The role of bone marrow -derived circulating vascular-and lymphatic endothelial progenitor cells in lung cancer

Doctoral thesis

Dr. Krisztina Bogos

Semmelweis University, School of Clinical Medecine
Respiratory diseases program
Lung tumors sub-group

Head of the graduate school:	Prof. Dr. Pál Magyar , medical science
Adviser:	Dr. Balázs Döme Ph.D.
Official examiners:	Dr. Gabriella Gálffy Ph.D. Dr. Fruzsina Gyergyay Ph.D.
Head of the Examination Comittee: Prof.	Dr. György Böszörményi Nagy
Members of the Examination Comittee:	Dr. Irén Herjavecz Ph.D. Dr. Pál Vadász Ph.D. Dr. András Kiss Ph.D.

Budapest
2009

I.Introduction

Lung cancer is the most common malignancy in terms of both incidence and mortality in the western world. Although modest survival benefit has been observed with surgery and chemo (radio)therapy, even in surgically resectable cases, more than 50% of patients develop metastases within 5 years and an efficacy plateau has been reached. Given the still dismal survival rates, attention over recent years has focused on novel molecular targeted therapies with different mechanisms of action and better toxicity profile. The tumor induced angiogenesis is one of the more exciting researching area. Until recently it was generally accepted that vascularization and lymphangiogenesis of tumor arises exclusively from endothelial sprouting.The bone marrow derived endothelial progenitor cells (EPCs) and the newly identified bone marrow derived cell population , called lymphatic/vascular endothelial porgenitor cells (LVEPCs) have been shown to contribute to vascular and to lymph capillary growth in experimental tumor systems.The circulating endothelial progenitor cells and the matured exfoliated endothelial cells have been thought to become biomarkers of the antiangiogenesis therapy in the future.

II. The aims of the study

Non–small-cell lung cancer (NSCLC) accounts for more than 80% of all lung cancers, and is responsible for more deaths from cancer than any other tumor type in the Western world. Despite surgical resection and the development of new chemotherapy regimens, many NSCLCs relapse and become fatal. Consequently, treatment for NSCLC is now moving beyond conventional chemotherapy with the advent of molecular targeted therapies, and a key therapeutic strategy is inhibition of specific cytokines essential for tumor vascularization. Currently, angiogenesis quantification to assess and predict the efficacy of antiangiogenic drugs is mainly based on the evaluation of microvascular density (MVD). However, this procedure is highly invasive and its association with the clinical outcome is uncertain in many tumor types, including NSCLC. Accordingly, clinical markers characterizing the angiogenic profile of a tumor and able to reflect the response to antiangiogenic drugs are still scanty.

Until recently, malignant tumors were thought to acquire their vasculature solely through angiogenesis, the mechanism by which new capillaries arise from preexisting ones. However, recent evidence suggests that tumor vasculature can also arise through vasculogenesis, a process by which bone marrow-derived endothelial precursor cells (EPCs) are recruited and differentiate in situ into mature endothelial cells to form new blood vessels. Moreover, recent studies have provided evidence that in addition to the physical contribution of EPCs to newly

formed microvessels, the angiogenic cytokine release of EPCs may be a supportive mechanism to improve neovascularization. Although these data suggest that there is a close interplay between EPCs and tumor vascularization, the exact role of these cells in the pathogenesis of NSCLC remains to be determined.

The aim of the study was to identify the role of the EPC cells in non small cell lung cancer (NSCLC). Further aim was to studying the role of the circulating LVEPC cells in small cell lung cancer (SCLC) and the relationship with the diseases progression..

1.subtopic Identification and clinical significance of circulating endothelial porgenitor cells in human non-small cell lung cancer (NSCLC)

Our aim was to determine the role circulating endothelial progenitor cells (EPC) in non-small cell lung cancer (NSCLC).

Small-cell lung cancer (SCLC) is an aggressive pulmonary malignancy comprising approximately 13% of lung cancers. Despite its sensitivity to chemo- and radiotherapy, SCLC is rarely curable with these treatment strategies. Consequently, new biological targets are needed to develop more effective therapies. Among the potential targets are haem- and lymphangiogenesis, which are thought to be fundamental to the progression of different solid tumors. However, because no specific markers for lymphatic endothelium were available until recently, our knowledge of the lymphatic system of malignant tumors lags far behind that of the vascular system , and the role of lymphangiogenesis in the growth and dissemination of SCLC remains unexplored. Nevertheless, based on recent observations, lymphangiogenesis appears to be a critical mechanism for the progression in a variety of human cancers. As part of the lymphangiogenic machinery, the newly identified bone marrow derived cell population, called lymphatic/vascular endothelial progenitor cells (LVEPCs) , has been shown to contribute to de novo lymphangiogenesis in human renal transplants, and more importantly, in experimental tumor systems . It is still unclear, however, whether LVEPCs participate in SCLC-induced lymph vessel growth. Nevertheless, since an analogous cell population (VEGFR2+ haemangiogenic endothelial progenitor cells, EPCs) has been demonstrated recently to have clinical significance in the haemangiogenic process of a wide range of human malignancies , including non-small cell lung cancer, we hypothesized that LVEPCs could be involved in the progression of human SCLC.

