Heparanase Expression Correlates with Angiogenesis and Lymphangiogenesis in Human Lung Cancer

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Abstract

Background and objective  Heparanase has been thought to be a good molecular marker of tumor, and the heparanase expression level was correlated closely with tumor metastasis. In this study, we investigate the effects of heparanase on angiogenesis and lymphangiogenesis of lung cancer and the relationship between heparanase expression and vascular endothelial growth factor (VEGF), vascular endothelial growth factor-C (VEGF-C).

Methods  Immunohistochemistry was used to detect the expression of heparanase, VEGF, VEGF-C protein and microvascular density (MVD), lymphatic vessel density (LVD) in 115 cases of non-small cell lung cancer (NSCLC) and 45 cases of adjacent normal tissue samples.

Results  Our results showed that heparanase expression was significantly increased in 91 (79.13%) of the 115 cases and correlated with lymph node metastasis (node positive rate 87.0%; node negative rate 36.8%; P=0.003). Heparanase positive expression cases have significantly higher concentration of microvascular density (MVD) and lymphatic vessel density (LVD) as compared with heparanase negative expression cases (P<0.01, P<0.01, respectively), heparanase expression was significantly correlated with VEGF, VEGF-C expression in NSCLC.

Conclusion  Heparanase overexpression was associated with angiogenesis and lymphangiogenesis of lung cancer; targeting of heparanase may represent a significant therapeutic potential for lung cancer.

Keywords  Lung Neoplasms; Heparanase; Vascular Endothelial Growth Factor-C; Lymphatic Metastasis

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Introduction

Metastasis is considered to be a major determinant of the malignant behavior of human lung cancer. The mechanisms of tumor metastasis are not well illuminated so far, but the degradation of extracellular matrix (ECM) and basement membrane (EM) has been found to be an essential prerequisite for metastases of malignant tumors. The consequence of degradation of heparin sulfate proteoglycans (HSPG), a chief component of ECM, is considered to play a dominant role in tumor metastasis. Heparanase (Hpa) is the only endoglycosidase in mammalian cell so far that degrades the heparin sulfate (HS) chains of HSPG[1]. Elevated levels of heparanase have been indicated to have a strong association with the invasive phenotype in many tumors. In addition, HSPG is also the reservoir for a variety of bioactive molecules including angiogenic growth factors and degradation of HSPG would result in releasing of these growth factors which involved in angiogenesis[12]. In the present study, we investigated the relationship between heparanase expression and VEGF, VEGF-C expression in NSCLC as well as its role in the angiogenesis and lymphangiogenesis.

Materials and methods

Patients and tissue sample

One hundred fifteen cases who were surgically resected with lymph node dissection for lung carcinoma at China Medical University, Shenyang, between 1991 and 2001 were included in the study, 45 cases of tumor nearby normal tissues were also included as a control group. The patients included 85 males and 30 females with a mean age of 56.7 years (range, 26-76 years). None of the patients had received preoperative
chemotherapy and radiotherapy. In these cases, 48 showed lymph node metastasis and 39 cases showed distant metastasis. The pathological types were 52 adenocarcinomas and 63 squamous cell carcinomas (well differentiated in 23, moderately in 37, and poorly in 55). The pathological stages were evaluated as stage I: 33, II: 21 and III: 61 according to the guidelines of International Union Against Cancer.

**Immunohistochemistry**

Four-micron thick sections were prepared from the paraffin-embedded tissues. Immunohistochemical staining was performed by the streptavidin-peroxidase (S-P) method (Ultrasensitive\textsuperscript{TM} MaiXin, Fuzhou, China). The primary antibodies used in this study were anti-heparanase rabbit polyclonal antibody (diluted 1:100, Santa Cruz, USA), anti-VEGF-C rabbit polyclonal antibody (diluted 1:150, Santa Cruz, USA), anti-VEGF rabbit monoclonal antibody (diluted 1:100, Santa Cruz, USA), anti-D2-40 and anti-CD34 monoclonal (ready to use, Maixin Company, China). For the negative control, the primary antibodies were replaced by PBS.

For evaluation of heparanase, VEGF and VEGF-C immunostainings in cancer cells: 0, no staining; 1, 1%-10% positive; 2, 11%-25% positive; 3, 26%-50% positive; 4, >50% positive. Sections were classified into two groups: negative expression (with a score of 0 or 1) and positive expression (with a score of 2, 3 or 4). Blood and lymphatic vessel staining was used as evaluation criteria as follows: brown-yellow stained endothelial cells with band or fissure-like isolated or clustered structures or with tubular lumen were counted as a single blood or lymphatic vessel. Within each section, we selected 3 tumor areas with the highest density of distinctly highlighted microvessels and lymphatic vessels when observed under low-power fields. Next, the average number of CD34- and D2-40-labeled tubular lumens was counted under high-power fields. MVD=mean (CD34-labeled tubular lumen number-D2-40-labeled tubular lumen number); LVD=mean D2-40-labeled tubular lumen.

