Topotecan treatment in combination with hypoxia resulted in a significantly greater tumor growth delay (time to reach a volume of 600mm^3) compared with controls (p=0.001), topotecan under normoxia (p=0.03), and hypoxia alone (p=0.002). The growth delay induced by hypoxia alone or topotecan alone in the dose used here did not induce a growth delay different from controls.

Our data shows that: (1) Topotecan has an increased growth inhibitory effect in tumors grown in a hypoxic environment; and (2) This effect is likely to be mediated through anti-angiogenesis by inhibition of HIF-1 transcriptional activity and a resultant suppression of VEGF.

584 POSTER

In vivo action of VEGF, bFGF and Angiopoietins in a quantitative angiogenesis assay

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A novel modification of the *in vivo* matrigel plug assay was used for measurement of angiogenic properties of the growth factors bFGF, VEGF and the Ang-1 & Ang-2.

This modification of the assay is based on a chamber of predefined volume and shape. The chamber is formed by a plastic ring with a porous membrane glued to either side of it. In this way, a chamber is delineated inside the ring.

Such chambers were filled with matrigel, containing different concentrations of the growth factors examined (alone as well as in combination). The chambers were then implanted subcutaneously on male nude NMRI mice.

Upon removal 12 days later, both sides of the chambers were photographed and angiogenic response quantified on basis of the ratio of red area versus total area occupied by matrigel.

Histologically (CD-31 immunostaining), numerous endothelial cells in mature as well as immature capillaries were found in most chambers, the degree of red coloration seeming approximately proportional to the number of mature capillaries found.

As expected, all three growth factors display angiogenic effects in this assay. Furthermore, our data indicate a strong synergistic effect of the growth factors bFGF and VEGF, displaying a much larger angiogenic potential than any of the two growth factors alone.

This modification of the *in vivo* matrigel assay circumvents some of the problems seen in other modifications. This assay has potential for widely different usages froim anti-angiogenic screening to investigation of angiogenic acitivty and of cells as well as substances.

585 POSTER

VEGF-C and VEGF-D mRNA expressions are rarely involved in the progression of esophageal squamous cell carcinoma

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Background: Lymph node metastasis is a major prognostic factor for esophageal cancer patients. However, the molecular mechanisms underlying node metastasis remain unclear. VEGF-C and VEGF-D, as ligands for VEGFR-3, have been reported capable of stimulating lymphangiogenesis under in vivo experimental conditions. The aim of the present study was to measure VEGF-C and/or VEGF-D mRNA expression in the clinical specimens of esophageal squamous cell carcinoma, and to examine the correlation between VEGF-C or VEGF- D gene expression and conventional clinicopathological parameters, especially lymphatic invasion of esophageal squamous cell carcinoma.

Materials and methods: Fresh tissue samples were obtained from 38 patients undergoing esophagectomy for esophageal squamous cell carcinoma. Total RNAs were isolated from 38 surgical specimens of esophageal carcinoma tissue and 28 normal esophageal mucosa. The relative mRNA abundance of VEGF-C and VEGF-D was measured by Quantitative real-time reverse transcription-PCR analysis was carried out to measure mRNA expression of both VEGF-C and VEGF-D by standardizing with GAPDH gene. Statistical analyses were performed using Mann Whitney test, chi-square test and Kruskal-Wallis test, and the statistical significance was defined as $p<0.05$.

Results: VEGF-C mRNA was expressed similarly in both esophageal carcinoma tissues and normal mucosa, however, VEGF-D mRNA expression was significantly decreased in carcinoma tissues compared to normal mucosa ($p<0.05$) and VEGF-C/VEGF-D ratio was significantly in creased in tumors compared with normal mucosa ($p<0.05$). However, neither mRNA expression of VEGF-C, VEGF-D or VEGF-C/VEGF-D ratio correlated with any clinicopathological factors such as lymphatic invasion, venous invasion, lymph node status or tumor stage.