2.subtopic Identification and clinical signification of circulating lymphatic/vascular endothelial progentitor cells in human small cell lung cancer (SCLC)

Our further aim was to studying the role of circulating lymphatic/vascular endothelial endothelial progenitor cells (LVEPCs) in non small cell lung cancer (SCLC).

III Methods

1.subtopic

To measure the number of circulating EPCs and the level of EPC specific genes at the time of diagnosis and following the appropriate anticancer therapy, peripheral blood (PB) was collected from 53 NSCLC patients. Control blood samples were also obtained from 14 healthy individuals. Flow cytometry was used to determine the numbers of CD34+VEGFR2+ double positive cells in the PB of NSCLC patients and healthy controls. CD34, CD133, VE-C and VEGFR2 mRNA levels in healthy controls and in 53 therapy-naïve NSCLC patients were determined by relative quantitative real-time RT-PCR. To characterize EPCs in the vasculature of NSCLC, we carried out immunostaining with the EPC-specific markers CD133 and VEGFR2, and the panvascular marker CD31 in serial sections of tumor tissues. EPCs in tumor samples were identified by confocal microscopy using CD31, CD34, CD133, and VEGFR2 antibodies.

2.subtopic

To measure the number of circulating LVEPC, peripheral blood samples were collected in from 88 patients with limited-disease SCLC before therapy. The control group was also included 32 individuals. There were determined the numbers of CD34-positive/VEGFR3-positive double-positive LVEPC in the peripheral blood of 32 control subjects and 88 SCLC patients by flow cytometry.The levels of VEGF-C in the peripheral blood of controls and patients with SCLC were carried out by using ELISA method.

IV.Results

1.subtopic

To characterize EPCs in the vasculature of NSCLC, we carried out immunostaining with the EPC-specific markers CD133 and VEGFR2, and the panvascular marker CD31 in serial sections of tumor tissues. Confocal microscopy revealed that CD31 antibody marks the

vasculature intensively. Because numbers of CD133+ and CD133+VEGFR2+ cells did not differ from each other significantly, EPC-positive and EPC-negative groups were established, based on CD133 labeling. Of 22 cases with surgically removed NSCLCs, 9 cases were positive for EPCs. No EPCs were observed in the normal lung tissue. In NSCLCs, EPCs were arrested mainly in small intratumoral capillaries, or, less frequently, were adhered to the endothelium of larger vessels or were located in the capillary walls. The mean number of EPCs within the tumor specimens of EPC positive patients was 2.4±1.1/mm^2 (mean±SD, n=9). However, there was no significant correlation between the presences or the number of EPCs identified in NSCLC vasculature and the circulating EPC levels as evaluated by FACS (data not shown). Although to date no clear definition of EPC exists, based on recent studies, using flow cytometry, we determined the numbers of CD34+VEGFR2+ double positive cells in the PB of NSCLC patients (Fig.1.A). Additionally, in 10 patients and 14 healthy controls we measured the number of VEGFR2+CD133+ cells, corresponding to a subfraction of immature EPCs. However, because cell counts of VEGFR2+CD133+ and CD34+VEGFR2+ EPCs did not differ from each other significantly (P>0.1 for all analyses, data not shown), in the further experiments levels of EPCs with the latter phenotype were evaluated, in accordance with previous studies. In healthy controls, the mean value of circulating EPCs was 345±54.8/mL of PB (mean±SEM, n=14, Fig.1.B). In NSCLC patients before anticancer treatment, the number of CD34+VEGFR2+ EPCs/mL of PB was significantly higher, with a mean value of 1162.4±242.4 (mean±SEM, n=53, P<0.002, Fig.1.B). Although responder and non-responder patients received the same therapy, circulating EPC numbers decreased in 74% of the responder population, while it increased in 93% of non-responders during anticancer treatment in case of all treated patients (P<0.001). Considering chemo-irradiated patients, a decrease in 89% vs. increase in 88% of patients was found in responders and non-responders, respectively (P<0.001).(Fig.1.B). Because 16 of the 53 NSCLC patients died within 30 weeks and the circulating EPC level of these patients at the time of diagnosis was significantly higher than in those who survived (2206.8±552 vs. 735.6±222.3, P<0.02), we established pre-treatment EPC cut-off values (500, 750, 1000, and 1250/mL of PB) which were tested for discriminating power in predicting disease outcome. This classification indicated that patients whose blood samples were categorized by a pre-treatment EPC level lower than 1000/mL (EPC-low) had significantly longer survival times than those with high levels of circulating EPCs (median survival time: 55.5 weeks vs. 26 weeks, P<0.001; Fig.1.C).

