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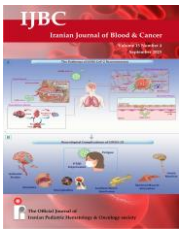

Review

Wnt7b as a novel candidate in silico analysis of angiogenesis-related expressed genes in non-small cell lung cancer patients

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Abstract

According to the latest WHO report, lung cancer ranks among the top cancer-associated mortalities. Moreover, it has been related to a high rate of metastasis, which indicates the importance of angiogenesis. Histologically, lung cancer is divided into NSCLC and SCLC, with NSCLC being the most common. Angiogenesis is essential for tumor development. Additionally, immune cells, soluble factors, and ECM play a crucial role in their formation. this study reviews the angiogenesis formation factors in previous studies as well as analyzes in silico angiogenesis-related genes in NSCLCs. It is reported that EPhB2, PIK3R2, HSPB1and Wnt7b were the most upregulated angiogenesis genes. Among them, Wnt7b is the most prevalent in NSCLC subtypes. Moreover, a decrease of 50% in overall survival in both low and high Wnt7b transcripts per million was observed. First, three high-throughput GEO data sets with 18 lung cancer and normal samples were adopted to achieve the study purpose. Then, the up-and-down-regulated genes with p-value <0.05 were isolated. Next, the genes were taken to the Enrichr and the KEGG databases. Lastly, our *in-silico* analysis confirmed the gene expression connection between angiogenesis and lung cancer invasion.

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1. Introduction

According to the World Health Organization (WHO) reports, in 2020, the cancer mortality rate took the first place with 1.80 million, and the cancer newly diagnosed cases in second place with 2.21 million rates related to lung cancer. In addition, the average survival of advanced cancer patients is up to 12 months (1-5). Some of the most significant factors known to be the main causes of lung cancer include smoking, environmental pollution, industrial air pollution (such as arsenic, radon, and asbestos in the workplace), and infection with the human papillomavirus (HPV). On the other hand, genetic changes are also key factors that lead to significant changes in cells that make people vulnerable to lung cancer. Meanwhile, this condition can be accelerated by smoking (6-13). The main traditional classification based on histology includes groups of non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC), which account for 85% and 15% of diagnoses, respectively, and are subdivided into squamous and non-squamous histology. Within NSCLC classifications, lung adenocarcinoma (LUAD) is the most common subtype of lung cancer, followed by lung squamous cell carcinoma (LUSC) (14-16). The main cause of genetic changes in LUAD is activating mutations in the epidermal growth factor receptor (EGFR) and Kirsten rat sarcoma virus (KRAS). Mutations in the EGFR gene account for approximately 15% of LUAD. EGFR gene mutation is the first molecular change associated with lung cancer in non-smokers. Recently, a high prevalence of somatic mutations in EGFR has been found in certain populations, including women, non-smokers, and Asians in patients with LUAD (6, 17-21). It has been reported to be associated with tumor angiogenesis and the expression of angiogenic factors such as transforming growth factor (TGF) and vascular endothelial growth factor (VEGF) in human cancers (22, 23). The results of a study investigating the relationship between EGFR mutations and VEGF in lung cancer cell lines and NSCLC tumor tissues, a significant increase in VEGF levels in the culture medium of lung cancer cells and NSCLC tissues with EGFR mutations was reported (24). This growth factor is the key stimulator of angiogenesis, which causes the proliferation, differentiation, and migration of endothelial cells and the initiation of tumor metastasis (25).

Angiogenesis or neovascularization, as ongoing procedure, leads to the formation of blood vessels, which plays a critical factor in many different normal processes, such as the production of a blood artery in the creation of the placenta and inner embryonic membranes during the initial growth of the fetus (26, 27). Moreover, since the vascular system, as a multi-branch network, carries oxygen and nutrients to body tissues, the value of this event and the presence of newly formed vessels can be seen in a variety of pathologic processes, such as various types of diseases (28). The word "vasculogenesis" refers to the growth of blood vessels from primary cells (angioblasts) during embryonic development. Hertig used the word "Angiogenesis" in 1935 to describe intraventricular blood vessel growth. Folkman used this word again in 1972 to describe neovascularization and solid tumors (29). Different phases of angiogenesis are characterized by specific endothelial functions, including reproduction, migration, lumen formation, differentiation, and maturation. Each phase involves a variety of growth factors, receptors, and molecules, and causes diversity in signaling pathways. This affects the pathogenesis of angiogenesis in various diseases. Furthermore, endothelial metabolism changes can initiate or inhabitant different steps in the angiogenic process (30-34).

