580 POSTER

Selective antimetastatic effect of low molecular weight heparin on human melanoma xenograft

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Background: The common use of heparin-derivatives in oncology revealed that anti-coagulant therapies have a beneficiary side-effect: delaying tumor progression. In this study we have used experimental metastasis models of human melanoma in SCID mice to analyse the effect of heparin and its low molecular weight derivative on tumor progression.

Materials and Methods: Two models have been used: lung colonization following i.v. injection of tumor cells and spontaneous liver metastasis model following intrasplenic injection of tumor cells. Heparin (Richter, Budapest) or its low molecular weight derivative (Fragmin, Pharmacia) were administered i.p. one day before tumor cell inoculation into the circulation and for 3 consecutive days (lung colonization) or one week after tumor cell inoculation into the spleen for 10 consecutive days (liver metastasis).

Results: Neither heparin nor its low molecular weight derivative influenced in vitro growth of human melanoma cells, HT168-M1, in the concentration range of 0.01-10 IU/ml. In the lung metastasis model low molecular weight heparin significantly inhibited lung colony formation from the concentration of 0.4 IU/animal, whereas heparin was proved to be much less effective even at 40 IU/ml dose. In case of the liver metastasis model neither heparin nor its derivative was able to influence the growth of the primary spleen tumor. However, low molecular weight heparin significantly inhibited the formation of liver metastases from the dose of 20 IU/animal which effect was achieved by heparin at a log higher concentration. There was no bleeding disorders detected throughout these experiments at the heparin concentrations applied. Hirudin treatment of SCID mice with metastatic human melanoma did not affect the process of metastatization suggesting that the effect of low molecular weight heparin is not mediated by thrombin.

Conclusion: The presented experimental data further support the clinical observations that low molecular weight heparin has specific antimetastatic effect in case of human melanoma, which is independent from the coagulation cascade. Studies are on the way to identify the molecular target of low molecular weight heparin in the metastatic cascade.

581 POSTER

Treatment with epoetin beta corrects anaemia and decreases transfusion use in patients with solid tumours or haematological malignancies

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Background: Anaemia is common in patients with cancer, affecting their quality of life and possibly impacting on long-term outcomes. The current study was performed to assess whether response to epoetin beta varied according to underlying disease.

Materials and methods: This was a 24-week multicentre, open-label study in which anaemic patients with solid tumours or haematological malignancies were enrolled. Patients were treated with subcutaneous epoetin beta (NeoRecormon®) at a starting dose of 2000 IU daily; this was increased to 5000 IU daily in patients with insufficient haematopoietic response after 4 weeks and to a maximum dose of 10000 IU daily after 8 weeks if necessary. Efficacy variables assessed included proportion of patients with haematopoietic response (increase in Hb from baseline of ≥2 g/dl, maintained without transfusion for 4 weeks before and after Hb increase), anaemia correction (Hb increase to ≥10 g/dl), and transfusion need as well as mean increase in Hb level.

Results: The per-protocol population included 259 patients with solid tumours (32% breast, 12% ovarian, 9% lung, 8% prostate, 39% others), and 361 patients with haematological malignancies (47% multiple myeloma, 45% malignant lymphoma, 8% acute or chronic myeloproliferative diseases). Mean baseline Hb was higher in patients with solid tumours (9.2 g/dl [range = 6-14 g/dl]) compared with patients with haematological diseases (8.8 g/dl [range = 4-13 g/dl]). The numbers of Hb responders (≥2 g/dl increase) at study end were 57% and 55% in the solid tumour and haematological disease groups, respectively. Mean Hb increases were 1.6 and 1.7 g/dl in the solid and haematological disease groups, respectively, and the proportions of patients with anaemia corrected to ≥10 g/dl at study end were 64% and 59%, respectively. Patients with solid tumours experienced a greater reduction in transfusion use compared with patients with haematological malignancy (82% vs 64%, baseline to study end). Epoetin beta was well tolerated. The presence of anti-erythropoietin antibodies was assessed in 503 patients after a median interval of 23 weeks from start of treatment. No antibodies were detected in any of the patients.

Conclusions: Epoetin beta increased and maintained Hb both in patients with solid tumours and haematological malignancies, thus reducing the need for blood transfusions and the associated risks.

582 POSTER

A phase I study of day 1 and 8 every three weeks docetaxel infusion.

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Docetaxel has demonstred activity in the treatment of patients with advanced cancers (breast, lung, head and neck, prostate, ovary and stomach). The conventional administration (75-100 mg/m^2 every three weeks) produces substantial myelosoppression and mucositis while a weekly docetaxel dosage of 36-40 mg/m^2 is less myelotoxic and has similar activity. However the weekly schedule is not very convenient in some patient and is associated with increased fatigue and pheripheral neuropathy. To develop a new convenient outpatient schedule with a favourable toxicity profile, we have designed a phase I study to determine the maximum tolerated dose (MTD) of docetaxel given as one hour infusion on day 1 and 8 every 3 weeks in patients with advanced cancer. The starting dose of docetaxel was 40 mg/m^2, in the first three patients, and was escalated by increments of 5 mg/m^2 on both days of administration, up to determine the MTD. In this study, MTD was defined as the dose level associated with the same dose limiting toxicity in at least 33% of treated patients. Sixteen patients entered the study and their characteristics were the following: 8 breast, 4 lung, 2 gastric, 1 esophageal cancer, 1 mesothelioma, median age 58 years (range 36-71), median ECOG PS 1 (range 0-2), M/F 6/10, twelve patients pretreated with chemotherapy for metastatic disease.

Dose level	Pts	DLT
40	3	–
45	6	1 pt. dermatitis G3/ 1 pt. diarrhea G3
50	6	1 pt. mucositis G3 / 1 pt. diarrhea G3
55	1	–

Patients accrual is continuing, at the dose level of 55 mg/m^2.

Up today MTD has not been reached and dose limiting toxicities (DLT) are summarized in the table.

Tumour biology

583 POSTER

Increased effect in hypoxic tumors and blocking of hypoxic upregulation of vascular endothelial growth factor by topotecan.

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The angiogenic factor VEGF is induced during hypoxia through stabilisation of the transcription factor Hypoxia Inducible Factor 1 (HIF 1). Camptothecin-analogues have been shown to inhibit HIF-1 transcriptional activity (Rapisarda *et al.*, Cancer Research, 62, 2002).

Here we examined how topotecan treatment interacts with hypoxia in modulation of the expression of VEGF and HIF-1alpha, and whether the effect of topotecan treatment is dependent on the oxygen partial pressure. Lewis Lung carcinoma (LLC) cells *in vitro* and grown as subcutaneous tumors in C57/bl mice were used.

Cell cultures were exposed to hypoxia (1.5% or 0.7% O) or normoxia for 24h. Media containing 0, 6, 30, 60 or 120nM topotecan was added immediately before exposure to hypoxia. The expression of cellular protein was measured by densitometry of western blots, and VEGF protein in culture media was measured by ELISA.

Four groups of mice with LLC tumors were treated with saline or 20mg/kg topotecan i.p, immediately followed by 72h exposure to hypoxic environment (10% O) or normal air. After 72h all mice were placed in normoxia until termination of the study at a tumor size of 1000mm^3. We found that the hypoxic upregulation of VEGF secretion to the culture medium could be completely abolished by the addition of topotecan at a dose of 30nM ($p=0.000$), while doses up to 120nM had no effect on VEGF secretion under normoxia.

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