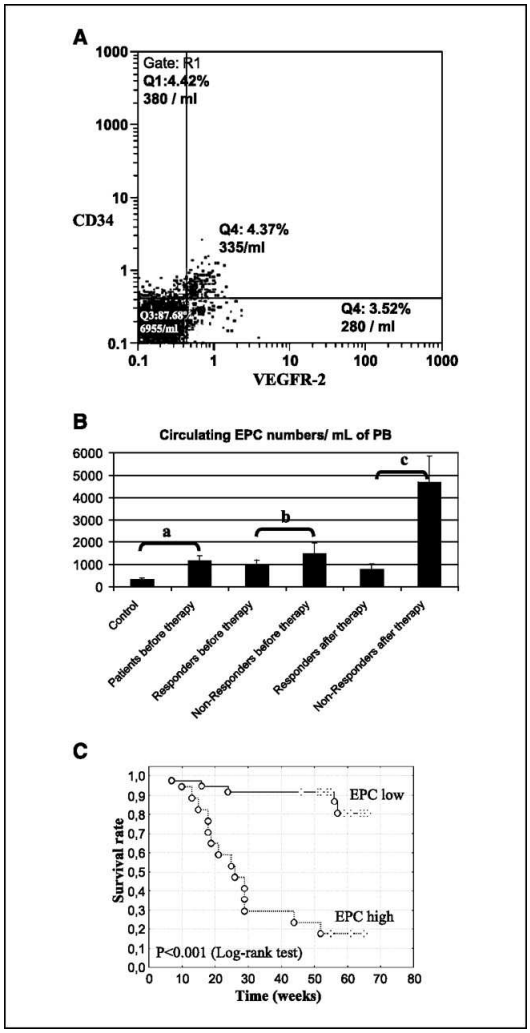

Figure 1. Quantitative evaluation of circulating EPCs by flow cytometric analysis.

(**A**) A representative flow cytometric analysis for determining the number of CD34/VEGFR2 double positive cells. Q1=CD34+, Q2=CD34+/VEGFR2+, Q3=CD34-/VEGFR2-, Q4=VEGFR2+ cells.

(B) Circulating EPC levels in healthy controls and different categories of patients. Data are mean EPC numbers/mL of PB±SEM. **a**= *P* value<0.002 (healthy controls vs. all NSCLC patients before therapy); **b**= *P* value=0.12 (responders vs. non-responders to treatment before therapy); **c**= *P* value<0.005 (responders vs. non-responders to treatment after therapy)

(C) Kaplan–Meier curves for the overall survival of the entire patient population with NSCLC, according to pre-treatment circulating EPC numbers as determined with CD34/VEGFR2 double labeling and flow cytometry. Cut-off value between low and high pre-treatment EPC levels was defined as 1000 EPCs/mL of PB.

CD34, CD133, VE-C and VEGFR2 mRNA levels in healthy controls and in 53 therapy-naïve NSCLC patients were determined by quantitative real-time RT-PCR (Fig.3). Pre-treatment levels of CD34, CD133 and VE-C (Fig.3.B-D) were not significantly altered in NSCLC

patients, whereas VEGFR2 expression was increased 80-fold (P<0.05, vs. healthy controls, Fig.2.A).Moreover, post-treatment VEGFR2 mRNA level in the PB was significantly higher in the subgroup of non-responding patients when compared with the level of patients responding to antitumor therapy (*P*<0.05, Fig.3.A). On the contrary, mRNA levels of CD34, CD133 and VE-C were not altered significantly either in pre- or in post-treatment samples of newly diagnosed or treated NSCLC patients as compared to healthy controls or to each other (Fig.2. B-D).

