mitogen for CFU-F were quantified by ELISA methodology (RD System). For CFU-F assay 2 × 106 BM mononuclear cells were cultured in alfa medium with 20% of fetal bovine serum (FBS) at 37 C and after 7 and 14 days the CM were harvested. We evaluated the effect of the CM from LCP and BCP CFU-F cultures on the growth of normal CFU-F. 2 × 106 normal BM mononuclear cells were incubated with alfa medium contained 20% of FBS and 20% of the CM of CFU-F cultures from patients and NV. The number of fibroblast colonies was counted at day 14. All the samples of the CM were incubated in duplicate with 5 normal BM.

Results: PDGF-AB (pg/ml, X ± ES) = 7 days: LCP = 47.0 ± 10.0; BCP = 95.0 ± 20.0 and NV = 77.0 ± 22.0 and 14 days: ± 31.2 in all the groups. bFGF (pg/ml, X ± ES) = 7 days: LCP = 6.0 ± 1.0; BCP = 5.0 ± 2.0 and NV = 16.0 ± 7.0 and 14 days: LCP = 6.0 ± 0.9; BCP = 7.0 ± 1.0 and NV = 6.0 ± 0.4.CFU-F assay: (number of CFU-F/2 × 106 normal BM mononuclear cells, X ± ES) = control without CM = 23.50 ± 2.98 (n = 5); with CM of LCP CFU-F cultures (7 days) = 9.74 ± 2.03 (n = 8, p < 0.002 vs. Control) and (14 days) = 8.63 ± 1.36 (n = 8, p < 0.0003 vs. Control); with CM of BCP CFU-F cultures (7 days) = 12.50 ± 2.22 (n = 7, p < 0.01 vs. Control) and (14 days) = 12.69 ± 3.08 (n = 7, p < 0.03 vs. Control); with CM of normal CFU-F cultures (7 days) = 19.50 ± 3.43 (n = 6) and (14 days) = 20.50 ± 2.66 (n = 6).

Conclusion: results show that CM from CFU-F cultures of LCP and BCP have inhibitory effect on fibroblast progenitors proliferation in serum supplemented cultures. Inhibition is not correlated with the PDGF-AB and bFGF levels.

384 PUBLICATION

Efficient autologous inhibition of tumor growth in a murine model of renal carcinoma

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Purpose: The murine RENCA-model simulates renal cell carcinoma growth and metastasis. Purpose of this study was to identify autologous inhibition of tumor growth in this model.

Method: 8 × 104 RENCA-cells were injected s.c. in either one or both hind feet of Balb/c mice. Animals were sacrifice, d 28 days after injection and weight, volume and vessel density of the s.c. tumors were determined. In a second experiment RENCA-cells were injected intrarenaly after flanc incision of anesthesized animals. Seven days after injection tumor nephrectomy was performed on half of the animals. On day 21 all animals were sacrificed and vessel density, weight and volume of the primary or recurrent tumor, lung metastasis count and weigth were measured.

Results: 50% of the animals with bilateral tumors had complete remissions of their established tumors, no remissions were observed in the unilateral group. On day 28, bilateral tumors were significantly smaller than unilateral tumors. In the second experiment tumor recurrence occurred in 4 of 10 nephrectomized animals. In the nephrectomy group lung metastases were reduced by 70% for animals with tumor recurrence. Lung metastasis of nephrectomized animals without recurrence was equal to controls. Recurrent tumors were of comparable size as primary tumors of the untreated group.

Conclusion: The experiments show that autologous inhibition can lead to strong antitumoral effects in the murine RENCA-model. The pronounced effects make autologous inhibition an interesting topic for further investigation of the underlying mechanism.

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K+-efflux modulation of cisplatin-induced apoptosis and cytotoxicity to cultured mesothelioma cells

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The efflux of potassium, plays a necessary role during the induction of apoptosis. Apoptosis with cell shrinkage due to potassium efflux is counteracted by increased inward pumping of potassium ions. Modulation of potassium pump activity could thus decrease or increase apoptosis due to the nature of the disruption of cellular potassium homeostasis and affect anticancer drug cytotoxicity.

