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

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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.

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Erythropoietin as an angiogenic factor in murine hepatic tumors

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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.

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Intense inflammation in bladder carcinoma indicate good prognosis

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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.

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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

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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.