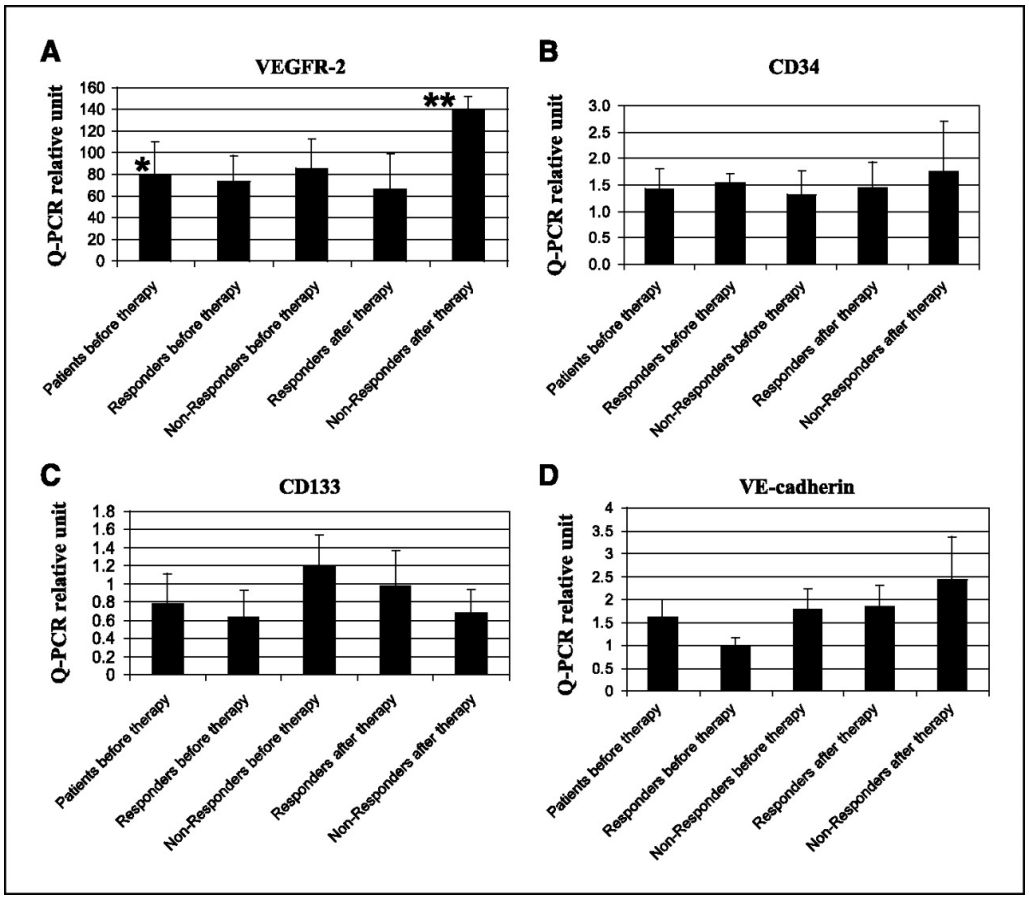

Figure 2. Relative quantification of VEGFR2 (**A**), CD34 (**B**), CD133 (**C**) and VE-C (**D**) mRNA in different categories of cancer patients. Results are relative to healthy controls and expressed as means±SEM. **P* value<0.05 (vs. healthy controls); ***P* value<0.05 (vs. responders after therapy)

No significant associations with age, gender, histologic type, smoking history, disease stage or therapy were detected (Table 1).

Table 1. Correlation of clinicopathologic features and circulating EPC number in patients with NSCLC

	No. of patients (%)	**EPC low[b] (%)**	**EPC high[b] (%)**	*P* value
All patients	53	36	17	
Age (years)[a]				
<58	26 (49.1%)	18 (50%)	8 (47%)	
≥58	27 (50.9%)	18 (50%)	9 (53%)	n.s.
Smoking history				
Non-smoker	19 (35.8%)	13 (36.1%)	6 (35.3%)	
Current or ex-smoker	34 (64.2%)	23 (63.9%)	11 (64.7%)	n.s.
Gender				
Male	28 (52.8%)	18 (50%)	10 (58.8%)	
Female	25 (47.2%)	18 (50%)	7 (41.2%)	n.s.
Histologic type				
Squamous cell	23 (43.4%)	15 (41.7%)	8 (47.1%)	
Adenocarcinoma	26 (49.1%)	18 (50%)	8 (47.1%)	
Adenosquamous	4 (7.5%)	3 (8.3%)	1 (11.8%)	n.s.
Pathologic stage				
Stage I	17 (32.1%)	10 (27.8%)	7 (41.2%)	
Stage II	9 (17%)	8 (22.2%)	1 (5.9%)	
Stage III	22 (41.5 %)	15 (41.7%)	7 (41.2%)	
Stage IV	5 (9.4%)	3 (8.3%)	2 (11.7%)	n.s.
Therapy				
Chemotherapy	18 (34%)	12 (33.3%)	6 (35.3%)	
Chemo-radiotherapy	10 (18.9%)	6 (16.7%)	4 (23.5%)	
Surgery	22 (41.5%)	15 (41.7%)	7 (41.2%)	
Palliative therapy	3 (5.6%)	3 (8.3%)	0 (0%)	n.s.

[a] Cut-off value is median value; [b] cut-off value between low and high pre-treatment EPC levels was defined as 1000 EPCs/mL of PB.
Data shown in parentheses are column percentages.

Multivariate analysis (including standard prognostic parameters such as tumor stage, smoking history, histologic type) also indicated that pre-treatment circulating EPC numbers predicted outcome independent of other variables (P<0.001; Table 2).

Table 2.