2. Angiogenesis as a normal cell's foundation

In adults, angiogenesis is considered a complex process that includes widespread reactions between cells, soluble factors, and extracellular matrix (ECM) components. Adult arteries are developed from a blood vessel network formed by vasculogenesis in the fetus. For this reason, angioblasts duplicate during vasculogenesis to develop a primary vasculature called primary capillary plexus. Once the plexus of the capillary network has been formed, it is restored by germination and branching of the pre-existing capillaries (35). To stimulate the formation of new capillaries, endothelial cells of the blood vessels should destroy the basement membrane and attack the stroma of the adjacent tissue (36-39). To begin, quiescent endothelial cells (less than 0.01% in the cell cycle) are released from arterial inhibitors and can migrate toward angiogenic stimuli. They are organized as a tissue, so their migration must be done through duplication. They move in the vessel wall and maintain their connection by adding new proximal endothelial cells. Next, they organize three-dimensional structures during movement and create an apparent curvature due to connections with existing vessels. For this, only a small number of cells remain in blood vessel terminal buds. Then, the primary membrane of endothelial cells and extracellular matrix are destroyed and regenerated by special proteases such as a metallurgical matrix. Finally, a new matrix is synthesized by stromal cells. Therefore, the "angiogenic cascade," as it is often referred to, consists of four stages of vascular contraction, cell migration, expansion, and structural reorganization (30, 40-42).

This process is regulated by using a wide range of angiogenic inducers, including growth factors (e.g., transforming growth factor (TGF), platelet growth factor (PGF), fibroblast growth factor (FGF), VEGF), angiopoietins, tumor necrosis factor-alpha (TNF-α), family members of interleukin (IL), chemokines, angiogenic enzymes, particular vascular receptors, adhesion molecules and also different endogenous angiogenesis inhibitors. Most stimulatory molecules are protein growth factors that trigger endothelial cells to divide and migrate toward the stimuli. In addition, they differentiate into tubular structures. As can be expected, specific angiogenic molecules can begin this process, and specific inhibitors can stop it. In the same way, most of them are produced by various cells, including endothelial cells, in response to an external or endogenous stimulus. They can also be produced locally and act as autocrine or paracrine systems. Maintaining a precise balance between these groups can ensure the physiological health of various organs, as any imbalance between angiogenic stimulators and inhibitory factors may cause disorder (43-46).

It is assumed that VEGF is a crucial stimulator of angiogenesis by which proliferation, permeability, and survival of endothelial cells are regulated. Increased permeability via loosening the connections between endothelial cells in a layer and the reconstruction of cadherin/catenin complexes by VEGF leads to the extraction of plasma proteins and the formation of an extracellular matrix suitable for the migration of endothelial cells and stroma. One of the factors affecting VEGF production is hypoxia, so that the binding of the hypoxia induction factor (HIF) leads to an increase in VEGF gene transcription and mRNA stability. Under these circumstances, VEGF stimulates the entire angiogenesis process through its various effects on endothelial cells. It also stimulates the angiogenesis process at low oxygen concentrations with HIF cooperation. Another point to consider is that angiopoietin and tyrosine kinase with immunoglobulin-like and EGF-like domain (Tie) receptors, which are mostly expressed in vascular endothelial cells and particular types of macrophages, play an essential role in angiogenesis. They are necessary for endothelial cell proliferation

and its maintenance. Tie1 and Tie2 appear to be specific for the vascular endothelium, although macrophages can also express Tie2. The expression patterns of both Tie receptors demonstrate their principal role in angiogenesis (36, 41, 47-50).

3. Lung tumors progress with angiogenesis via enslaving normal tissue components in the TME

Angiogenesis is one of the prominent features of malignancies that occur at different stages of tumor development. It is regulated by the balance between pro-angiogenic and anti-angiogenic factors. In most cases, disruption of this balance causes the pathogenesis of several disorders, such as cancer (28). It is an essential process in malignant tumor development. Tumors cannot grow more than 1-2 mm in diameter without angiogenesis because the tumor metastasizes and obviously needs more vital substances (51, 52). During cancer development, the lack of enough oxygen or glucose in the tumor microenvironment (TME) can stimulate the formation of new capillaries. This phenomenon could make the TME a unique and complex site in which cancer cells anchor to survive, grow, invade, and sustain metastasis. It is generally accepted that abnormal tumor arteries are the main problem in cancer therapy. This is due to their contribution to growth, expansion, and chemotherapy resistance. Increased vascular permeability supports cancer growth, metastasis, and oxygen and nutritional requirements. Vascular endothelial cells provide oxygen to the TME by creating newly formed blood vessels. In addition, VEGF secretion increases endothelial cell proliferation and tubule formation (53, 54).

In several clinical studies on lung cancer, intratumoral microvessel density (IMVD), angiogenesis criteria of a tumor, has been linked to tumor growth and prognosis. In that case, it is measured indirectly in tumors by counting small vessels. This includes immunohistochemical staining of vascular endothelial cells using antibodies against factor VIII, CD31, or CD34. For this purpose, tumor margin sections are used because they usually have the most microvessels and the least necrosis. The indicated evidence of the association between angiogenesis and tumor cell metastasis are derived from the elevated microvessel counts that are related to the increased shedding of tumor cells into the blood circulation and metastasis pre- and during surgery (55). Studies in NSCLC patients show that LUADs have higher vessel counts than LUSCs. This may be the reason for the increased likelihood of metastasis in NSCLC histology types. Lung cancer is composed of microvascular and oxygen-rich TME, which plays an essential role in tumor progression. Hence, targeting the TME provides a fantastic opportunity to develop novel emerging therapies (56-60). Malignant lung tumor cells recruit normal cells around tumor tissue to construct a complex structure for their demands. This complex structure consists of cells, namely immune cells, mesenchymal cells and fibroblasts, endothelial cells, and soluble factors such as growth factors and cytokines. In addition, it contains the ECM surrounding or infiltrating tumor tissues. ECM is composed of intercellular material and a basement membrane. It consists of tumor cells, cytokines, growth factors, and various MMPs. These substances are secreted by tumor cells and other cells in the tumor microenvironment. Tumor cell metabolites contain acidic substances because they maintain the tumor microenvironment's acidity in tumor tissues. This, in turn, causes the epithelial-mesenchymal transition (EMT) of tumor cells and tumor metastasis (61).

