories, small cell and non-small cell lung cancer (NSCLC), NSCLC is more common and affects 85% of patients.[5] Various factors cause lung adenocarcinoma. Factors such as air pollution, gender, age, smoking, occupation and eating habits play a role in the expression of genes involved in lung cancer.[6, 7] In a study, mutations in the HER2, BRAF, NF1, MEK1, RET and ROS1 genes were linked to NSCLC adenocarcinoma.[8] Homeobox (HOX) genes are large gene sequences that encode transcription factors that contain 61 long amino acids as part of their functions.[9] There are two groups of mammals' Homeobox genes. A conserved 180-183 nucleotide sequence encoding a homologous domain of 60-61 amino acids is present in class I HOX genes arranged in series on different chromosomes. This domain recognizes DNA sequences with a 5'-TAT-3' core motif and controls the transcriptional activity of downstream genes. Class II HOX genes, also called non-HOX or para-HOX genes, are heterogeneous in their molecular structure and are not distributed tandemly on the chromosomes. These gene families include the PAS, DLS, Hes, and MSX gene families.[10] HOXA, HOXB, HOXC and HOXD are the four clusters of the 39 human HOX genes that can be distinguished based on chromosomal location and sequence similarity. The following are known as HOX loci and are located along a 3'→5' DNA sequence: HOXA, 7p15.3; HOXB, 17p21.3; HOXC, 12q13.3; and HOXD, 2q31. Each cluster has 1-13 loci, including 9-11 genes. Although the precise functions of HOX genes in adult cell differentiation are less understood, they are closely linked to regulating physiological processes and serve as important regulators of vertebrate embryonic development.[10, 11] Nevertheless, increasing data suggest that HOX genes play a critical role in the development and progression of cancers.[12-14] The present study aimed to evaluate the expression of HOXB8 in lung cancer and its effect on the biological functions of lung cancer cell.

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2. Material and methods

Search strategy

Until September 2024, the international databases MEDLINE (PubMed and Ovid), Embase and Cochrane were searched for scientific evidence using relevant keywords to evaluate the level of Homeobox B8 and its association with cell lung cancer proliferation downregulation (Table 1). Scopus Wiley Online Library, Web of Science, Cochrane Central Register of Controlled Trials, EBSCO, ISI, Elsevier and the Google Scholar search engine were also used. The present study is based on the 27-point checklist PRISMA 2020.[15]

Table 1. The search strategy used for each database.

NO.	Search Terms
	The search strategy used in MEDLINE (via PubMed)
1	((("Lung Neoplasms"[Mesh]) OR "Small Cell Lung Carcinoma"[Mesh]) OR "Carcinoma, Non-Small-Cell Lung"[Mesh]) OR ("Lung Neoplasms/blood"[Mesh] OR "Lung Neoplasms/classification"[Mesh] OR "Lung Neoplasms/diagnosis"[Mesh] OR "Lung Neoplasms/prevention and control"[Mesh]).
2	Neoplasms, Pulmonary OR Neoplasm, Pulmonary OR Pulmonary Neoplasm OR Pulmonary Neoplasms OR Neoplasms, Lung OR Lung Neoplasm OR Neoplasm, Lung OR Lung Cancer OR Cancer, Lung OR Cancers, Lung OR Lung Cancers OR Cancer of Lung OR Pulmonary Cancer OR Cancer, Pulmonary OR Cancers, Pulmonary OR Pulmonary Cancers OR Cancer of the Lung.
3	"Genes, Homeobox"[Mesh].
4	Gene, Homeobox OR Homeobox Gene OR Homeobox Genes OR Genes, Homeo Box OR Gene, Homeo Box OR Homeo Box Gene OR Homeo Box Genes OR Genes, Homeotic OR Gene, Homeotic OR Homeotic Gene OR Homeotic Genes OR Hox Genes OR Gene, Hox OR Genes, Hox OR Hox Gene OR Homeo Box Sequence OR Homeo Box Sequences OR Sequences, Homeo Box OR Homeo Box OR Homeo Boxes OR Homeobox OR Homeobox Sequence OR Homeobox Sequences OR Sequence, Homeobox OR Sequences, Homeobox OR Homeoboxes OR Sequence, Homeo Box.
	The search strategy used by Cochrane
1	Lung OR Lung Neoplasms OR lung cancer or Small Cell Lung cancer, Non-Small-Cell Lung cancer.
2	Genes, Homeobox.
	The search strategy used in Embase
1	(lung) OR (lung cancer) OR (lung Neoplasms) OR (Small Cell Lung cancer) OR (Non-Small-Cell Lung cancer): ab, ti, kw.
2	Genes, Homeobox: ab, ti, kw.
3	Chapter' OR 'conference abstract' OR 'conference paper' OR 'conference review' OR 'editorial' OR 'erratum' OR 'letter' OR 'note' OR 'preprint' OR 'short survey'/it (Filter).