Multivariate analysis of various prognostic factors in patients with NSCLC

Prognostic factor	**RR**	**95% CI**	***P***
Gender (female vs. male)	0.719	(0.289-1.789)	0.478
Smoking history (non-smoker vs. smoker)	1.602	(0.576-4.453)	0.366
Histologic type (non-adenocarcinoma vs. adenocarcinoma.)	0.781	(0.367-1.665)	0.523
Stage (I-II. vs. III-IV.**)**	1.277	(0.811-2.013)	0.291
Circulating pre-treatment EPC number (low vs. high)[a]	8.41	(3.333-26.610)	<0.001

[a] Cut-off value between low and high pre-treatment EPC levels was defined as 1000 EPCs/mL of PB.

2.subtopic

In the study we determined the numbers of CD34+/VEGFR3+ double positive LVEPCs in the PB of 32 control subjects and 88 SCLC patients by flow cytometry (Fig. 3.A.). In the control group, the median value of CD34+/VEGFR3+ circulating LVEPCs was 455/mL (interquartile range, 370-530/mL) of PB (n=32; Fig. 3.B.). In patients with SCLC, this level was significantly higher, with a median value of 1625 (interquartile range, 600-2750/mL; n=88; $P<0.01$).

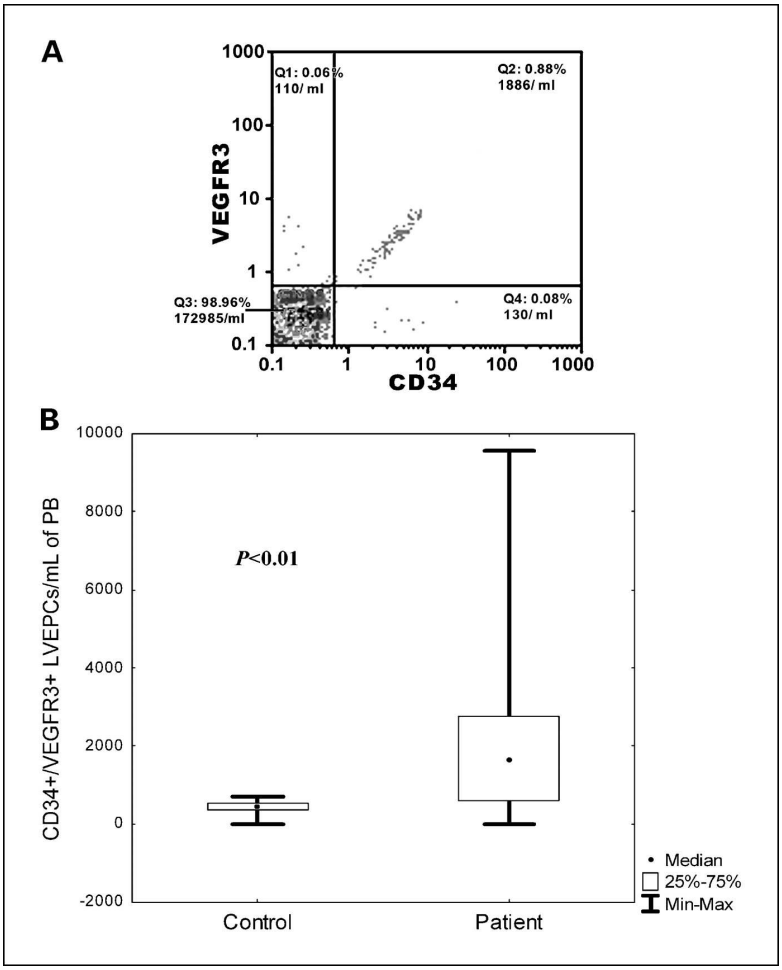

Figure 3. Quantitative evaluation of circulating LVEPCs by flow cytometric analysis (**A-B**).

(**A**) Representative flow cytometric analysis for determining the number of CD34+/VEGFR3+ LVEPCs (Q1=CD34-/VEGFR3+, Q2=CD34+/VEGFR3+, Q3=CD34-/VEGFR3-, Q4=CD34+/VEGFR3- cells). (**B**) Box plots showing median (central dots), 25%-75% quartile ranges (boxes) and minimum/maximum levels (whiskers) of circulating CD34+/VEGFR3+ LVEPC levels in control subjects (n=32) and patients with SCLC (n=88).

Because lymphatic involvement of SCLCs was associated with increased LVEPC counts, we next used Kaplan-Meier analysis to calculate the overall survival rates for patients with low and high PB LVEPC levels (Fig. 2.). We found that patients whose PB samples were categorized by low pretreatment CD34+/VEGFR3+ LVEPC levels (based on median value, <1625/mL of PB) had significantly longer survival times than those with high levels of circulating LVEPCs (median survival time was 20 versus 11.5 months; P<0.01, Fig. 4.).

