Small cell lung carcinoma (SCLC): the angiogenic phenomenon

Marco Lucchi a,*, Alfredo Mussi a, Gabriella Fontanini b, Pinuccia Faviana b, Alessandro Ribechini c, Carlo Alberto Angeletti d

a Cardiac and Thoracic Department, Division of Thoracic Surgery, University of Pisa, Via Paradisa 2, Pisa 56124, Italy
b Oncology Department, Division of Pathology, University of Pisa, Pisa, Italy
c Oncology Department, Division of Pathology, University of Pisa, Pisa, Italy

d Cardiac and Thoracic Department, Division of Thoracic Surgery, University of Pisa, Via Paradisa 2, Pisa 56124, Italy

Abstract

Objectives: Tumor angiogenesis, expressed by the microvessel count (MVC), and its mediators (i.e. vascular endothelial growth factor) significantly correlate with metastases in surgically treated non-small cell lung carcinoma/cancer (NSCLC). SCLC is rarely treated by surgery, as a consequence, few specimens are available to perform a biological characterization. We reviewed our experience in the surgical treatment of SCLC with particular reference to the angiogenetic expression and its correlation to the stage of disease and prognosis.

Methods: We retrospectively investigated a homogenous cohort of 87 patients with SCLC, who were primarily operated on and then underwent adjuvant chemotherapy between 1980 and 1998. Their median age was 62 years (range 34–73). All the patients were completely staged. The surgical procedures included: 32 pneumonectomies and 55 lobectomies. There were 46 N0, 17 N1 and 24 N2-disease. The adjuvant chemotherapy consisted of four to six courses of cyclophosphamide, epirubicin and etoposide. The MVC was determined highlighting the microvessels with anti-CD34 monoclonal antibodies. Immunostaining for VEGF was performed using the ABC method with anti-VEGF monoclonal antibodies. The p53 protein expression was assessed by NCL-DO7 anti-p53 monoclonal antibody.

Results: With a median follow-up of 109.6 months (range 25–238), 37 patients are alive and well, two are alive with systemic metastases. Forty-four patients died of local (n = 5) or systemic (n = 39) relapse, while four patients died from other causes. The median MVC was 59 (range 18–145). Among the clinico-pathological parameters, metastatic nodal-involvement (P = 0.002) and advanced stage of disease (P = 0.005) were associated with a worse overall survival (OS). MVC and VEGF protein expression significantly affected the survival (P < 0.001 and P = 0.0008, respectively). No statistical association was found between p53 alterations and OS as well as no association was found among p53 alterations, MVC and VEGF expression. On multivariate analysis only the VEGF expression (P = 0.003) was an independent prognostic factor.

Conclusions: Angiogenesis plays a role in the metastatic process of the SCLC as well as NSCLC. SCLC has a higher vascularization than NSCLC as results from the higher number of microvessels; however, tumor angiogenesis tested by the MVC and the VEGF protein expression correlates with the prognosis also in SCLC. SCLC may be an ideal field to test new antiangiogenic drugs associated to chemotherapy. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Small cell lung cancer; Surgery; Prognosis; Neoangiogenesis; Vascular endothelial growth factor

1. Introduction

Surgery can be applied as part of a combined treatment including chemotherapy and radiotherapy in patients with limited small cell lung carcinoma/cancer (SCLC), selected on the basis of the TNM staging [1–8]. Recent studies on non-SCLC (NSCLC) have shown that the number of microvessels in the tumor area, as an index of neoangiogenesis, and vascular endothelial growth factor (VEGF) expression correlate to an increased risk of metastatic disease and a worse survival [9–11]. Moreover, some interesting data indicate a putative genetic control of neoangiogenesis by genes involved in the proliferative/apoptotic balance (i.e. p53 and bcl-2) [12–14].

The fact that SCLC is rarely treated by surgery has produced scanty data for its biological characterization. We investigated the angiogenic phenomenon in SCLC, analyzing the microvessel count (MVC), the VEGF expression and p53 pattern in a cohort of resected SCLC focusing on the relation with the stage of disease and with the prognosis.

