406 POSTER

Induction of angiogenesis and tumour progression by irradiated C6 glioma cells implanted on the chicken embryo chorioallantoic membrane

A. Parthymou[1], P. Katsoris[2], D. Kardamakis[3], E. Papadimitriou[1].
[1] University Of Patras, Department Of Pharmacy, Patras, Greece; [2] University Of Patras, Department Of Biology, Patras, Greece; [3] University Of Patras, Department Of Medicine, Patras, Greece

Purpose: Malignant gliomas present a remarkable degree of invasiveness into surrounding tissues and are critically dependent on angiogenesis. In the present work, we studied the effects of irradiation of C6 glioma cells on their proliferation, protease production and angiogenesis induced after implantation on the chicken embryo chorioallantoic membrane (CAM).

Methods: C6 glioma cell cultures were irradiated at doses of 2.5, 5, 10, 20 and 40 Gy. 48 h post-irradiation, cell proliferation changes were assessed by MTT assay. Metalloproteinase expression of irradiated C6 cells was detected by zymography in non denaturing SDS-PAGE. C6 glioma cells were implanted randomly on CAMs and induction of angiogenesis and tumour growth were confirmed in paraffin sections stained with haematoxylin and eosin

Results: When C6 cells were irradiated, their proliferation was decreased in a dose-dependent manner. The decrease was marginal at the dose of 10 Gy and statistically significant at 40 Gy. Interestingly, metalloproteinase expression increased with elevated doses of radiation and was mostly evident at the higher doses. Implantation of C6 cells onto the chicken embryo CAM induced angiogenesis, effect further increased when cells were irradiated prior to their implantation. The induction of angiogenesis was mostly evident when C6 cells were irradiated with a 40 Gy dose of X rays.

Conclusion: High dose irradiation inhibits cell proliferation but stimulates protease expression, a crucial event in tumour progression. Furthermore, it induces angiogenesis by glioma cells and this may be at least partially responsible for the low effectiveness of radiation therapy.

407 POSTER

Erythropoietin as an angiogenic factor in murine hepatic tumors

K. Nakamatsu[1], M. Suzuki[1], S. Kanamori[1], Y. Nishimura[1], Y. Yasuda[2].
[1] Kinki University, Radiology, Osakasayama, Japan; [2] Kinki University, 1st Department Of Anatomyf, Osakasayama, Japan

Purpose: Erythropoietin (Epo) is known as a hematopoietic factor, which induced by hypoxia. However, it has also been suggested that Epo has an angiogenic activity. To clarify the role of Epo in tumor angiogenesis, concentration and localization of Epo and Epo-receptor (Epo-R) were investigated using a chemical-induced rat hepatic tumor model.

Methods: To induce cirrhosis and hepatic tumors, diaminobenzidine was given for 8-week-old Wister rats for 5 months. In total, 30 hepatic tumors of more than 3 mm in diameter were induced among the 12 rats. Histological type of the 30 tumors were, hepatocellular carcinomas; 13, cholangio-cell carcinomas; 8, poorly differentiated carcinomas; 7, and hamartomas; 2. The 30 hepatic tumors were resected with the surrounding hepatic tissues. Half of a tumor and surrounding hepatic tissues was fixed with liquid nitrogen to measure the concentration of Epo and western blotting for Epo-R. Concentration of Epo was measured by the RIA method. The remaining half of a tumor was fixed by Zambony solution for immunohistochemical staining. Vascular endothelial cells were stained with Factor...± (F8) to count vascular density. Number of tumor vessels was counted at x200 on 100 fields for each tumor. Vascular density was defined as a number of vessel per a field. To demonstrate the presence and localization of Epo-R in tumors or surrounding liver tissues, western blotting for Epo-R and immunohistochemical staining for Epo-R were performed.

Results: Epo was detected in all the 30 tumors with a range of 6.1 and 97.8 mU/ml with a median of 21.8 mU/ml, although Epo was not detected in the normal liver tissues or cirrhotic tissues. Concentration of Epo in a tumor was significantly higher than that of the adjacent cirrhotic tissues. For hepatocellular carcinomas, significant correlation between Epo-concentration and vascular density was noted. Epo-R was detected in hepatic tumors by western blotting, and immunohistochemical staining revealed Epo-R in the endothelium of tumor vessels.