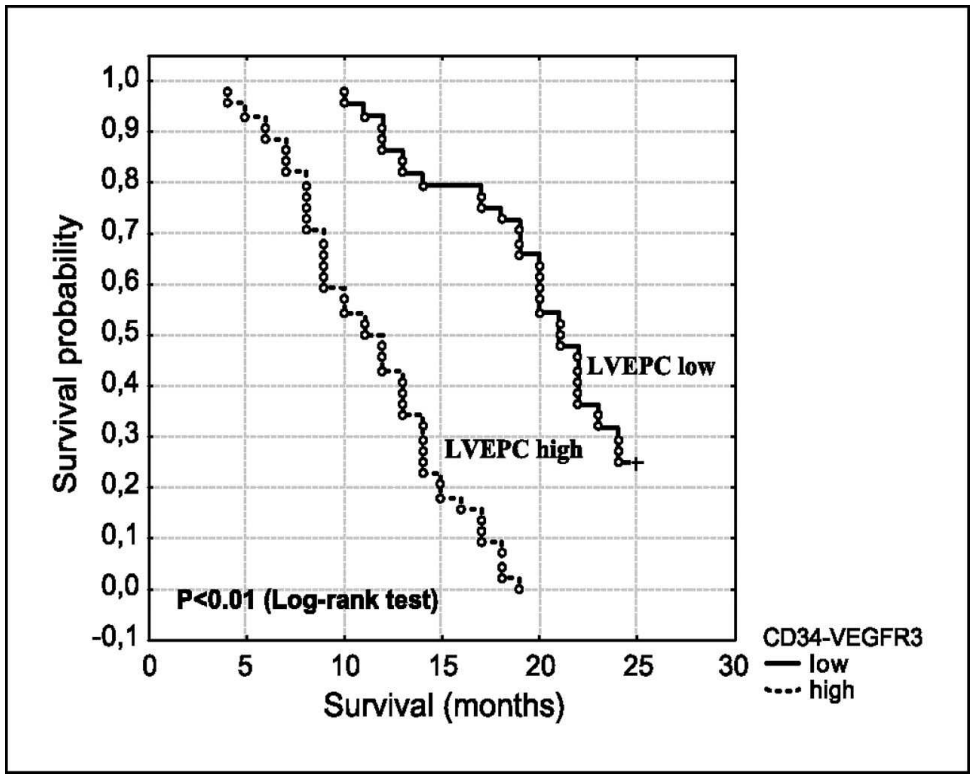

Figure 4. Kaplan–Meier curves for the overall survival of the patient population with SCLC, according to PB circulating CD34+/VEGFR3+ LVEPC numbers as determined with flow cytometry. Cut-off value between low and high pre-treatment CD34+/VEGFR3+ LVEPC levels was 1625 LVEPCs/mL of PB.

Although VEGF-C serum levels of patients were significantly elevated as compared with those of control subjects (4931±881 vs. 3992±462 pg/mL, respectively, $P<0.01$; Table 3), we were unable to detect a significant relationship between the concentrations of the key lymphangiogenic molecule, VEGF-C, and circulating CD34+/VEGFR3+ LVEPC counts ($P=0.74$, data not shown). Moreover, when VEGF-C levels were evaluated according to the clinicopathological factors of our patients, no significant associations with age, smoking history, gender, or more interestingly, with lymph node status, tumor stage or survival were detected (data not shown).

Table 3. Characteristics and VEGF-C levels of patient and control groups

	Patients (n=88)	**Controls (n=32)**	***P***
Gender (male/female)	54/34 (61.4% vs. 38.6%)	19/13 (59.4% vs. 40.6%)	0.5 [a]
Age (years)	63 (range, 44-77)	61 (range, 48-70)	0.62 [b]
Smoking status (current or ex-smoker/non-smoker)	75/13 (85.2% vs. 14.8%)	26/6 (81.2% vs. 18.8%)	0.39 [a]
Lung function (spirometry) (normal/mild or moderate COPD) [c]	74/14 (84% vs. 16%)	27/5 (84.4% vs. 15.6%)	0.61 [a]
VEGF-C (pg/mL)	4931$\pm$881[d]	3992$\pm$462[d]	<0.01[e,f]

[a] Fischer's exact test; [b] Mann-Whitney test; [c] according to the GOLD (Global Initiative for Chronic Obstructive Lung Diseases) classification of COPD severity (ref. 19); [d] mean$\pm$SD; [e] Student t-test; [f]Significant difference between patient and control groups

LVEPC numbers were also evaluated according to the clinicopathological factors of our patients. There was a statistically significant relationship between LVEPC levels and lymph node involvement ($P<0.01$, Table 2). However, no significant associations with age, smoking history, gender or tumor (T) stage were detected (Table 4).