2. Patients and methods

Eighty-seven SCLC patients out of 135 who had under-
gone curative surgical resection at the Department of Thoracic Surgery, University of Pisa, between 1980 and 1998, were analyzed. They were homogenously treated by surgery and adjuvant chemotherapy while patients who underwent surgery alone or neoadjuvant chemotherapy were excluded from the study. There were 79 males and eight females (mean age 61.7 years, median 62 years, range 34–75 years). All the patients underwent a complete preoperative staging. This included: a detailed history and a physical examination, the evaluation of the Performance Status according to Karnofsky, a complete blood count and biochemical profile, cardiac and pulmonary function tests, chest X-ray, bronchoscopy, computed tomography of chest, the upper abdomen and brain, abdominal ultrasonography, bone scan, Gallium-67 scan, bilateral bone marrow biopsy and aspiration. Preoperative mediastinoscopy was not routinely performed. The surgical procedures included: 32 pneumonectomies and 55 lobectomies. Tumors were classified as adenocarcinomas (n = 161) or squamous cell carcinomas (n = 19). According to Tumor—Node—Metastasis (TNM) classification and within the guidelines of the American Joint Committee for Cancer Staging [16], according to Tumor—Node—Status, there were 17 T1, 58 T2, 12 T3 and 46 N0, 17 N1, 24 N2-disease, respectively. The adjuvant chemotherapy consisted of four to six courses of cyclophosphamide, epirubicin and etoposide. Post-operative radiotherapy (45–60 Gy) was administered to the mediastinum after chemotherapy to patients with nodal involvement.

2.1. Immunohistochemistry

2.1.1. Microvessel detection and counting

Intratumor microvessels were highlighted with anti-CD34 monoclonal antibodies (Mab) (QB-END 10, Novocastra Laboratories, Newcastle, UK) diluted 1:100 and detected using the ABC method. In all cases, MVC was determined independently by two pathologists. Each pathologist evaluated the slides without any knowledge of the counts made by the other pathologist and of the clinical outcome of the patients. When conflicting data were obtained, we used mean values. Each sample was examined by each pathologist, who identified the three most intense regions of neovascularization under low microscopic power (×10 objective lens and ×10 ocular lens). A ×250 field (×25 objective lens and ×10 ocular lens; 0.74 mm² per field) in each of these three areas was then counted, and the average count of the three fields was recorded. MVCs were categorized as a dichotomous variable (low vs. high MVC). In the dichotomous categorization a count of 59 microvessels (the median value obtained in this series) was used as the cut-off point to distinguish a low MVC from a high MVC.

2.1.2. VEGF expression

Immunostaining for VEGF was performed using the ABC method with anti-VEGF Mab (SantaCruz Biotechnology, Inc., Santa Cruz, CA, USA; dilution 1:50). After the primary antibodies, biotinylated anti-rabbit IgG (Vector Laboratories, Burlingame, CA, USA) were applied and followed by detection using the ABC method (Vector Laboratories, Burlingame, CA, USA). Light counterstaining was performed with hematoxylin. Normal rabbit IgG substituted the primary antibody as negative controls. VEGF expression was evaluated as percentage of positive cells in a total of at least 1000 tumor cells. Tumor sections with no VEGF immunoreactive cells were considered as negative. The median values of the series (50% of positive) was used as cut-off values to distinguish low from high VEGF expressing tumors.

2.1.3. p53 expression

NCL-DO7 anti-p53 Mab (Novocastra Laboratories, New Castle, UK) was used to detect the p53-altered protein (1:250 of dilution). The avidin–biotin peroxidase method was used by developing immunoreaction with diaminobenzidine. Simultaneous staining of a known p53 positive case was employed as positive control for p53. Incubation of parallel slides omitting the first antibody was performed as negative control. The count of p53 immunoreactive cells was made by scoring a minimum of five high-power fields (HPFs) (40× objective lens) and counting the number of immunoreactive cells out of the total of epithelial cells analyzed in each field. The 5% of positive cells was used as a cut-off value to distinguish negative from positive tumors.