4. Soldier cells follow orders in the lung TME

Immune cells play a crucial role in ensuring defense against foreign pathogens and eradicating damaged and tumor cells from the body. However, in malignant tumor tissues, the lung tumors TME leads to the mechanisms of immune system suppression and development of tolerance towards the tumor, causing the growth and metastasis of lung cancer cells to reinforce. Therefore, immune cells sustain their survival instead of eliminating the disturbing cells (62). Adoptive defense functions via T and B lymphocytes are the most important responses of the human immune system. Regulatory B cells and regulatory T cells are the main suppressor cells of the immune system. Generally, the presence of these cells in the lung TME is associated with the production of TGF-β, IL-10, IL-35, IL-37 and other cytokines to suppress lymphocyte immune responses. Similarly, myeloid-derived suppressor cells (MDSCs) produce reactive oxygen species and nitric oxide synthase and suppress cytotoxic T-cell responses (63, 64), resulting in tumor survival. Large amounts of matrix metalloproteinase (MMP) and growth factors secreted by neutrophils such as MMP9 and VEGF enhance tumor cell proliferation, invasion, and metastasis. Contrary to the protective roles of M1 macrophages, activated M2 macrophages produce IL-4, IL-10, IL-19, IL-33, TGF-β, and epithelial growth factors in lung tumor tissues that result in tumor growth and metastasis (65, 66). Mesenchymal cells and fibroblasts are also present in large numbers in tumor tissues. These cells increase tumor cells' growth, invasion, and metastasis by secreting FGF, VEGF, MMP2, and chemokine (C-X-C motif) ligand-12 (CXCL-12). In addition, adipose tissue secretes IL-6, adiponectin, and leptin to promote malignant tumor growth (67, 68).

5. The weapons of soldier cells in the lung TME

One of TME's specific features is hypoxia, which guides cancer progression and angiogenesis. The rapid growth of tumor cells requires a lot of energy. Although high energy consumption increases the capacity of oxidative phosphorylation to fulfill the demands of cell proliferation, the speed of vascular regeneration in tumor tissues often hardly matches the growth speed of tumor cells. Therefore, the tumor microenvironment is typically hypoxic (69). Healthy human lung tissue contains 5.6% O2. In comparison, it is reduced to 1.9%–2.2% in NSCLC patients, indicating a relative dependence of oxygen levels on the source tissue of origin. At low oxygen concentrations, lung tumor cells can initiate and develop aerobic metabolism and angiogenesis. This helps them survive or escape from their hypoxic environment. On the whole, the HIF transcription factor accumulates in tumor cells in response to reduced oxygen levels and regulates tumor cells' adaptation to hypoxia. It has been found that HIF-1, HIF-2, and HIF-3, members of the human HIF family, activate other transcription factors. They are critical in hypoxic signaling pathways, which result in tumor cell survival in hypoxic conditions. In particular, they can cause the expression of VEGF, FGF, and PDGF, which theses growth factors are the crucial protein polypeptide factors in promoting neovascularization. Moreover, they play a significant role in regulating normal and abnormal angiogenesis (57, 58, 70, 71).

As mentioned, endothelial cells play an essential role in tumor angiogenesis. Therefore, regulation of signal transduction is crucial to angiogenesis and tumor development. VEGF binds to (vascular endothelial growth factor receptor 1-3) VEGFR1-3 then intracellular signaling pathways are activated, which then regulate tumor angiogenesis. For endothelial cells' proliferation, VEGFR can interact with Grab/Src/Gab1/Shb/PKCγ to stimulate

RAF/MEK/MAPK and PI3K/AKT signaling pathways. In addition, it can activate PI3K/AKT by binding to cdc42, Rho, and RacGTPases, and increases their migration and invasion. Moreover, VEGFR can enhance blood vessel permeability by increasing NFAT/β-catenin/VE-cadherin and eNOS and promote EMT-induced vasculogenic mimicry by upregulating the expression of EMT-related genes (72-77). VEGFA, as one of the central intermediaries of angiogenesis, contributes to the growth of newly formed arteries from the old arteries and changes in the tumor's environment (78). It has been recognized as the primary agent of angiogenesis in lung cancer. Tumor-derived VEGFA secretes VEGFA via the signaling pathway of VEGFR-2/ PI3K/ mTOR, enhancing the pro-angiogenic signal. When receptors are activated, VEGFRs are dimerized and phosphorylated, triggering the downstream regulatory pathway. This helps endothelial cells survive, duplicate, and migrate (79, 80). VEGF also causes vasodilation and acts as a vascular permeability agent. However, tumor cells can make various angiogenic agents during development. Tumor cells' ability to secrete various angiogenesis factors is linked to recent tumor progression stages. VEGFA signaling can induce the formation of a network of growth factors that can maintain angiogenesis and, potentially, other phenomena involved in the tumor progression (81). VEGFA helps lung cancer progress by secreting several factors that exacerbate angiogenesis. Increasing the density of small arteries and increasing VEGF-A expression in circulation has been significantly associated with undesirable lung cancer survival. Therefore, the angiogenesis pathway has been considered an essential therapeutic target in lung cancer and other types of cancer (58, 82).