Conclusion: It is suggested that Epo has an important role in the angiogenesis of murine hepatic tumors.

408 POSTER

Intense inflammation in bladder carcinoma indicate good prognosis

B.V. Offersen[1], M.M. Knap[1], S. Hamilton-Dutoit[2], J. Overgaard[1].
[1] Department of Experimental Clinical Oncology, University Hospital of Aarhus, Aarhus, Denmark; [2] Institute of Pathology, University Hospital of Aarhus, Aarhus, Denmark

Introduction This study was initiated to investigate the prognostic impact of angiogenesis in patients diagnosed with bladder carcinoma. As estimates of angiogenesis are not performed lege artis in areas of inflammation, and inflammation was encountered in all these bladder carcinomas, we established a semiquantitative grading of inflammation.

Method Bladder carcinomas from 113 patients were investigated. Tumor specimens from TUR-B were immunostained for CD34 highlighting vessels and VEGF. Inflammation in the invasive parts of the carcinomas was scored as no, slight/moderate and intense inflammation, and microvessel density was estimated in these areas. VEGF intensities were scored semiquantitatively depending on staining intensity.

Results Thirty-two (25%) tumors had areas if intense inflammation, whereas the rest had areas of no and moderate inflammation in the invasive carcinoma. Median vascular scores in areas of no, moderate, and intense inflammation were 32, 62, and 105, respectively, ($P<0.0001$). Ninety-eight (87%) of the tumors had areas of moderate inflammation, and in this group high vascular scores were correlated to a good disease-specific survival. However, after eliminating tumors with either no or intense inflammation from this group, angiogenesis was not a prognostic factor. Inflammatory cells were equally or even more intense of VEGF than the carcinoma cells. In a Cox multivariate analysis muscle-invasiveness, absence of intense inflammation and high tumor grade were found to be independent indicators of poor disease-specific survival, the relative risks being 3.5, 2.5 and 1.5, respectively.

Conclusions Intense inflammation in invasive bladder carcinoma is an independent parameter of good prognosis. An association between increasing degree of inflammation and increasing estimates of angiogenesis was found. High vascular density was identified as an indicator of good prognosis, but when stratified for degree of inflammation the estimates of angiogenesis lost information. VEGF staining revealed both carcinoma cells and host cells as contributors and therefore both as active players in the stimulation of angiogenesis.

409 POSTER

In vitro HUVECs proliferation activity after the influence of the recombinant TNF-α and the supernatants of the primary culture of the tumors and serum of gastric cancer patients

I. Hipp, N. Volodko, V. Barylka, V. Piddubnjak, B. Bilynsky. *Lviv Medical University, Division of Oncology and Medical Radiology, Lviv, Ukraine*

Purpose: Vascular endothelium proliferation plays an important role in a tumor progression. This cytokine's regulated process. We have investigated the influence of the recombinant TNF-α (rTNF-α) (various concentrations), supernatants of primary tumors culture of and serum of 21 gastric cancer patients on the HUVECs proliferation response and it's correlation with tumors pathomorphological features.

Methods: HUVECs were obtained by adaptation of the method of Jaffe (J. Clin. Invest. 1973. 52: 2745–2756.) 72 hours incubation of HUVECs with stimulation factors (rTNF-α (Sigma, USA), supernatants or serum) was performed. [methyl-H^3]-Thymidine (Amersham) for radiolabeling was used (in 12 last hours of incubation, in dose 1 μci per well). The proliferation index (PI) was calculated by dividing number of cells of stimulated endothelium, by number of cells of nonstimulated HUVECs. The bioassay for TNF-α concentration in serum and supernatants was performed

Results: The rTNF-α in concentration from 0.033 ng/ml until 1.5 ng/ml inhibited the HUVECs proliferation. In concentrations higher then 1.5 ng/ml stimulation of HUVECs proliferation was revealed. The rTNF-α in concentrations higher then 13.6 ng/ml decreased stimulation influence on the HUVECs.

After the HUVECs treating *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. We have observed the strong correlation between TNF-α concentration in serum and PI.

After *tumor supernatants* influence PI of the HUVECs was for G1–G2 tumors - 2.4 ± 0.2, for G3–G4 - 2.3 ± 0.2. For I–II Bormann types PI was – 2.59 ± 0.2, for III - 2.55 ± 0.2, for IV - 2.12 ± 0.2. The strong correlation between TNF-α concentration in serum and PI was observed.