Selection criteria

Articles published in English were the inclusion criteria for this research. The answers to the questions in the current study were based on the PICO strategy: Population (P): patients with lung cancer; Intervention (I): Homeobox B8 (C): adjacent normal tissues; Outcome (O): HOXB8 expression. Review studies and books, qualitative studies, animal studies, studies without comprehensive and relevant data, and Data not reported on breast cancer were excluded from the study.

The process of selection and data collection

Two researchers used a standard data collection form, previously created to minimize reporting, data collection errors, and omissions, to collect data from individual subjects. The original form prepared by the research team contained the following information: the author's name, the year of publication, the number of patients, the mean age, Clinical stage, Tumor size, and Lymph node metastasis.

Heterogeneity and publication bias

The heterogeneity across studies was examined using the Chi-square ($\chi 2$) test and quantified by the I2 statistic. According to the I2 value, heterogeneity was classified as low (less than 50%), between 50 and 74% means moderate heterogeneity, and above 75% is considered high heterogeneity.

Data analysis

The effect measure of choice was the Hazard Ratios with 95% confidence intervals. The results were reported based on a Random-effects model with the RMEL method. The data were analyzed at a significance level 0.05 using Stata software (version 17).

3. Results

Description of studies

The initial search found 183 articles. Based on the article titles, 31 articles were removed in the first phase because they contained duplicate records. In the second step, the abstracts of 137 publications were screened to eliminate studies that did not meet the inclusion criteria (n=103). In the third step, the full texts of 34 articles were analyzed to eliminate 29 articles that needed more data or did not meet the inclusion and exclusion criteria. In summary, five articles were used for this study (Fig. 1).

Study characteristics

In the present study, three studies reported the number of patients. Table 2 provides a summary of study characteristics.

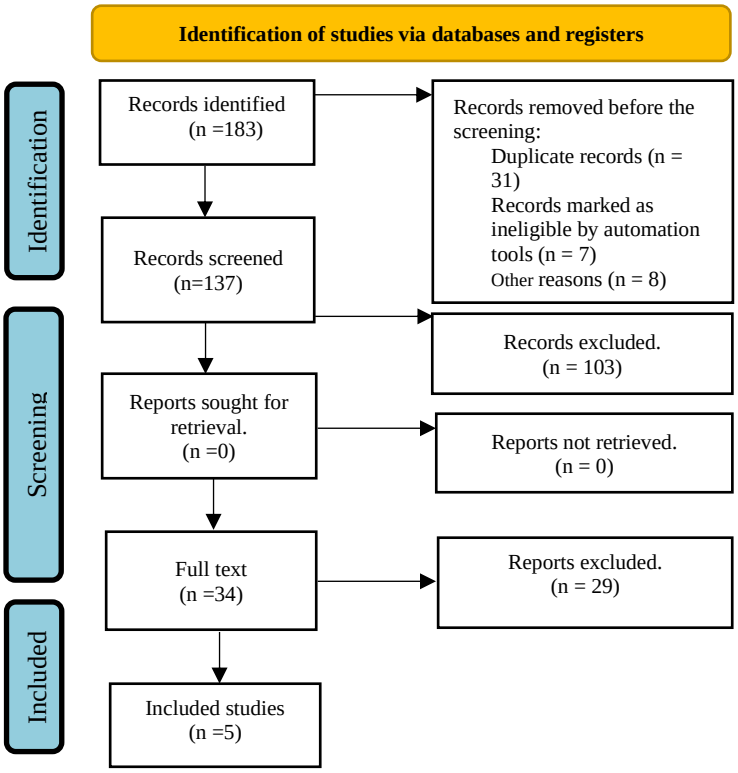

Fig. 1. PRISMA 2020 Checklist.

Table 2. Summary characteristics of studies.