It has been shown that FGF can mediate neovascular formation and maturation-related signal transduction via endothelial cell proliferation and ECM degradation, as well as altering intercellular adhesion molecules expression. The secretion of FGF2 by neutrophils in the lung TME can increase angiogenesis in metastatic tumors, according to studies. In fact, FGF binding to its receptor induces a conformational change from inactive to functional. Activated fibroblast growth factor receptor (FGFR) further induces FGFR substrate two and recruits PLCγ, which consequently recruits growth factor receptor binding 2 to stimulate PKC, RAS/RAF/MEK/MAPK signaling, and PI3K/AKT signaling. Moreover, it activates the p38 MAPK and JNK kinase pathways, STAT-mediated pathways, and ribosomal protein S6 kinase 2. Furthermore, FGF2 interacts with membrane-bound integrinV3, leads to signal transduction and increasing angiogenesis (83-85).

PDGF plays an essential role in embryonic development, cell growth and differentiation, and tissue repair. Increased aberrant expression of PDGF and its receptors has been reported in several cancers and pathological conditions. PDGFA enhances tumor angiogenesis in ovarian and hepatocellular carcinoma cells by promoting MEK/ERK signaling. PDGF-BB increases not only VEGF expression and proliferation but also migration and tube formation in vascular endothelial cells. It can also facilitate the peripheral migration of pericytes to surrounding tumors to elevate tumor angiogenesis and vascular mimicry formation. PDGF-D can cause colorectal cancer tumor angiogenesis by activating Notch1/Twist1 signaling and recruiting macrophages to tumor tissues (86-90).

Cytokines play a vital role in the maturation, activation, proliferation, and regulation of immune cells but also participate in various physiological and pathological processes. IL-1 is a crucial inflammatory cytokine for tumor angiogenesis. It induces angiogenesis by activating JNK or p38

MAPK and NF-κB signaling, and an IL-1 receptor antagonist inhibits tumor angiogenesis by blocking IL-1 signaling (91). IL-33 can activate endothelial cells, increasing vascular permeability and angiogenesis via ST2/TRAF6-Akt-eNOS signaling. IL-18 promotes Src and JNK signaling pathways. However, several studies have shown that IL-33 and IL-18 can exert anti-angiogenic aspects in different tissues according to the local microenvironment (92, 93). TGF-β can enhance IL-37's ability to bind to the activated protein receptor-like kinase one receptor complex and upregulate angiogenesis-related genes. IL-37 induces endothelial proliferation and migration, capillary formation, and endothelial survival through ERK1/2 and AKT signaling (94, 95). IL-17 can increase tumor angiogenesis in non-small-cell lung cancer and glioma cells with VEGF expression via the STAT3 signaling pathway and also in lung adenocarcinoma cells with IL-6, IL-8, and VEGF by STAT1 signaling activation. In addition, IL-17 stimulates beta-fatty acids in endothelial cells. Besides, several studies have shown that IL-22 has pro-angiogenic activity. It seems that ILs function in the tumor microenvironment and can promote tumor angiogenesis (59, 96-98).

In this review, in addition to reviewing previous studies summarized in **Figure 1**, we also investigated the signal transduction mechanisms of lung tumor cells under in silico analysis. This was to fully comprehend them. Hence, we adopted three high-throughput Gene Expression Omnibus (GEO) data sets classified as up-and down-regulated genes. The differentially expressed genes related to angiogenesis impact lung cancer patients' overall survival.