We explored the role of enhanced potassium efflux as an explanation to cisplatin cytotoxicity and apoptosis. Amphotericin B, known to potentiate cisplatin cytotoxicity, is a potassium ionophore inducing cellular potassium efflux. Modulation by inhibition of the K+ influx pumps ATPase and Na+, K+, Cl−-cotransport on a mesothelioma cell line with activity of only theses K+ influx pumps was performed. To determine the cytotoxicity we incubated the cells with amphotericin B, ouabain a Na+, K+, ATPase blocker, bumetanide a Na+, K+, Cl−-cotransport blocker and cisplatin, alone or in combination for 1 h.

The number of surviving clones were compared with untreated controls. For apoptosis we first analysed the DNA integrity by DNA-ladder formation on agarose gels.

To quantify free nucleosomes we used a ELISA-kit.

Combination of cisplatin (16.7 umol/L) with amphotericin B (3.2 mmol/L), led to an additive reduction of the percentage of surviving clones. When 100 umol/L ouabain was added a potentiation of the cytotoxicity was seen. Ouabain however, reduced apoptosis of the amphotericin B/cisplatin combination. Bumetanide (100 umol/L) did not affect the cytotoxicity of amphotericin B and cisplatin combined but significantly increased apoptosis of the mixture.

We conclude that cisplatin cytotoxicity and ability to induce apoptosis is influenced by cellular potassium flux modulation.

386 PUBLICATION

In-vivo growth modulation of FaDu-xenografts in nude mice with 2-methoxyoestradiol and oestradiol

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Introduction: Recently, significant in-vivo growth inhibition has been reported for the treatment of B16 melanoma and Meth-A-sarcoma in mice with oral 2-methoxyoestradiol (ME). As the underlying mechanism, antiangiogenic properties of ME have been postulated. We therefore investigated whether ME or oestradiol (ED) exert an antiproliferative effect on a human squamous cell carcinoma xenograft in the nude-mice model.

Methods: FaDu human squamous cell carcinoma was transplanted as single cell suspension subcutaneously (10^6 cells/0.05 ml medium) on nu/nu athymic mice. Oral treatment with ME (100 mg/kg per day, 7 days per week) or ED (600 mg/kg per day, 7 days per week) suspended in olive oil was started on the day of transplantation. Tumour volume was measured daily. Animals were killed when the tumour volume reached 1000 mm^3. The in-vivo tumour doubling time of ME or ED treated animals and controls as well as the specific growth delay were calculated.

Results: Daily oral treatment was well tolerated without obvious toxicity. However, no difference in tumour doubling time in ME or ED treated animals as compared to controls could be observed.

Conclusion: Neither ME nor ED exert a significant growth inhibition on FaDu xenografts in nu/nu-mice.

387 PUBLICATION

p53 protein expression and proliferative activity in renal cell carcinomas

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Objective: Renal cell carcinoma is an heterogeneous group of neoplasia with unpredictable biological behavior. The aim of this study was to investigate the p53 protein expression and the proliferative activity in a group of 55 renal cell carcinoma cases in relation to the histological grade of malignancy.

Patients and Methods: Fifty five unselected patients with renal cell carcinoma (RCC) undergoing radical nephrectomy in the Department of Urology were prospectively studied. All patients were operated curatively. In order to investigate the p53 protein and the proliferative activity of tumor cells an immunohistochemical ABC technique was applied in histological sections DO-7 and MIB-1 (Ki67) are used as primary antibodies correspondingly.

Results: Immunostaining of p53 suggested the presence of mutant p53 was found in 30% of cases. The p53 immunoreactivity was confined to the tumor cell nuclei. Tumors did not stain homogeneously. The percentage of MIB-1 positive cells ranged between 1% and 90%. Positive statistical correlation was seen between the tumor grade and MIB-1. In some tumors, an inverse trend of p53 protein expression and proliferative activity was observed.

Conclusion: Our results suggest that mechanisms related to cell cycle control and cell proliferation play a role in the biology of renal cell cancer. More detailed analysis is needed to elucidate the role of the tumor suppressor gene p53 and cell proliferation in renal cell carcinoma and prognosis.