Study. Years	Study design	Number of patients	Mean age	method
Jiang et al., 2024[16]	In-vitro	75	63.1	Western blot assays and immunohistochemistry (IHC) examined the expression of HOXB8 in NSCLC tissues. HOXB8 was also knocked down in NSCLC cells to evaluate its biological role in this context. The invasive and migratory potential of cells was assessed using Transwell inserts with 8 μm pores (BD, Franklin Lakes, NJ, USA). Furthermore, the possible influence of HOXB8 on epithelial-mesenchymal transition (EMT) was examined using Western blotting.
Yan et al.., 2022[17]	In-vitro	574	NR	This study used the Cancer Genome Atlas (TCGA) database to thoroughly investigate the transcription levels and prognostic values of HOXB genes in Lung adenocarcinoma (LUAD). To identify apoptosis, three different methods were used for proliferation and migration: flow cytometry, CCK-8 and Transwell assays. When analyzing LUAD tumor tissues, we found dysregulation of eight genes belonging to the HOXB cluster (HOXB2, HOXB3, HOXB4, HOXB6, HOXB7, HOXB8, HOXB9 and HOXB13).
Yan et al., 2019[18]	In-vitro	NR	NR	The expression of microRNA-27a-3p in non-small lung cancer cell lines was determined using quantitative real-time polymerase chain reaction, using a standard cell line as a benchmark. The influence of microRNA-27a-3p on cell growth and death was assessed using the Cell Counting Kit-8 assay and flow cytometry analysis. Luciferase activity reporter assay and Western blot were then performed to confirm the potential targets of miR27a3p identified through preliminary screening using Target Scan.
Xianxu et al., 2014[19]	In-vitro	NR	NR	Three different methods, flow cytometry, CCK-8, and Transwell assays, were used to identify apoptosis, proliferation and migration, respectively.
Inamura et al., 2007[20]	In-vitro	38	NR	Grossly isolated samples were used and screened for the transcript levels of three upregulated genes (HOXB2, HOXB8, NEGF, and MAGED1), three down-regulated genes (SFRS11, VAPA, and ZFP36), and IQGAP1.

Effect of HOXB8 on the survival rate

According to Figure 2, the association between HOXB8 expression and patient survival was statistically significant (p<0.001). The results suggested a shorter survival time for patients with high HOXB8 expression. high HOXB8 level was a risk factor for unfavorable lung cancer patient overall survival (Fig. 2, HR = 3.32; 95% CI 2.78, 3.86; p<0.001). This means that HOXB8 was expressed at significantly higher levels in cancer tissue than in adjacent normal tissue. The heterogeneity was low (I^2 = 0%, p = 0.74).

Proliferation, migration, and invasion ability of lung cancer cells

HOXB8 can promote lung cancer cell migration and invasion ability (Fig. 3, HR = 2.78; 95% CI 2.31, 3.26; p<0.001). The heterogeneity was low (I^2 = 45%, p = 0.13).

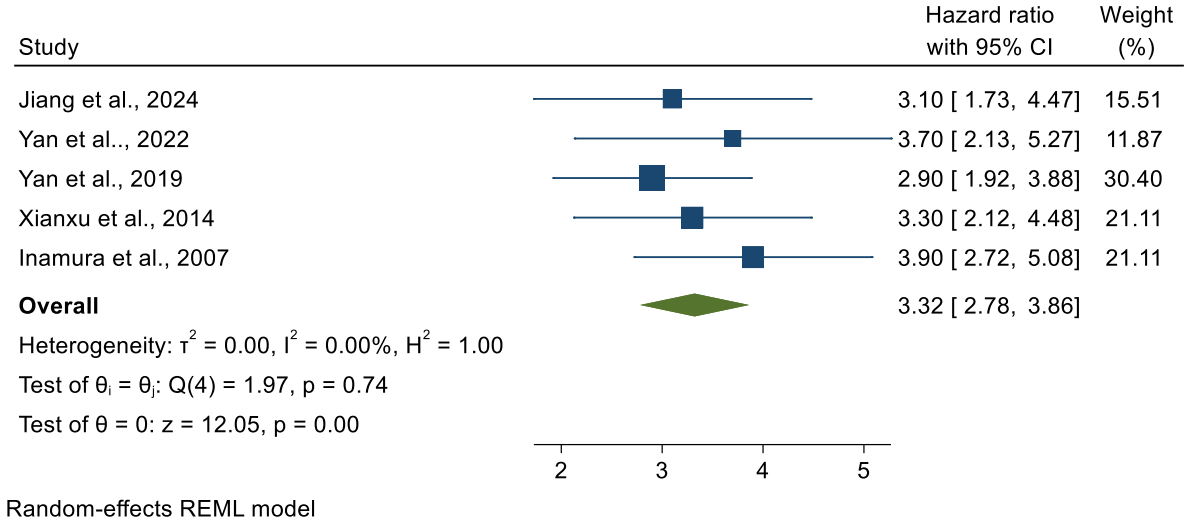

Fig. 2. The forest plot showed the Effect of HOXB8 on the survival rate.