**Statistical analysis**

The statistical package SPSS 13.0 (SPSS incorporated, Chicago) was applied to complete data processing. The Chi-square test was used to determine the correlation between heparanase expression and clinicopathological factors. All data were expressed as Mean±SD, Values of \( P < 0.05 \) were considered statistically significant.

**Results**

**Heparanase, VEGF, VEGF-C expression in NSCLC**

Heparanase and VEGF, VEGF-C were mainly expressed in the cytoplasm of carcinoma cell (Fig 1), heparanase protein expressed positively in NSCLC cases (91/115 cases, 79.13%) compared with the adjacent normal tissue samples (2/45 cases, 4.44%), and VEGF, VEGF-C expression were positive in 88 of 115 (76.52%) and 83 of 115 (72.17%) of the NSCLC patients, respectively. 89.6% tumors with lymph node metastasis were positive for heparanase expression, and heparanase was positive expression in 71.6% tumors without lymph node metastasis.

![Fig 1](image-url)

**Fig 1** Correlation of the expression of heparanase, VEGF, and VEGF-C with MVD and LVD in NSCLC (immunohistochemical S-P method, \( \times 400 \)) Relationship of the expressions of heparanase (A, D), VEGF (B) and VEGF-C (E) in NSCLC with MVD (C) and LVD (F). When the expression levels of these molecules was positive, the corresponding CD34-labeled MVD and D2-40-labeled LVD were also higher.
metastasis ($P<0.05$). Moreover, heparanase staining intensity correlated with the distant metastasis ($P<0.05$). There was a significant positive correlation between heparanase expression and VEGF, VEGF-C expression in clinical samples of lung carcinoma (both $P<0.01$). (Tab 1, 2).

**Correlation of the expression of heparanase, VEGF, and VEGF-C with MVD and LVD**

The average MVD in 115 NSCLC cases was 43.94±11.89, and the average LVD was 24.97± 6.54. Tab 2 shows that the LVD in the tumors with lymph node metastasis was significantly higher than that without them ($P<0.05$); and the MVD is closely related to distant metastasis ($P<0.001$). Heparanase positive expression cases have strikingly higher concentration of MVD and LVD as compared with cases negative for heparanase expression (both $P<0.001$).

**Discussion**

Previous studies have demonstrated that heparanase gene expression is closely associated with tumor invasion, metastasis and angiogenesis. Overexpression of heparanase has been shown in many malignant neoplasms, such as bladder, oesophageal, gastric, breast, colon, prostate, pancreatic acute myeloid leukemia and the lung[^3][^12]. However, the effects of the expression of heparanase on human lung cancer have not been fully evaluated. Cohen E showed that heparanase is overexpressed in lung cancer and correlates inversely with patient survival[^13]. In this study, we detected the heparanase expression in 115 cases of NSCLC, our results show that heparanase expression is significantly higher in tumor cells compared with its adjacent, the overexpression of heparanase was strikingly associated with distant metastasis and lymph node metastasis of NSCLC.

Lymph node metastasis and blood metastasis are the major metastasis pathways of lung cancer. Vascular endothelial growth factor (VEGF) is a potent mediator of angiogenesis which regulates vascular permeability and endothelial cell migration[^14]. VEGF-C is a member of VEGF family which plays a key role in tumor lymphangiogenesis[^15][^16]. We also detected VEGF, VEGF-C expression levels of 115 cases lung cancer tissues, the results demonstrated that heparanase expression was positive
correlated with VEGF and VEGF-C expressions in tumor tissues. VEGF expression level was positive correlated with MVD, and VEGF-C expression level was positive correlated with LVD (both \(P<0.001\)). And MVD and LVD were positive correlated with distant metastasis and lymph node metastasis respectively. Tian\(^{17}\) reported that overexpression of heparanase and VEGF-C protein in lung cancer tissues perhaps participate in regulation of tumorigenesis, progression in lung cancer. Our results also showed that heparanase overexpression was associated with vascular endothelial growth factor (\(P=0.002\)), vascular endothelial growth factor-C (\(P=0.002\)); in addition, the MVD and LVD in heparanase positive expression cases were significantly higher than those in heparanase negative expression cases. These results suggest that high heparanase expression is associated with the degree of malignancy and metastatic potential of lung cancer cells. Therefore, we supposed that heparanase might induce microvascular and lymphatic vessel formation by up-regulating VEGF and VEGF-C in lung cancer tissues, thus promoting the distant and lymph node metastases and being implicated in the progression of non-small cell lung cancer.

Taken together, our findings suggest that heparanase protein expression was closely involved in lung carcinoma metastasis, there was a significant positive correlation between heparanase expression and VEGF, VEGF-C expressions in NSCLC. Heparanase is involved in multiple biological activities that include angiogenesis and lymphangiogenesis, but further studies are necessary to reveal the exact mechanism of heparanase in lung cancer progression.

**References**


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