Lung Cancer (2007) **55**, 53—60

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Prognostic significance of vessel architecture and vascular stability in non-small cell lung cancer

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Received 14 March 2006; received in revised form 12 July 2006; accepted 20 September 2006

KEYWORDS
Angiogenesis;
Angiopoietins;
VEGF;
Pericytes;
Vascular stability;
Coopted vessels

Summary To evaluate characteristics and prognostic impact of different structure types of intratumoural blood vessels, tissue samples of 72 patients with primary stages I and II non-small cell lung cancer (NSCLC) were analysed. Performing immunohistochemistry, 45 of 56 analysed tumours (80%) demonstrated an obvious alveolar vascular pattern with tight coverage with perivascular cells in atleast parts of the sample. After an overall median follow-up of 139 months for surviving patients, tumours with an alveolar vascular pattern showed a significantly better overall survival (OS) compared to those with an entirely angiogenic vascular pattern (108 months versus 63 months; $p < 0.05$). Furthermore, high expression of angiopoietin-1 (Ang-1) correlated with OS ($p < 0.05$). In contrast, expression of Ang-2 or vascular endothelial growth factor was not significantly associated with survival. Collectively, alveolar vessel architecture and angiopoietin expression appear to be common phenomenons in early stage NSCLC and may serve as prognostic factors.

1. Introduction

Angiogenesis, the formation of a neovascular blood supply derived from preexisting blood vessels, plays a central role in tumour growth, maintenance and metastasis [1] in several solid tumour systems including non-small cell lung cancer (NSCLC). However, the prognostic value of the vessel number remains controversial for NSCLC. While some studies

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doi:10.1016/j.lungcan.2006.09.025

have reported microvessel density to be a prognostic factor atleast in a subset of patients [2], several previous studies have failed to detect the vessel number as a prognostic factor independent from already established prognostic factors [3,4]. Besides inducing angiogenesis, some authors have proposed that, in particular circumstances, tumours could acquire blood supply in alternative ways such as vascular mimicry [5] and by exploration of the already established vasculature of the respective organ [5,6]. Particularly the lung provides a dense vasculature system which derives from the pulmonary arteries and — potentially even more relevant — from the aorta via bronchial arteries. However, besides structural differences a deeper analysis of preexisting and newly formed vessels has not been undertaken.

In general, newly formed vessels have been characterized as structurally distinct from the preexisting vasculature [7,8]. After the formation of new endothelial cell sprouts, stabilization factors such as the vascular endothelial growth factor (VEGF) or attachment by perivascular cells such as pericytes and vascular smooth muscle cells (VSMCs) are needed for endothelial cell maintenance [9]. Besides VEGF, one of the most potent endothelial cell survival factors [1,10], the angiopoietins (Ang-1 to Ang-4) have been shown to be important regulators of neovascularization and endothelial cell survival in malignant and non-malignant tissues [11]. Ang-1 has been recognized as the major activating ligand to the tyrosine kinase receptor Tie2, thereby promoting endothelial cell survival and vessel stabilization by recruiting and sustaining peri-endothelial supporting cells [5,12]. Ang-2 is a naturally occurring antagonist to Ang-1 and prevents Tie2 activation. However, the effects of the angiopoietins on angiogenesis remain controversial [13,14]. Some studies suggest that Ang-1 may be pro-angiogenic [13], whereas others have shown that Ang-1 inhibits angiogenesis, tumour growth and vascular permeability [14—16]. In this regard, it has been hypothesized that the presence of VEGF may determine the effect of the angiopoietins. For example, in the presence of VEGF, vessel destabilization by Ang-2 has been hypothesized to induce an angiogenic response; however, in the absence of VEGF, Ang-2 leads to vessel regression [17].

To evaluate characteristics and prognostic impact of different vessel structure types as possible indication of preexisting versus newly formed (i.e. angiogenic) blood vessels, we investigated the vascular pattern and microvessel density on 72 NSCLC patients with stages I and II disease. Besides a structural analysis of tumour vessels, we questioned whether the different vessel types may be further characterized by differential vascular stability as exerted by pericyte coverage and distinct expression patterns of known stabilization factors such as VEGF and angiopoietins.

2. Patients and methods

2.1. Patients

Vessel density and expression levels of Ang-1, Ang-2 and VEGF proteins were determined by immunohistochemistry in 72 patients with primary NSCLC surgically treated with curative intention from February 1993 to June 1994. After written informed consent, patients underwent thoracotomy with the objective of achieving complete resection of the tumour (resection margin microscopically free of tumour cells) and extensive mediastinal lymph node dissection. Diagnosis of malignant disease was confirmed pathologically and classified according to World Health Organization criteria. The postsurgical stage of each tumour was determined according to the revised International System for Staging of Lung Cancer [18]. Inclusion criteria for this study were surgical stages I and II, complete resection of the tumour (resection margin microscopically free of tumour cells) and perioperative survival (within 30 days after surgery). Patient characteristics are shown in Table 1. The median age was 62 years (range 34—79 years).

Table 1 Patient characteristics (SQC, squamous cell carcinoma; AC, adenocarcinoma; LCC, large cell undiffereniated carcinoma)

Characteristics	No. of patients (%)
Gender	
Male	60 (83.3%)
Female	12 (16.7%)
Histology	
SQC	30 (41.6%)
AC	20 (27.8%)
LCC	22 (30.6%)
Stage	
I	57 (79.2%)
II	15 (20.8%)
Surgical procedure	
Lobectomy	55 (76.4%)
Bilobectomy	4 (5.6%)
Pneumonectomy	13 (18.1%)
Local relapse	
Yes	17 (23.6%)
No	55 (76.4%)
Distant relapse	
Yes	16 (22.2%)
No	56 (77.8%)
Vascular pattern	
''Alveolar'' entirely	27 (37.5%)
''Angiogenic'' entirely	11 (15.3%)
Mixed	18 (25.0%)
Absent	16 (22.2%)
VEGF expression	
High	31 (43.0%)
Low	37 (51.4%)
Absent	4 (5.6%)
Ang-1 expression	
High	44 (61.1%)
Low	26 (36.1%)
Absent	2 (2.8%)
Ang-2 expression	
High	23 (32.0%)
Low	43 (59.7%)
Absent	6 (8.3%)