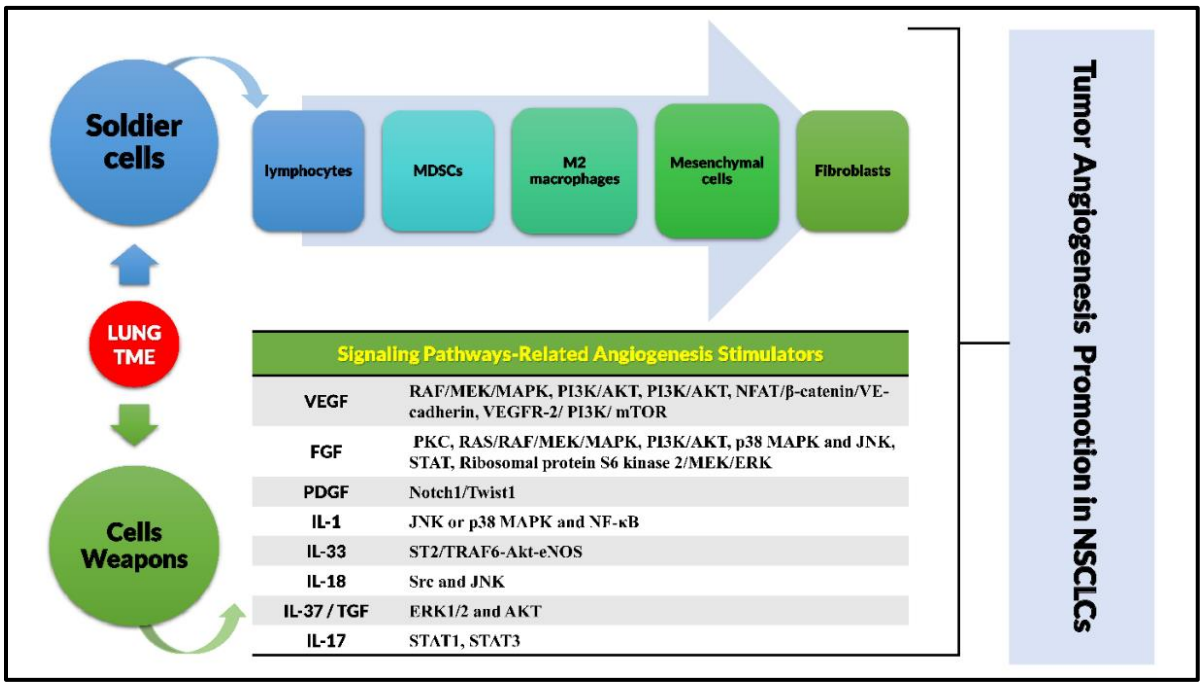

Figure 1. **Summary of the tumor microenvironment significant components in promoting angiogenesis via various signal transductions in non-small cell lung cancer.** Tumor cells can create a complex structure for themselves by recruiting normal cells around the tumor tissue to fulfill their demands. This complex structure includes the tumor's soldier cells, such as immune cells, mesenchymal cells, and fibroblasts. The weapons of them are soluble factors such as growth factors (VEGF, FGF, PDGF) and cytokines (IL-1, IL-33, IL-18, 1L-37, 1L-17, and TGF beta), which lead to endothelial cells proliferation and migration, increase angiogenesis, epithelial to mesenchymal transition of tumor cells and metastasis via various signaling pathways.

6. In silico analysis sheds light on the war strategies in the lung TME

Recently, high-throughput platforms have been developed to deal with massive genetic data. With bioinformatics methods, high-throughput platforms, such as microarrays and next-generation sequences, can be analyzed to identify cancer biomarkers and therapeutic agents. GEO database (https://www.ncbi.nlm.nih.gov/geo/) serves as a public genetic expression profile, especially suitable for a wide range of high-throughput experimental data. As a result, we used three high-throughput GEO data sets with 18 lung cancer samples with different stages and control groups for this study. In this study, we used the GSE21933 dataset from the GEO (https://www.ncbi.nlm.nih.gov/gds) database. We extracted the gene expression profile and saved it as an Excel file. Then we isolated the up and down genes with p-value <0.05. Next, we took the genes to the Enrichr (http://amp.pharm.mssm.edu/Enrichr/) database and separated their significant signal pathways from the KEGG (https://www.genome.jp/kegg/). We uploaded the genes most associated with cancer pathways to the GEPIA (http://gepia.cancer-pku.cn/) database and plotted the body map, Kaplan Mir, violin, and box plot diagrams of gene expression between cancer and normal groups. To highlight the importance of angiogenetic-related genes in tumor progression, the obtained table must be refined. Hence, **Table 1** represents the four most upregulated genes in lung cancer patients, filtered based on their regulatory role in angiogenesis. The mentioned genes play multiple regulatory roles in carcinogenesis and cancer progression via signal transduction.

Eph receptors and their ligands constitute the largest family of tyrosine kinase receptors in the human genome. They are primarily found in cell-cell interactions in the nervous system and vascular systems. Various cancers can activate these complex' bidirectional signals. Especially, B-type Eph receptor 2 (EphB2) is thought to be a target of Wnt signaling, which is regularly hyper-activated in cancer progression (99). Additionally, pharmacological activation of EphrinB2 manifests its contribution to angiogenesis which leads to blood vessel formation (100). EphB2 has an interesting dual role. This is in such a way that increased expression of EphB2 protein can lead to less survival in breast and lung cancers. It should be noted that loss of EphB2 expression is also associated with decreased survival in colorectal and gastric carcinomas (100, 101). However, EphB2 expression pattern and clinical significance in lung cancer remain unknown. However, our analysis showed 1.67 logic in lung cancer compared with healthy patients. Phosphoinositide-3-Kinase Regulatory Subunit 2 (PIK3R2) catalyzes phosphatidylinositol formation of phosphatidylinositol (3,4,5)-trisphosphate (PIP3), which activates a signaling cascade involving protein kinase B (PKB). Cell survival is induced by phosphatidylinositol-3 kinase (PI3K) activation, and it contributes to cell cycle progression promotion, cell migration, and cancer-associated metabolic switch (102).

