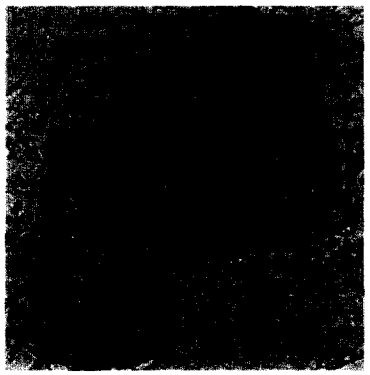
of angiogenesis. To reveal clinical significance of GMPs in non-small cell lung cancer (NSCLC), the present study was conducted.

**Materials and Methods:** A total of 236 patients with completely resected pathologic (p-) stage I-IIIA NSCLC were retrospectively reviewed. ECs were highlighted with immunohistochemical staining using an anti-CD34 antibody, and GMPs were defined as focal glomerulus-like aggregates of closely associated and multi-layerer CD34-positive ECs (Figure). Expression of vascular endothelial growth factor (VEGF) was also examined immunohistochemically, and the grade of expression was quantitatively represented from 0 to 6 (VEGF-score).



**Results:** GMPs were positive in 60 (25.4%) patients, and the presence was not correlated with age, gender, histologic type or p-stage. The mean MVDs for GMPs-negative tumor and GMPs-positive tumor were 178 and 184, respectively, showing that GMPs were not associated with MVD (p=0.690). In addition, there was no correlation between VEGF expression and the presence of GMPs; the mean VEGF-scores for GMPs-negative tumor and GMPs-positive tumor were 3.5 and 3.8, respectively (p=0.330). The 5-year survival rate of GMPs-positive patients was 54.3%, which was significantly lower than that of GMPs-negative patients (72.3%; p=0.016). The 5-year survival rate of higher-MVD patients (71.5%) seemed to be lower than that of the lower-MVD patients (63.7%), but the difference did not reach a statistical significance (p=0.137). A multivariate analysis confirmed that the presence of GMPs was a significant prognostic factor (p=0.003) whereas MVD was not.

**Conclusions:** GMPs may indicate an aggressive angiogenic phenotype associated with a poor prognosis in NSCLC.

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## HARP peptides modulate the in vitro angiogenic activity of VEGF

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**Background:** VEGF (vascular endothelial growth factor) is a growth factor with an established angiogenic activity, which promotes tumor growth and metastasis. HARP (heparin affin regulatory peptide) is a relatively new growth factor with a potential role on angiogenesis *in vitro*. We have recently found that HARP interacts directly with VEGF and modulates its angiogenic activity. In the present work, we studied if peptides derived from different regions of HARP could affect the *in vitro* VEGF-induced migration and differentiation of endothelial cells.

**Material and methods:** Endothelial cells (HUVEC) were isolated from human umbilical cords. In order to study the effect of different agents on the migration and differentiation of HUVEC, the Boyden chamber and the matrigel assay were respectively performed. HARP or HARP peptides were incubated for 30 min with VEGF prior to addition in the cell culture medium.

**Results:** Degradation of HARP with plasmin results in five peptides that have different effects on endothelial cell functions. The peptides that correspond to one of the heparin binding central regions of HARP partially abolish the VEGF-induced migration and differentiation of HUVEC, while the peptides that contain both heparin-binding domains totally inhibit VEGF actions. Similarly, recombinant peptides of HARP that correspond to one or both of the heparin-binding domains of the whole molecule partially or totally abolish the VEGF-actions on HUVEC, in a way similar to the plasmin-derived peptides.

**Conclusions:** Our results indicate that the effect of HARP on angiogenesis *in vitro* could partially be attributed to the modulation of the activity of VEGF.

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## The dependence of the in vitro HUVECs proliferation of the TGF-beta concentration in the serum of gastric cancer patients.