Table 4. Correlation of clinicopathologic features and circulating LVEPC numbers in 88 SCLC patients

	No. of patients (%)	CD34+/VEGFR3+ LVEPC		*P value*
		low [a] (%)	high [a] (%)	
Age (years) [a]				
63<	43 (48.9%)	22 (50%)	21 (47.7%)	
63≥	45 (51.1%)	22 (50%)	23 (52.3%)	0.83
Smoking history				
Non-smoker	13 (14.8%)	6 (13.7%)	7 (15.9%)	
Current or ex-smoker	75 (85.2%)	38 (86.3%)	37 (84.1%)	0.77
Gender				
Male	54 (61.4%)	25 (56.8%)	29 (65.9%)	
Female	34 (38.6%)	19 (43.2%)	15 (34.1%)	0.38
N stage				
N0-1	24 (27.3%)	21 (47.3%)	3 (6.8%)	
N2-3	64 (72.7%)	23 (52.7%)	41 (93.2%)	<0.01
T stage				
T1	8 (9.1%)	6 (13.6%)	2 (4.5%)	
T2-T4	80 (90.9%)	38 (86.4%)	42 (95.5%)	0.14
VEGF-C level [b]				
High	44(50%)	22 (50%)	22 (50%)	
Low	44(50%)	22 (50%)	22 (50%)	1
CT regimens				
EP	68 (77.3%)	37 (84.1%)	31 (70.5%)	
EP+CEV	20 (22.7%)	7 (15.9%)	13 (29.5%)	0.13

[a] Cut-off value is median value; [b] cut-off value is mean value; Data shown in parentheses are column percentages; CT, chemotherapy; EP, cisplatin and etoposide; CEV, cyclophosphamide, epirubicin and vincristine

Multivariate analysis (including standard prognostic variables, such as age, gender, tumor and lymph node stage) also indicated that pretreatment circulating LVEPC levels predicted outcome independent of other variables (P<0.01, Table 3). In accordance with the latest IASLC (International Association for the Study of Lung Cancer) analysis of clinical staging

for SCLC (23), a further independent prognostic factor related to poor survival was N2-3 disease (vs. N0-1 stage, P=0.014, Table 5).

Table 5. Multivariate analysis of various prognostic factors in patients with SCLC

Prognostic factor	**RR**	**95% CI**	***P***
Age in years (<63 versus ≥63)	1.213	(0.747-1.969)	0.434
Gender (female versus male)	1.081	(0.655-1.782)	0.761
T stage (T1 versus T2-4)	2.024	(0.725-5.65)	0.178
N stage (N0-1 versus N2-3)	2.634	(1.215-5.711)	0.014
CD34+/VEGFR3+ LVEPC level (low versus high)[a]	5.379	(2.659-10.882)	<0.01
VEGF-C serum level (low versus high)[b]	1.221	(0.76-1,961)	0.408

[a] Cut-off value is median value; [b] cut-off value is mean value; RR, relative risk, CI, confidence interval

V. Discussion

1.subtopic

Although immunofluorescent labeling of microvessels made clear that incorporation of EPCs is a rare phenomenon in NSCLC tissue (9/22 cases), circulating EPC levels before therapeutic intervention were increased in NSCLC patients (P<0.002, vs. healthy controls), and high pre-treatment circulating EPC numbers correlated with poor overall survival (P<0.001). Furthermore, in the subgroup of responders to treatment, the post-treatment EPC numbers in the PB were significantly lower as compared with non-responding patients. Interestingly, pre-treatment mRNA levels of CD133, VE-cadherin, and CD34 were not significantly increased in NSCLC patients, whereas VEGFR2 expression was increased by 80-fold. Moreover, post-treatment VEGFR2 mRNA level in the PB was significantly higher in the subgroup of non-responding patients when compared with post-treatment level of patients responding to antitumor therapy.

2.subtopic

CD34+/VEGFR3+ LVEPC levels were significantly increased in patients (vs. controls; P<0.01), and there was also significant relationship between LVEPC counts and lymph node metastasis (P<0.01). High pre-treatment circulating LVEPC numbers correlated with poor overall survival (P<0.01). Although we observed significantly elevated VEGF-C concentrations in patients (vs. controls; *P*<0.01), there was no significant correlation between VEGF-C and LVEPC levels. Moreover, no significant differences in PB VEGF-C levels were seen between patients subgrouped by clinicopathological variables including tumor and lymph node stages and survival.

VI. Conclusions

Circulating levels of bone-marrow derived EPCs are significantly increased in NSCLC patients, and correlate with clinical behavior. This was the first study that demonstrates evidence of an increased number of EPCs in the PB of patients with NSCLC. In addition to the observation of significantly higher pre-treatment numbers of circulating EPCs in NSCLC patients as compared with healthy controls, this prospective study presents the novel finding that a single measurement of CD34+VEGFR2+ EPCs by flow cytometry is a useful tool to predict outcomes in patients with NSCLC. Our data suggest that those patients with lower pre-treatment EPC numbers, presumably having more “normal” tumor vessels, respond better, while those with higher EPC numbers (with tortuous intratumoral capillaries) do not respond well. Consequently, patients with high pre-treatment EPC numbers could be treated with anti-VEGF therapy to lower EPCs (normalizing the vasculature) prior to chemotherapy, thus potentially improving therapeutic responses.