Conclusion: Supernatants of tumors primary culture of and serum of gastric cancer patients can stimulate the endothelium proliferation *in vitro*. PI correlated with TNF-α concentration. Serum stimulation showed the correlation between PI and pathomorphological features of tumors.

410 POSTER

Overexpression of angiogenic growth factors, VEGF, PDGF, and bFGF in head and neck squamous cell carcinoma in the betel quid chewing prevalent area

J.T. Chang[1,2], A.J. Cheng[1,3], C.T. Liao[1,4], H.M. Wang[1,5], I.H. Chen[1,4]. *[1] Taipei Chang Gung Head & Neck Oncology Group, Taoyuan, Taiwan; [2] Chang Gung Memorial Hospital, Radiation Oncology, Taoyuan, Taiwan; [3] Chang Gung Unisversity, Medical Technology, Taoyuan, Taiwan; [4] Chang Gung Memorial Hospital, Otolarynology Head & Neck Surgery, Taoyuan, Taiwan; [5] Chang Gung Memorial Hospital, Medical Oncology, Taoyuan, Taiwan*

Purpose: Head and neck squamous cell carcinoma (HNSCC), including cancers of the pharynx, larynx and oral cavity (ICD 140-149, except 147), is one of the ten most frequent cancers in the world. Angiogenic growth factors have been found associated with the aggressiveness of HNSCC. In this study, we evaluated the level of angiogenic growth factors, VEGF, PDGF and bFGF in paired normal and cancerous tissues from HNSCC patients to assess the association of these factors with clinical characteristics of the tumors.

Methods: A reverse transcription-quantitative PCR method was used to measure mRNA levels of VEGF, PDGF, bFGF in 112 paired (normal and cancerous) tissues from HNSCC patients. Clinical information was available for all patients. Analysis for correlation of these factors with clinical and histopathological parameters was done using the Pearson Chi-square test.

Results: Two-fold over-expression of VEGF, PDGF and bFGF were found 65%, 60% and 60% in the matched tumor tissue samples. Levels of VEGF and bFGF have clinical correlation with tumor differentiation ($P \approx 0.022$ and 0.05, respectively). Levels of these growth factors with other clinical and histopathological parameters were less associated.

Conclusion: Over-expression of angiogenic growth factors VEGF, bFGF, and PDGF were commonly found in head and neck cancer. This information may be further applied in clinical applications of diagnosis or therapeutics.

411 POSTER

Inhibitory effect of sulindac and sulindac sulfone (Exisulind)on tumour growth and angiogenesis

E. Rogala[1], E. Skopinska-Rozewska[1], H. Skurzak[2], E. Sommer[1], B. Bialas-Chromiec[1], M. Filewska[1], W.J. Thompson[3], R. Pamukcu[3]. *[1] Nat.Inst.Tuberc.Lung Dis., Lab.Diagn.Immunol., Warsaw, Poland; [2] Oncology Center, Immunology, Warsaw, Poland; [3] Cell.Pathways INC, cell pathways inc, Horsham, USA*

Inhibition of tumour angiogenesis presents new method for cancer therapy in combination with conventional treatment.The aim of our study was to evaluate the effect of NSAID sulindac and its derivative EXISULIND, which lacks ant-COX activity, on: a) tumour-induced angiogenesis(TIA)test, which shows the earliest stage of angiogenic response, recruitment of endothelial cells from non-tumour vessels, similar to the process of metastases vascularisation, b) production of angiogenic growth factors in in vitro cultures of human cancer cells, c) growth of L-1 sarcoma tumour in syngeneic mice and its haemoglobin(Hb) content.

Results: Sulindac and EXISULIND suppressed mice skin cutaneous TIA induced by human lung, ovary, kidney and urinary bladder cells, suppressed bFGF production in lung and kidney cancer cell cultures, have delayed and diminished growth of L-1 sarcoma tumours.Hb content of tumours was lowered by EXISULIND only.