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**Background:** Vascular endothelium plays an important role in many physiological and pathological processes. The progression of the tumor may depend of its vascularisation. Vascular endothelium growth is regulated by many cytokines. We have investigated the correlation between TGF-beta concentration in the serum of gastric cancer patients and HUVECs proliferation activity.

**Methods:** HUVECs were obtained by the method of Jaffe (J. Clin. Invest. 1973. 52: 2745-2756.) 72 hour incubation of HUVECs with stimulating factor (serum of gastric cancer patients) was performed. [methyl-H3]-Thymidine (Amersham) for radiolabeling was used (in 12 last hours of incubation, in dose 1 1/4 Ci per well). The proliferation index (PI) was calculated by dividing number of stimulated endothelium cells, by number of nonstimulated HUVEC cells. The bioassay for TGF-beta concentration in the serum was performed.

**Results:** The TGF-beta serum concentration of the patients with the poorly differentiated tumors is higher than one of the patients with well differentiated carcinomas: 2,3±0,2 ng/ml and 0,5+0,08 ng/ml accordingly p<0,001.

Accordingly to the type of growth of the tumor the concentration was: I-II Bormann type - 0,5±0,09AB ng/ml; III Bormann type - 1,8±0,5A; IV Bormann type - 2,5±0,3B ng/ml (A-p < 0,05; B-p<0,001) .

The highest TGF-beta serum concentration was in the group of the patients with the antral tumors (2,0+0, 36 ng/ml) then in the group of patients with the tumors of the middle third of stomach (1,9±0,4 ng/ml) or upper third (1,8±0,45 ng/ml) . After the treating of the HUVEC by serum the PI was for G1-G2 tumors - 2,5±0,1 for G3-G4 - 3,0±0,1. For I-II Bormann types PI was 2,34±0,1, for III - 2,67±0,2, for IV - 3,2±0,1.

The correlation index between PI and TGF-beta concentration in serum of the patients with well differentiated tumors was r= -1.

In group of poorly differentiated tumors the correlation index was r = 0,127.

In group I-II Bormann tumor types the correlation indices were: for serum r=-1.In group III Bormann tumor types these correlation indices were: for serum r= 0,249956.

In group IV Bormann tumor types these correlation indices were: for serum r= 0,42781.

**Conclusion:** The proliferation activity of the HUVEC in vitro is inversely proportional to the TGF-beta serum concentration of gastric cancer patients.

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## Amifostine modulates endothelial cell proliferation and migration

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**Background:** Amifostine is a broad-spectrum selective cytoprotective agent for normal tissues. It is a pro-drug metabolised to the free thiol WR-1065 that may act as a scavenger of free radicals, generated in tissues exposed to chemotherapeutic agents or irradiation. WR-1065 can be further oxidized to its symmetric disulfide WR-33278 or degraded to $H_2O_2$. Both WR-1065 and WR-33278 resemble endogenous polyamines. Although amifostine is used in some cases in the clinic, there are only few studies concerning its actions at the cellular level. We have previously shown that amifostine inhibits angiogenesis *in vivo*, affecting the expression of several angiogenic genes.

**Material and Methods:** In the present work, we studied the effect of amifostine on human umbilical vein endothelial cell (HUVEC) functions *in vitro*. We used MTT and Boyden chamber assays to study HUVEC proliferation and migration, respectively. Also, we used Western blot analysis for detection of 3-nitrotyrosine.

**Results:** Amifostine increased HUVEC proliferation, an effect that was reversed by the intracellular $H_2O_2$ scavenger pyrouvic acid and agents that increase intracellular cAMP levels and inhibit the $H_2O_2$-induced signalling pathways. Moreover, valine that inhibits polyamine synthesis, reversed HUVEC proliferation induced by amifostine. This is in line with studies showing that amifostine increases the levels of spermidine in mammalian cells. On the other hand, amifostine decreased HUVEC migration, an effect that was reversed by valine or excess L-arginine. This is in line with the