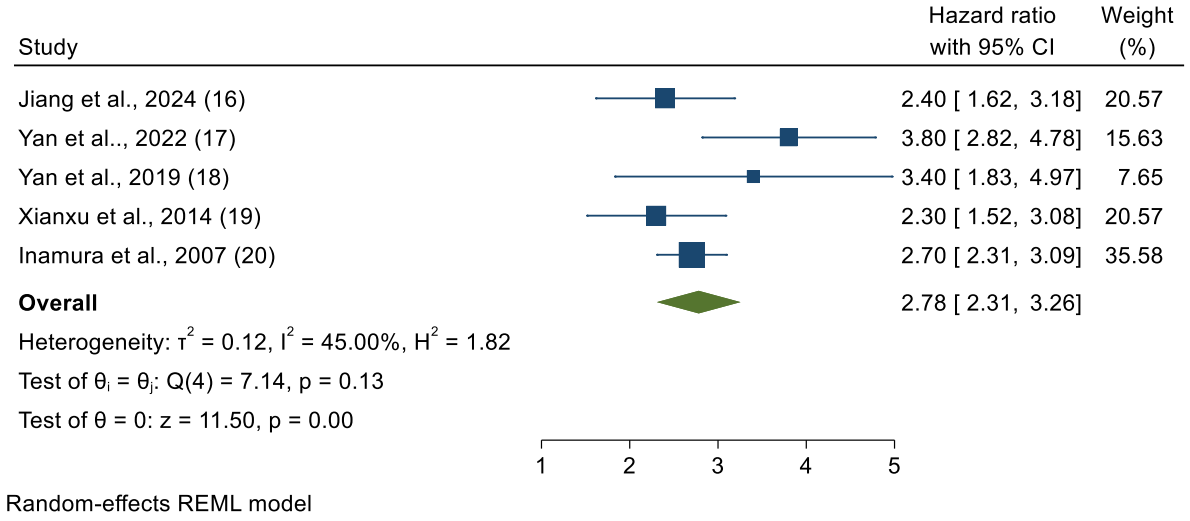

Fig. 3. Forest plot showed HOXB8 expression attenuated the proliferation, migration, and invasion ability of lung cancer cells.

4. Discussion

To our knowledge, this is the first meta-analysis study that examines the abundance of Homeobox genes and their association with the downregulation of lung cancer cell proliferation. Unfortunately, repeated searches found few articles on this topic reporting nearly similar results. In the present study, the results found that HOXB8 was significantly upregulated in lung tissue compared with adjacent normal tissues. Numerous tumors have been found to express HOXB8, including breast cancer,[21] pancreatic cancer,[22] cholangiocarcinoma,[23] gastric cancer,[24] osteosarcoma,[25] colon cancer,[26] and pancreatic cancer.[27] Higher HOXB8 expression levels were associated with worse outcomes in most of these reports. According to the current study, there was a significant increase in HOXB8 expression in lung cancer and a negative correlation was found between HOXB8 expression levels and overall survival - shorter survival time was associated with higher HOXB8 expression. Other researchers have also published results in this direction.[23, 24] Li et al., 2019 showed that the colon cancer cell line HCT116's proliferation slowed when HOXB8 expression was turned off. The apoptosis of HCT116 cells was also supported by the knockdown of HOXB8, and the invasiveness

of the cells was significantly reduced.[28] Wang et al., 2019 pointed out that the knockdown of HOXB8 slowed the growth and invasion of colon cancer cells in vitro as well as the initiation of cancer and its spread in living organisms.[29] Yan et al., 2022 emphasized that in lung adenocarcinoma, increased expression of HOXB3 is associated with tumor immunity and predicts poor prognosis.[17] The previous studies reported the expression levels of HOXB2, HOXB7, HOXB9 and other HOXB genes in lung cancer using limited samples.[20, 30] Yan et al.., 2022 (17) showed that HOXB genes had two different expression states in LUAD tumors. In other words, two members HOXB4 and HOXB6 are weakly expressed in LUAD tumors, while six members HOXB2, HOXB3, HOXB7, HOXB8, HOXB9, and HOXB13 are highly expressed in LUAD tumors. This suggests that although each member of the HOXB family plays a different role, the family may be involved in developing LUAD. Creating useful biomarkers for cancer monitoring and prognosis assessment will improve clinical outcomes and treatment.[31, 32] In the current study, HOXB8 was found to be independently associated with patient overall survival, indicating the potential utility of HOXBs in lung cancer treatment. Studies have shown that HOXB3 is an active factor in the development and progression of several types of cancer.[33, 34]

5. Conclusion

A meta-analysis revealed an association between HOXB8 levels and overall survival and an increase in HOXB8 levels in lung cancer tissues and cells. Lung cancer cell invasion, migration, and multiplication are all aided by HOXB8.

Conflict of Interest

The authors declared that there is no conflict of interest.

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