Table 1. **The most angiogenesis-related upregulated genes in NCSLC progression**

Gene Symbol	Gene Title	P.Value	adj.P.Val	logFC
EPHB2	EPH receptor B2	9.25E-04	0.05346	1.672074
WNT7B	Wnt family member 7B	2.37E-03	0.08249	2.339257
HSPB1	heat shock protein family B (small) member 1	5.34E-03	0.11933	1.265713
PIK3R2	phosphoinositide-3-kinase regulatory subunit 2	7.96E-03	0.14383	0.749138

It has been determined that PIK3R2 could serve as an interfering tool for lung squamous cell carcinoma therapy due to its increased expression in this carcinoma (103).

Wnt7b is a Wnt family ligand necessary for the proper formation of several organs and tissues, including the eye, the bones, the lungs, and many other tissues. Wnt7b regulates vasculature development in the eye, the brain, and the lungs. This regulation is made by different cellular mechanisms (104). Furthermore, the Wnt pathway is a highly conserved signal transduction pathway that plays a crucial role in the pathogenesis of many development-related lung diseases. Additionally, Wnt7b's protection role has been confirmed in lung injury (105). In that case, the various critical functions of Wnt7b prompted us to select the Wnt7b gene among our candidates for further investigation. **Figure 2** illustrates Wnt7b median expression in non-tumor and tumor samples. It highlights the widespread expression of Wnt7b in both normal and tumor samples. This indicates the importance of Wnt7b in various healthy and disease-related mechanisms. It is determined that the expression of Wnt7b and also TGF-β1 in disease and normal conditions in different lung cell types hardly depends on Wnt7b and TGF-β1 concentration in their microenvironments. Moreover, they may be affected by their neighboring cells' changes in expression of these and other factors with the progression of the disease (106).

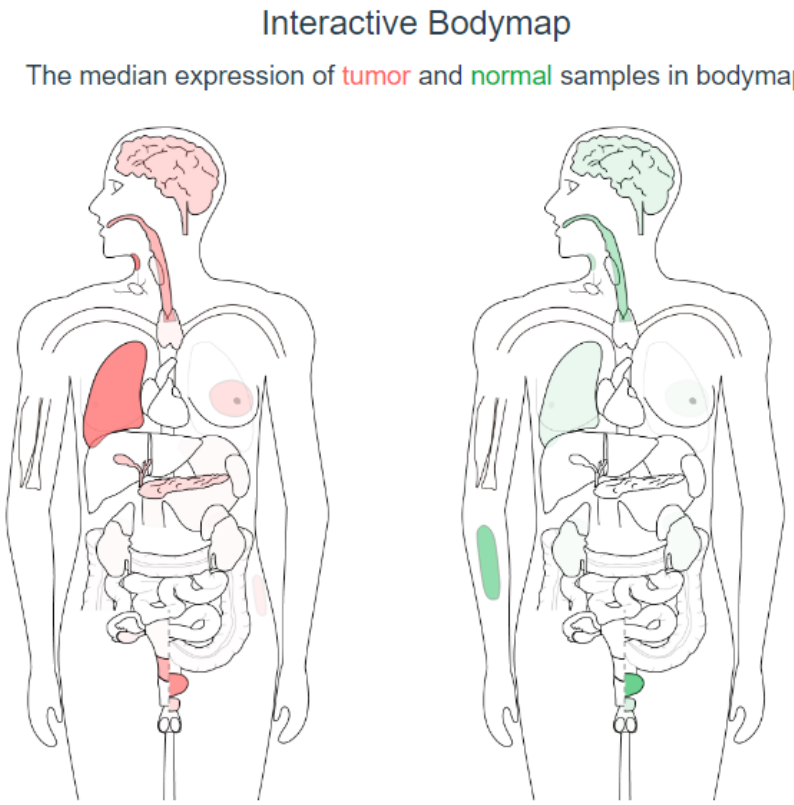

Figure 2. **The median expression of Wnt7b in normal and tumor samples.** This figure highlights Wnt7b's widespread expression in both normal and tumor samples. WNT7B expression in the Brain and Testis is considerable. [http://gepia.cancer-pku.cn/]

As we described, LUSC and LUAD are subtypes of NSCLC which are the most common lung cancer type. There is much similarity between the gene expression of these subtypes. However, pan-cancer studies have revealed that carcinogenesis molecular mechanisms could be highly heterogeneous between LUAD and LUSC (107). Here, in silico, our analysis of Wnt7b showed a higher expression of Wnt7b in both LUSC and LUAD compared with normal tissue, which indicates the importance of Wnt7b in carcinogenesis (**Figure 3**). The higher number of samples combined with the distribution of expression data simply offer a validated conclusion.

One of the main causes of lung cancer mortality is distant metastasis. Detecting molecular changes in the early stages of primary tumors, such as gene expression differences, will reveal to scientists the prognostic value of detecting tumor metastasis. This will increase survival rates (108). A multistage analysis of lung cancer has revealed that the genes associated with stage I NSCLC patients are quite different from stage II (109). There have been some specific genes identified that are valuable in diagnosing patients with stage I or II squamous cell

