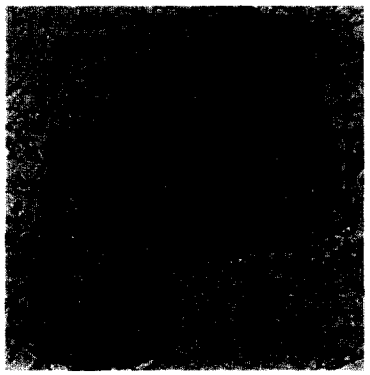
of angiogenesis. To reveal clinical significance of GMPs in non-small cell lung cancer (NSCLC), the present study was conducted.

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

hypothesis that amifostine increases polyamine synthesis in these cells, reducing the amounts of L-arginine that can be metabolised by nitric oxide (NO) synthase to NO. It is well known that spermidine does not affect migration, while we have previously shown that decreased levels of NO inhibit HUVEC migration. Therefore, the decrease in migration seems to be due to a decrease in NO production by these cells. Finally, amifostine reduced tyrosine nitration of the cytoskeletal proteins actin and tubulin, in a time dependent manner. This last action could be due to the reduced amounts of NO or to other, not yet identified mechanisms.

Conclusions: Collectively, our results suggest that amifostine acts on endothelial cells through pathways that affect the redox status of the cells, either by producing H_2O_2 or by modulating NO production.

591 POSTER

Microvessel density of bone metastasis is dependent on the cancer type and therapy

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Background: Bone may provide an extremely fertile microenvironment for angiogenesis. Experimental investigations indicate angiogenesis as a major regulator of bone metastasis development. However, no studies have investigated angiogenesis in bone metastases of human cancers.

Methods: We have evaluated microvessel density in bone metastases of various cancer types and compared to their primary tumors in paraffin samples of 39 patients. Microvessel density (MVD) was determined by using the hot spot method and the endothelial marker, CD34. Patients were chemotherapy-naïve except a subgroup of breast cancer cases.

Results: Two patterns of modulation of the angiogenic phenotype in the bone emerged in this study which seem to be cancer type specific: decreased angiogenic potential characterizing 45% of renal cell cancers and breast cancers of high vascularity in their primary, and increased angiogenic potential characterizing 40% of lung adenocarcinomas and breast cancers of low vascularity in their primary lesion. Analysis of the breast cancer cases indicated no differences in VEGF expression, hormone receptor status or histology between the two groups of primary tumors. However, when we have analysed these cases for possible cause for the different angiogenic responses we found that those cases where MVD decreased in bone metastases were all but one have been treated by chemo- or hormone therapy.

Conclusions: Our data demonstrate that 1. the vascularization of cancer metastases is different from that of the primary tumors, 2. patterns are different in the case of various cancer types. Among factors modulating MVD in bone metastases the unique microenvironment as well as the therapeutic interventions both have to be considered.

592 POSTER

1p chromosomal deletion and candidate genes mapping in liver fluke related cholangiocarcinoma.

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Background: We have characterized genetic alteration in the development of liver fluke related cholangiocarcinoma which is commonly found in northeastern region of Thailand. Genomic wide aberration have been previously examined in 30 cases of cholangiocarcinoma patients (Uchida K,et al. unpublished data). They found the most frequent chromosomal loss at 1p36-qter with the frequency of 35%. To identify the possible candidate gene on this region, deletion mapping were investigated in cancerous tissues using quantitative PCR.

Material and methods: Five Sts markers covering 1p36-pter were firstly screened in 23 cancerous tissues using lightcycler- DNA Master SYBR GreenI (Roche). All samples were run in triplicates with an acceptable CV of less than 10%.

Results: Large deletion was spanned between these markers with 48-60% of all 23 cases. Nine out of 23 cases were selected as representatives for further fine mapping study. Gene copy number was quantitated using 6 gene markers designed in accordance with candidate gene present on this region. At least 4 gene markers demonstrated high deletion frequency. The most frequent deletion (7/9cases) was found at p73 locus.

Conclusions; Our data provided deletion information on 1p36-pter in liver fluke related cholangiocarcinoma. The possible candidate gene was represent as potential marker for further investigation. The expression of p73 will be investigated for the involvement in malignant progression of cholangiocarcinoma.

593 POSTER

Gene expression profiling in papillary thyroid carcinoma: Are there different pathways of carcinogenesis?

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The aim of the study was to evaluate the expression profiles in papillary thyroid carcinomas (PTC) by means of high density DNA microarrays. The molecular mechanisms leading to different types of thyroid tumors are not completely understood. RET protooncogene activation, as a consequence of chromosomal rearrangement, is regarded at present as the most important initiating event in the development of papillary carcinomas. However, in those papillary thyroid tumors which are RET-negative the molecular mechanisms of carcinogenesis are unknown. Nine samples of PTC together with the corresponding normal tissues were frozen immediately after excision. Total RNA was isolated using RNeasy Total RNA Midi and Mini Kits. The RET gene rearrangements were found in four cases by RT-PCR. All samples were hybridized to Human Genome U133A arrays as recommended by Affymetrix. Three different approaches have been used to analyze gene expression data. In the first four methods of gene selection and Support Vector Machine technique with a linear kernel for classification were applied. In the second Singular Value Decomposition and hierarchical clustering algorithm and in the third Affymetrix Data Mining Tool software were used. Very similar results were obtained by all three methods giving a clear separation of gene expression profiles in tumors and normal samples. There were 99 genes overexpressed and 93 genes underexpressed in tumor samples, among them genes previously indicated by Huang et al (2001), particularly SCYA21, TFF3, CITED1, FABP4, LAMB3, SCEL, DPP4. Also, other differentially expressed genes, unreported so far, were found: EVA1, LRB1B, CDH3, gastrointestinal tumor-associated antigen GA733-1, prostate differentiation factor, low density lipoprotein receptor-related protein, TMPRSS, CDH16, PCSK2 and solute carrier NaPiIIb. Expression patterns observed in RET-positive and RET-negative tumors exhibited some differences which were related to expression of both thyroid-specific genes (i.e. PDS), cancer-related (i.e. CAXII, PCNA, PICOT) and still unknown genes (i.e. FLJ10044, FLJ10359). This differences may represent distinct pathways of carcinogenesis Our results obtained so far corroborate the rather stable molecular profile of PTC as postulated by Huang and form a starting point for the further studies of molecular markers in RET-negative thyroid cancers.

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The role of XPD exon 10 polymorphism in susceptibility to ovarian and breast cancer

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Background: Breast and ovarian cancer are two neoplasia with important incidence and mortality in women all over the world. The mechanisms involved in the carcinogenesis of these cancers are not well understood. Nucleotide excision repair (NER) is a crucial pathway in the maintenance of genome stability. Variants of several DNA repair genes, including gene *XPD*, have been described. This protein has a dual function, both in nucleotide excision repair and in basal transcription. The *XPD* exon 10 polymorphism is characterized by a G to A change, being responsible for aspartic acid to asparagine amino acid substitution in the coding region of the *XPD* gene.The purpose of this study was to evaluate the role of *XPD* exon 10 polymorphism as genetic indicator of susceptibility to breast and ovarian cancer.

Materials and Methods: We have used a case-control study. We analysed DNA samples from 499 unrelated individuals, 199 breast cancer