2.2. Statistical analysis

All statistical analyses were carried out using the Statistica software. Univariate analysis was performed by modeling Kaplan–Meier survival curves [17]. The log–rank test was used to evaluate differences in survival distributions among prognostic groups. Multivariate analysis was carried out by use of the Cox proportional-hazard model. The Cox model was first used to select from among variables that significantly affected survival in univariate analysis, and then from among those variables whose prognostic role was independent.

3. Results

With a median follow-up of 109.6 months (range 25–238), 37 patients are alive and well, two are alive with metastases. Forty-four patients died of local (n = 5) or systemic (n = 39) relapse, while four patients died from other causes. The median MVC was 59 (range 18–145).

3.1. Clinical–pathological parameters and overall survival

Among the clinical–pathological parameters, metastatic nodal-involvement (P = 0.002), and advanced stage (P = 0.005) were significantly associated with a worse overall survival (OS). Figs. 1 and 2 show Kaplan–Meier survival
Fig. 1. Survival curves according to the nodal status.

Fig. 2. Survival curves according to the stage of disease.
plots generated on the basis of nodal status and stage, respectively.

3.2. Vascular count and outcome in SCLC

MVC, analyzed as a dichotomous variable (using the median value of 59 microvessels as the cut-off point), was a highly significant predictor of OS (Table 1; $P < 0.001$). Fig. 3 shows Kaplan–Meier survival plots generated on the basis of low and high vascular count.

3.3. VEGF and p53 expression and outcome in SCLC

VEGF protein expression, evaluated as low and high on the basis of median value of the series, significantly affected OS ($P = 0.0008$). Fig. 4 shows Kaplan–Meier survival plots generated on the basis of low and high VEGF expression. No statistical association was found between p53 alterations and OS. Furthermore, no association was found between p53 alterations, vascular count and VEGF expression.

3.4. Vascular count, VEGF expression and clinicopathological parameters

When we analyzed the relationship between clinicopathological parameters, vascular count and VEGF expression we were unable to find any statistical association among these variables.

3.5. Multivariate analysis

A multivariate analysis was performed to evaluate the independent prognostic role of MVC after adjusting for other significant covariates. All variables that significantly affected survival in univariate analysis were introduced into a Cox proportional-hazard model (Table 2). At the end of the stepwise process, only the VEGF expression ($P = 0.003$) maintained its independent prognostic influence on OS.

4. Discussion

Several studies stress the importance of tumor angiogenesis in tumor development and progression [18]. MVC (as a measure of tumor angiogenesis) is a significant predictor of increased risk of metastatic disease and worse OS in

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Table 1

<table>
<thead>
<tr>
<th>Features</th>
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<th>Overall survival $P^a$</th>
</tr>
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<tbody>
<tr>
<td>Sex</td>
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</tr>
<tr>
<td>Male vs. female</td>
<td>79/8</td>
<td>NS</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>62 vs. &gt;62</td>
<td>46/41</td>
<td>NS</td>
</tr>
<tr>
<td>Tumor status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
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<td>NS</td>
</tr>
<tr>
<td>T2</td>
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<td></td>
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<td>T3</td>
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<td></td>
</tr>
<tr>
<td>Node status</td>
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<td></td>
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<tr>
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<td>0.002</td>
</tr>
<tr>
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<td></td>
</tr>
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<td></td>
</tr>
<tr>
<td>Stage</td>
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<tr>
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</tr>
<tr>
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</tr>
<tr>
<td>S3</td>
<td>29</td>
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</tr>
<tr>
<td>MVC</td>
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<td></td>
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<tr>
<td>Low vs. high</td>
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<td>&lt; 0.001</td>
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<tr>
<td>P53 expression</td>
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<td></td>
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<tr>
<td>Positive vs. negative</td>
<td>51/36</td>
<td>NS</td>
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<td>VEGF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low vs. high</td>
<td>38/49</td>
<td>0.0008</td>
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</table>

$^a$ Log-rank test.