Peripheral blood levels of bone-marrow derived LVEPCs are significantly increased in patients with SCLC and correlate with lymphatic involvement and prognosis. This was also the first study that demonstrates evidence of increased numbers of circulating LVEPCs in patients with a malignant tumor. LVEPCs play a role only in the lymphatic spread of the tumor, or whether they also facilitate primary tumor growth and the development of blood-borne metastases via the enhancement of blood capillarization.

Moreover, it has yet to be determined if EPC and LVEPCs can be used as a surrogate marker to monitor the efficacy of standard or future antivascular and anti(lymph)angiogenic therapies in lung cancer Further research is also needed on whether EPC and LVEPCs can be targeted to treat patients with NSCLC and SCLC, or alternatively—as they are endowed with the capacity to home to the tumorvasculature and lymphatic network— can be manipulated to deliver toxins or haem/lymph vessel-targeting agents.

VII. Acknowledgements

Herewith I would like to thank the help of Balázs Döme for the possibility to join his researching team and for the professional help. I am grateful for my head of clinical department, Dr Gábor Kovács providing me the background of the researching work. Thank for Dr Judit Moldvay and Dr József Tímár helping me becoming familiar in molecular procedures. Thank for Dr János Strausz as director of the National Koranyi Institute of Tb and Pulmonology and for Dr Pál Magyar as Head of the graduate school of Semmelweis University, School of Clinical Medecine allow me the researching work beside the clinical work. I am very appreciate of the great help of my colleagues : Dr Gyula Ostoros, Dr Erzsébet Gergely-Farnos, Dr Éva Mihály, Dr Éva Tóth, Dr Gyöngyi Gyökeres , Dr Krisztina Tóth . Special thank for the coworkers of the Departments of Tumor Biology, Thoracic Oncology and Bronchology, Department of Surgery, Department of Pathology. Dr Ibolya Soltész and colleagues. Thanks for the kind help of Dr Éva Kánitz for critical reviewing the dissertation.

There remains no more but to thank my husband and to my family for their tolerance, understanding, and support me to reach my purpose.

VIII. References

Publication serve as a basis of the dissertation:

1. **Bogos K**, Renyi-Vamos F, Dobos J, Kenessey I, Magyar M, Tovari J, Timar J, Strausz J, Ostoros G, Klepetko W, Ankersmit HJ,Lang G, Hoda MA, Nierlich P, Dome B. High VEGFR-3 positive circulating lymphatic/vascular endothelial progenitor cell level is associated with poor prognosis in human small cell lung cancer. Clin Cancer Res, 2009, 1;15:1741-6.
2. Dome B**,** Timar J, Ladanyi A, Paku S, Renyi-Vamos F, Klepetko W, Lang G, Dome P, **Bogos K**, Tovari J. Circulating endothelial cells, bone marrow-derived endothelial progenitor cells and proangiogenic haematopoietic cells in cancer: from biology to therapy. Crit Rev Oncol Hematol, 2009, 69:108-24.
3. Dome B, Timar J, Dobos J, Meszaros L, Raso E, Paku S, Kenessey I, Ostoros G, Magyar M, Ladanyi A, **Bogos K**, Tovari J. Identification and clinical significance of circulating endothelial progenitor cells in human non-small cell lung cancer. Cancer Res, 2006; 66:7341-7.

Other references :

1**. Bogos K**, Renyi-Vamos F, Kovacs G, Tovari J, Dome B. Role of retinoic receptors in lung carcinogenesis. J Exp Clin Cancer Res, 2008; 27:18.

2. Derecskei K., Moldvay J., **Bogos K**., Tímár J.: Protocol modifications influence the result of EGF receptor immunodetection by EGFR pharmDx^{TM} in paraffin-embedded cancer tissues. Pathol. Oncol. Res. 2006, 12, 243-246.

3**.Bogos K.** Pain control of the lung cancer patient. Családorvosi Fórum 2006,11:2933

4. **Bogos K**., Tóth K., Máthé A., COPD and infection. Orvostovábbképző Szemle. 2006: Suppl, 31-36.

5. Moldvay J, Jackel M, **Bogos K**, Soltész I, Agócs L, Kovács G, Schaff Z.
The role of TTF-1 in differentiating primary and metastatic lung adenocarcinomas. Pathol Oncol Res. 2004,10,85-8.

6**. Bogos K**, Ostoros G. Tüdődaganatos betegek szupportív kezelése, Magy Onkol. 2000, 44, 227-233.