412 POSTER

Angiopoietins and the carcinogenesis of renal cell carcinoma (RCC)

W.-D. Beecken, J. Jones, C. Blumenberg, W. Glienke, J. Binder. *University of Frankfurt/Main, Clinic for Urology, Frankfurt/Main, Germany*

Introduction: Renal cell carcinoma (RCC) is the most common cancer developing in the kidney. Angiogenesis, the sprouting of preexisting capillaries is essential for tumor growth and progression. Angiogenesis is regulated by an orchestra of different angiogenic mediators. Angiopoietins are important regulators of angiogenesis. The angiopoietins 1 and 4 are active as maturation factors of the vascular wall, while angiopoietin 2 and 3 activity desintegrates the vascular wall and therefore leads to the initiation of vascular sprouting. In this study the role of the angiopoietins in the carcinogenesis and progression of RCC was investigated.

Methods: By RT-PCR we investigated the expression of the angiopoietins 1 to 4 in five different RCC cell lines, in human endothelial cells, in human fibroblasts and in tumor samples of 15 patients with RCC.

Results: None of the RCC cell lines produced any of the angiopoietins. Human endothelial cell produced the angiopoietins-2, -3 and -4. Human fibroblasts produced angiopoietin-1. Three of the tumor samples from patients with RCC produced angiopoietin-2. None of the tumor samples expressed any of the other angiopoietins.

Discussion: RCC cell lines did not produce any of the investigated angiopoietins. Based on these results we assume that angiopoietin-2 expressed in human RCC derives from tumor endothelium. However, the expression of angiopoietin-2 by tumor endothelium seemed to be downregulated in the majority of cases. Furthermore, expression of angiopoietin-1 by tumor infiltrating fibroblasts and expression of angiopoietin-3 and -4 by tumor endothelium was down regulated in all tumor samples investigated. Therefore we hypothesize that the down regulation of angiopoietins is an important step in the carcinogenesis of the majority of renal cell carcinomas. The co-expression of vegf and the tie-2 receptor is currently under investigation in our laboratory.

413 POSTER

Intratumoral vessels and proliferative index in lung adenocarcinoma

Y. Slavova[1], A. Popov[2], D. Petrov[3]. *[1] University Hospital of Lung Diseases, Pathology, Sofia, Bulgaria; [2] Alexsander Hospital, Pathology, Sofia, Bulgaria; [3] University Hospital Of Lung Diseases, Thoracic Surgery, Sofia, Bulgaria*

Goal: To research and explore the intratumotal vessels, to examine the connection beteen tumor vascularization and proliferative index, as well as the expression of some biological markers.

Methods: Tissue sections from resected tumor specimens from 20 patients with lung adenocarcinomas are examined immunohistochemically for CD31,Ki-67(MIB-1), p53 and Synaptophysin. Diagnoses were confirmed by HE,PAS,Alcian blue.

Results: Out of 20 adenocarcinomas 12 were highly differentiated (7 with mixed subtypes,3 papillary and 2 acinar),2 were moderately differentiated and 6-low-differentiated solid adenocarcinomas. Availability of intratumoral microvasculature was confirmed immunohistologically by CD31 expression in all cases. For the highly differentiated adenocarcinomas networks of interconected angiogenic vessels were found, distributed in the tumor stroma around the acinar structures, in papillary axis and/or along the alveolar walls. In these cases all intratumoral vessels consist of and are covered by endothelial cells. In 3 low-differentiated adenocarcinomas with solid structures, thin-wall vessels were found, for which, in part of their wall, no endothelial cells were identified immunohistochemically. In these cases, tumor cell of adenocarcinoma exist as a vessel wall. Such vessels of microvasculature, for which there is no endothelial cell lining in part of their wall, and tumor cell perform the function of a vessel wall in this part of vessels wall, are found among tumor cells in solid adenocarcinoma. The proliferative index in 6 low-differentiated adenocarcinomas is higher than 50%, these tumors also express in a large extent the p53-from 45% to 95% of tumor cells population. Neuroendocrine divergent differentiation was established in 7 adenocarcinomas-4 highly differentiated and 3 low-differentiated adenocarcinomas.

Conclusion: Intratumoral vessels in highly differenriated adenocarcinomas are built of and covered by endothelial cells.In low-differentiated solid adenocarcinomas thin-wall intratumoral vessels of the microvasculature are found, in part of their wall there is a lack of endothelial cells, and tumor cells perform the function of vessel wall.The proliferative index in this low-differentiated adenocarcinomas is higher than 50%.