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Fig. 3. Survival curves according to the MVC (low vs. high).
Neoangiogenesis is under the control of several growth factors and cytokines [19], and the imbalance between stimulating and inhibiting factors is responsible for activating the angiogenic potential of the tumors. VEGF plays a main role in the control of angiogenesis both in physiological and pathological situations [20], including tumor development and progression. It is mitogenic and angiogenic for endothelial cells, and it can also increase vascular permeability.

An elevated VEGF expression has also been correlated to a worse prognosis in cancers such as gastric [21], colonic [22] and lung carcinomas [10,11,23].

SCLC represent a subgroup of neuroendocrine lung tumors with a particular clinical behavior and an ominous prognosis despite its initial sensitivity to chemotherapy. An exhaustive biological characterization of SCLC is predictable but not available because it is rarely treated by surgery and only few specimens have been disposable for the pathologists. Previously, we published our surgical experience and our policy in the clinical selection of patients for a multimodality treatment including surgery in SCLC limited disease [1,2,5].

As already reported in previous studies [1,2] we again observed that nodal metastasis and tumor stage were important predictors of poor prognosis. This confirms and stresses the importance of a careful clinical selection of patients with SCLC limited disease before proposing a patient to surgery. As regards vascular count, we observed that a higher number of microvessels (higher than median value of the series) in the most intense area of neovascularization significantly affected OS. The median value of microvessels in SCLC is higher than in same stage NSCLC. SCLC differs from NSCLC both from the pathologic and from the angiogenic point of view.

Interesting findings concern the expression of VEGF, which represents one of the most important factors of vascular development and growth, in SCLC. Previously we demonstrated that both protein and mRNA VEGF expression were strictly associated with prognosis in the NSCLC subgroup [10,11]. In our current study, we observed that a high VEGF expression was significantly associated with poor prognosis in SCLC and, most important, that in multivariate analysis VEGF expression retained its prognostic significance on OS. Recent data from Salven et al. [24] on SCLC showed that pre-treatment serum levels of VEGF were associated with poor response to treatment and unfavorable survival in patients treated with combination chemotherapy with or without interferon [24]. Our results provide further evidence that neoangiogenesis modulators

<table>
<thead>
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<th>Variables</th>
<th>$\beta$</th>
<th>$t$</th>
<th>$P$</th>
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<td>Node status</td>
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<tr>
<td>Stage</td>
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<td>0.433</td>
<td>NS</td>
</tr>
<tr>
<td>MVC</td>
<td>0.465</td>
<td>1.197</td>
<td>NS</td>
</tr>
<tr>
<td>VEGF expression</td>
<td>1.311</td>
<td>3.095</td>
<td>0.003</td>
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Fig. 4. Survival curves according to the VEGF expression (low vs. high).
may play some role in the metastatic cascade and their dosage in the serum or in the surgical specimen may add prognostic informations.

As regards to p53 protein alterations, we did not observe any association between these alterations and vascular count and/or VEGF expression, although a high percentage of p53 mutations have been reported to be very frequent also in SCLC [25]. Probably in this type of cancer, the genetic regulation of neoangiogenesis is different from what we observed in the NSCLC subgroup, suggesting the possibility of more complex mechanisms and multiple interactions of several mediators.

Overall, our data suggest the necessity of further analyzing the complex phenomenon of neoangiogenesis also in SCLC, in order to determine if in this model the vascular network and/or its regulators may modify in some way the behavior of this, otherwise aggressive, cancer, and whether, in SCLC, neoangiogenesis may represent an ideal field in which to test new antiangiogenic drugs in association with chemotherapy.

References


Appendix A. Conference discussion

Dr G. Friedel: (Gerlingen, Germany): We have some problems with the MVC, because the counts differ significantly between different persons who count the microvessels. So I would ask you how many counters you have and did you match the number of counts?

Dr Lucchi: I know that in the literature there is a discrepancy among the pathologists, but it seems now clear that if you have standardized the method to count the microvessels, there should not be such a difference. In any case, we collaborate with two pathologists who don’t know the clinical outcome and the pathological characteristics of the patients. When conflicting data are obtained, we use the mean value.