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