Conclusions: These results suggest that VEGF-D mRNA expression, significantly down-regulated in tumor specimens comparing to normal mucosa, might have an association with carcinogenesis in esophageal carcinoma. However, VEGF-C or VEGF-D gene expression seems to be rarely involved in the progression of esophageal carcinoma.

586 POSTER

Expression of a novel MMP inhibitor, RECK, in relation with expression of MMPs and angiogenic factors in non-small cell lung cancer

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Objectives: RECK is a novel matrix metalloproteinase (MMP) inhibitor (Cell 107; 789-800, 2001), and it has been experimentally shown that RECK suppresses tumor invasion, metastasis, and angiogenesis. We have already revealed that enhanced RECK expression is correlated with a reduced tumor angiogenesis and a favorable prognosis in non-small cell lung cancer (NSCLC). The present study was conducted to reveal the correlation between RECK status and expression of MMPs or angiogenic factors.

Material and Methods: A total of 166 patients with pathologic stage I-IIIA NSCLC were reviewed. Expression of RECK, MMP-2, MMP-9, vascular endothelial growth factor (VEGF), angiopoietin (Ang-) 1 and Ang-2 in tumor cells was examined immunohistochemically.

Results: RECK expression was high in 76 patients (46%) and low in 90 patients. High-RECK patients had a significantly lower MVD (158.1) than low-RECK patients (194, p=0.02), whereas high-RECK patients showed a higher VEGF-score. In addition, high-RECK patients showed significantly higher scores of tumoral MMP-2 and MMP-9 expression. There was no difference in interstitial MMP-2 expression score between high-RECK and low-RECK patients. There was no significant correlation between RECK status and Ang-1 or Ang-2 expression. When RECK status was combined with tumoral MMP-2 expression, MVDs for low-RECK/low-MMP-2, high-RECK/low-MMP-2, low-RECK/high-MMP-2, and high-RECK/high-MMP-2 tumors were 188, 161, 222, and 155, respectively; low-RECK/high-MMP-2 tumor showed a extremely high MVD and the poorest prognosis (5-year survival rate, 44%).

	Low-RECK tumor	High-RECK tumor	p-Value
5-yr survival	57%	74%	0.03
VEGF score (tumor)	3.6	4.0	0.07
MMP-9 score (tumor)	2.4	3.2	<0.01
MMP-2 score (tumor)	1.5	2.3	<0.01
Interstitial MMP-2 score	1.1	1.3	0.14
Ang-1 positive (tumor)	36/90(40%)	41/76(54%)	0.09
Ang-2 positive (tumor)	17/90(19%)	15/76(47%)	1.00

Conclusions: Positive correlation was observed between RECK status and expression of MMP-2, MMP-9, and VEGF in NSCLC. A poor prognosis was observed where expression of MMPs and/or VEGF are enhanced without RECK expression, suggesting the balance between these angiogenic factors and RECK plays important roles in tumor progression.

587 POSTER

Glomeruloid microvascular proliferations are superior to microvessel density as a marker of angiogenesis in non-small cell lung cancer

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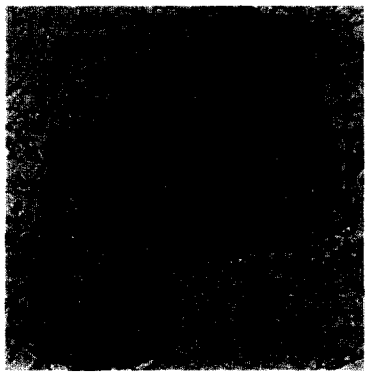
Objectives: Exact evaluation of tumor angiogenesis is important in the diagnosis and therapy of malignant tumors, and microvessel density (MVD) is usually used as a marker of tumor angiogenesis. However, some clinical studies did not document the prognostic significance of MVD whereas others did, and the clinical significance of MVD remains controversial. Glomeruloid microvascular proliferations (GMPs) are focal proliferative buddings of endothelial cells (ECs) resembling a renal glomerulus, and recent studies have suggested that GMPs are superior to MVD as a marker

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.

588 POSTER

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.

589 POSTER

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.

590 POSTER

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