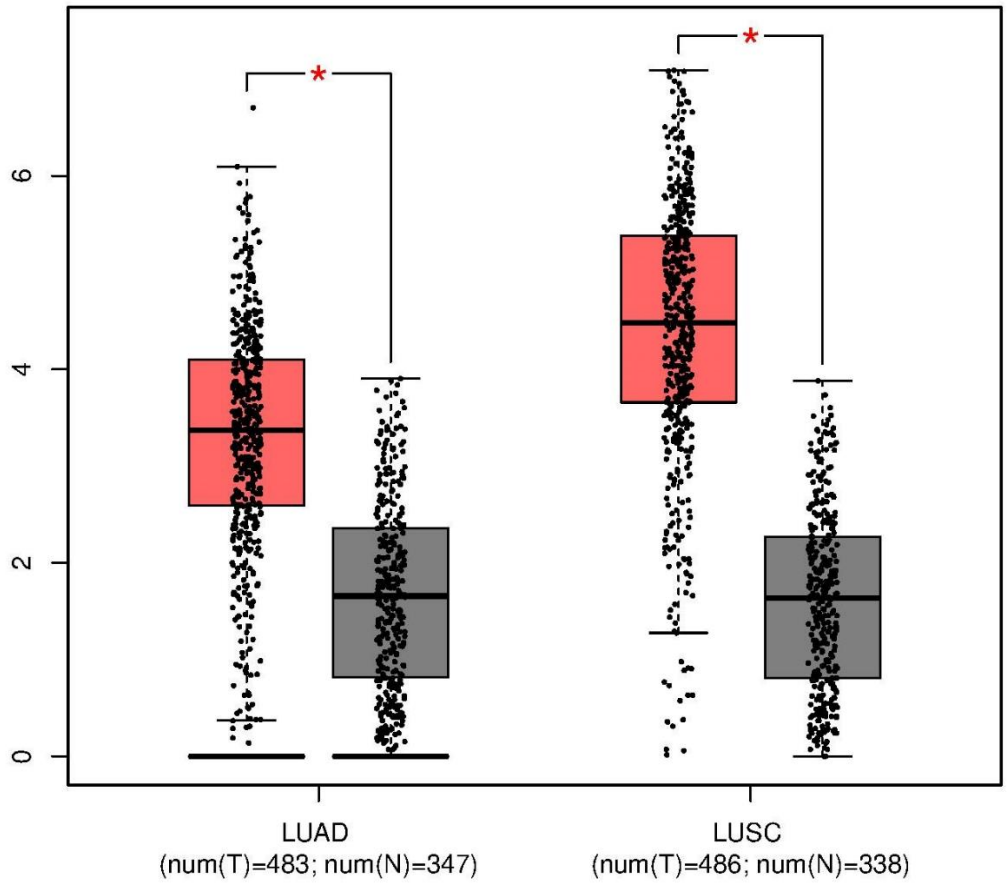

Figure 3. **In silico analysis of Wnt7b in non-small cell lung cancer subtypes.** It shows a higher expression of Wnt7b in both LUSC and LUAD compared with normal tissue. This indicates the importance of Wnt7b in the carcinogenesis process. The "red box" represents WNT7B in cancer, and the "gray box" represents a normal sample. [http://gepia.cancer-pku.cn/]

lung cancer and predicting prognosis (110). To highlight this issue, the differential expression level of Wnt7b in LUAD was analyzed by a violin plot (**Figure 4**). As can be seen in **Figure 3**, the median Wnt7b expression and interquartile range are the same in stages I, II and III. Only stage IV showed differential expression, which has no clinical and diagnostic value. **Figure 3** shows the importance of the Wnt7b gene in cancer processes and mechanisms. In **Figure 5**, a Kaplan-Meier analysis of overall survival in both high and low Wnt7b transcripts per million (TPM) in LUAD has been investigated. It simply illustrates a 50% decrease in overall survival percent within 50 months in both low and high Wnt7b TPM. Also, this figure highlights the significance of Wnt7b expression on long-term survival rates.

The administrative role of Wnt7b in various crucial mechanisms in lung tissue and its function as a Wnt pathway and angiogenesis regulator, makes this gene a potential candidate to increase the survival rate of lung cancer patients. Nevertheless, the potential role of Wnt7b in overall survival needs more consideration. Moreover, the current study identifies WNT2B and WNT7A as potential prognostic indicators for LUAD patients and crucial prognostic factors for LUSC patients. Furthermore, the research suggests that these genes might be associated with immune cell infiltration in both types of lung cancer, but further experimental validation is necessary to understand the exact mechanisms. The findings offer valuable insights for future research on the biomarker and prognostic significance of the WNT gene family in NSCLC (111). In the tumor microenvironment of

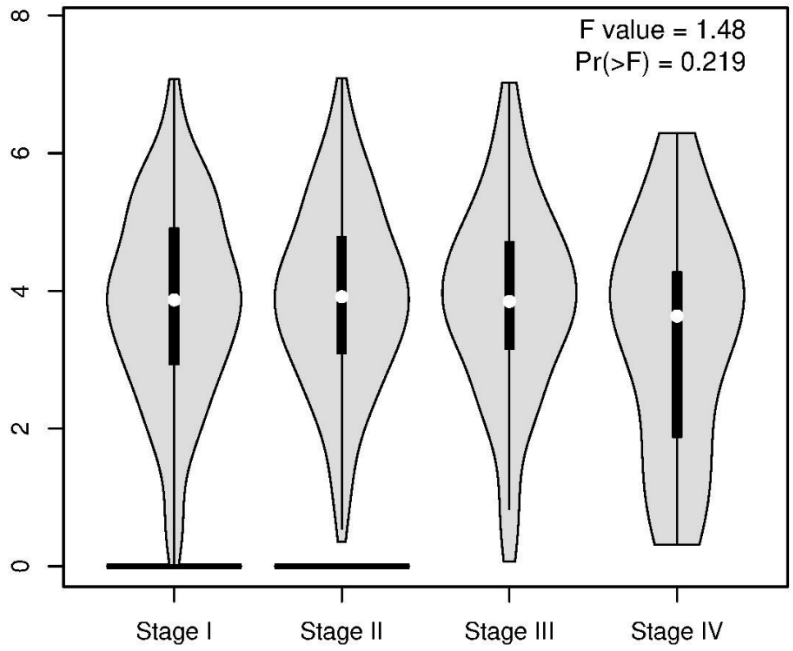

Figure 4. **The differential expression level of Wnt7b in LUAD has been visualized by a violin plot.** It illustrates that the median Wnt7b expression and its interquartile range are the same in stages I, II, and III. [http://gepia.cancer-pku.cn/]

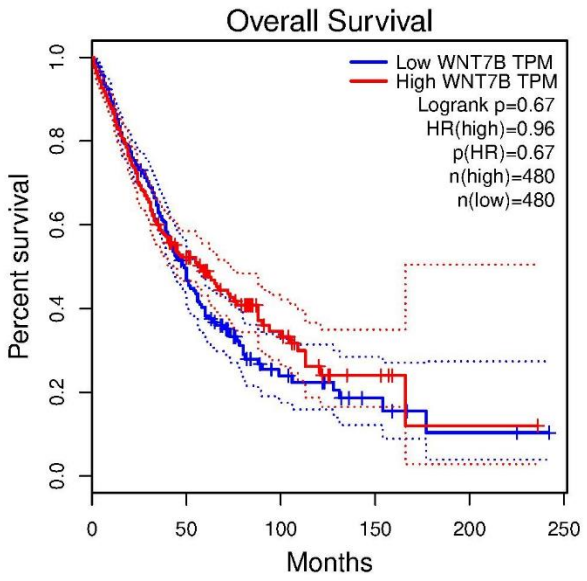

Figure 5. **Kaplan-Meier analysis in LUAD patients.** They show overall survival both with high and low Wnt7b TPM. [http://gepia.cancer-pku.cn/]

lung cancer, tumor-associated macrophages (TAMs) play a critical role in promoting angiogenesis, which is essential for tumor growth and metastasis. Consequently, targeting TAMs to block angiogenesis has the potential to be a significant breakthrough in controlling lung cancer growth and spread. another study investigated the effects of Sanguinarine (Sang) on lung cancer in mice. The researchers found that Sanguinarine effectively inhibited tumor growth and tumor angiogenesis in mice with subcutaneously transplanted tumors of Lewis lung cancer. In vitro, experiments also showed that Sanguinarine suppressed the proliferation, migration, and lumen formation of human umbilical vein endothelial cells (HUVECs), along with reducing the expression of CD31 and VEGF. These effects were achieved by regulating the polarization of M2 macrophages. Interestingly, even after the removal of macrophages using small-molecule drugs, Sanguinarine still displayed an inhibitory effect on angiogenesis in vivo. High-throughput sequencing results indicated that the WNT/β-Catenin signaling pathway might be the underlying mechanism responsible for the beneficial effects of Sanguinarine. Further experiments with the β-Catenin activator SKL2001 demonstrated that it counteracted the effects of Sanguinarine, implying that Sanguinarine regulates M2-mediated angiogenesis through the WNT/β-Catenin pathway (112).

The exploration of Wnt7b as a potential target in NSCLC clinical trials opens new avenues for personalized and effective cancer treatments. While the preclinical data is encouraging, it is essential to conduct well-designed and adequately powered clinical trials to establish the safety and efficacy of Wnt7b-targeted therapies. The results from these trials could pave the way for novel precision medicine approaches, offering hope to NSCLC patients and potentially changing the landscape of lung cancer treatment.

7. Conclusion

In conclusion, the gene expression association between the development of angiogenesis and lung cancer invasion has been proven by in silico analysis. The most upregulated genes which showed high logfc in lung cancer in comparison with the healthy patient have been identified. Among them, Wnt7b, due to its high logfc, distinct regulation, and carcinogenesis, was chosen as the candidate. Furthermore, in silico analysis highlights Wnt7b's importance in tumor cells. This regulates angiogenesis, and carcinogenesis, and is critical for normal lung tissue development. The violin plot (As shown in **Figure 4**) and overall survival graph (As shown in **Figure 5**) have demonstrated that the distinct role of Wnt7b in lung cancer needs more consideration. Our result has fully illuminated the significance of angiogenesis-related genes in lung

cancer progression. In particular, Wnt7b plays a crucial role in regulating survival rates. In silico our analysis has shed light on the potential of Wnt7b in enhancing the overall survival rate. Finally, future in vitro and in vivo studies should provide a more reliable understanding of its regulatory function.

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Ethical approval statement

This study does not have any ethical approvals.

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Competing Interests

The authors have no competing interests to declare that are relevant to the content of this article.

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