p = 0.007 respectively). Moreover, all the types of tumour patients had the tendency to high occurrence of DRB-11 allele; however, only in BC group this difference reached the level of statistical significance (25% vs. 11.7%; p = 0.007). Other peculiarities in distribution of DRB alleles were less universal. In particular, there was a decrease in frequency of DRB-1 allele ($p = 0.018$) and an increase of DRB-3-1 allele occurrence ($p = 0.003$) in lung cancer patients. Further, the increase in frequency of DRB-2 allele in leukemia individuals was revealed ($p = 0.048$).

Conclusions: The data imply the role of individual features of HLA class II genotype in the determination of susceptibility to neoplastic diseases.

43 POSTER

Long-term assessment of dysplasia, DNA ploidy, proliferation and p53 alteration in ulcerative colitis

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Purpose: Malignant transformation in long-standing ulcerative colitis is a rare event. The onset of this process is difficult to predict. The specific aim of the present study was to find additional cellular characteristics with possible predictive value.

Methods: The retrospective study comprised 22 patients with long-standing ulcerative colitis. The average length of disease was 10 years. 6 patients developed a colorectal carcinoma. All patients had between 4 and 7 colonscopies within at least 5 years. At those instances biopsies were taken at 8 different locations. 5 tissue sections were cut from each paraffin-embedded specimen and stained for the following purposes: Hematoxylin-eosin, Feulgen (DNA), MIB 1 (Ki-67), p53, WAF1. The latter 3 were stained by means of the ABC-technique. DNA assessment were performed in an image cytometry manner.

Results: Dysplasia of grade 3 was mainly observed in those patients, that later developed a colorectal carcinoma. The grade of dysplasia did not correlat with DNA-aneuploidy. Several specimens with low grade dysplasia were highly aneuploid, especially in those patients who later had high grade dysplasia and a carcinoma subsequently. All 6 carcinoma patients had highly aneuploid lesions up to 4 years before the cancer was diagnosed. The histological grade of inflammation correlated well with the proliferative activity (MIB1). P53 levels were low, whereasWAF1 was highly elevated in most lesions, indicating the presence of wild-type p53 function.

Conclusion: All carcinoma patients had highly aneuploid lesions years ahead of the final diagnosis, that were macroscopially and microscopially unsuspicious. Nuclear DNA evaluation in ulcerative colitis patients appear thus to be of additional value in the individual risk assessment.

44 POSTER

DNA damage in blood lymphocytes of former uranium miners

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Purpose: Uranium miners who were employed during the fourties and fifties in mines of the former Wismut AG were highly exposed to radon The intension of our study was to investigate the degree of DNA damage in blood lymphocytes of these former uranium miners.

Methods: We investigated a group of 41 former uranium miners and compared the results with those of several control groups with different deseases of the lung (lung cancer in smokers and former asbestos workers, lung fibroses, inflammatory lung deseases) and a group of healthy persons. Frequencies of DNA single-strand breakage and cross-linking in lymphocytes were determined by alkaline filter elution. The number of chromosomal aberrations was also determined for blood lymphocytes.

Results: The uranium miners had a significantly lower DNA elution rate for PC-filters and digestion with proteinase K than the control groups. This result could indicate an elevated rate of DNA-DNA cross-linking for the former uranium miners. The number of chromosomal aberrations was significantly elevated for the former uranium miners compared to the patients with lung cancer and lung fibroses.

Conclusions: The results of alkaline filter elution and chromosomal aberrations point to a higher genotoxic damage in lymphocytes of former uranium miners even after decades of exposure cessation.

45 POSTER

Cancer genetics knowledge of doctors treating cancer patients

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Purpose: The cancer genetics information is growing up during the recent years. We performed a study which was designed to evaluate the view of doctors treating cancer patients on the following issues: genetic predisposion to cancer, genetic testing and cancer screening. Their knowledge on common Mendelian inheritance was also determined.

Methods: Two approaches for obtaining the relevant information were used. Anonymous questionnaire was sent to 46 doctors. It was arranged to cover main practical topics of cancer genetics as well as management of the patients with regard of genetic counseling and predictive testing. The other methodology step comprised search through the patients' data and assessment of whether or not the information about family history and related problems had been collected and exploited.

Results: Questionnaire was returned by 41 doctors. About 80% of them knew about familial cancer syndromes and half was able to specify some isolated genes. Most of them considered genetic testing and cancer screening just as a research work yet not applicable in the clinical practice. Only two physicians were aware of cases with familial cancer. 60% of specialists had a good knowledge of Mendelian pattern of inheritance. Looking through 850 patients' data we found out that a family history was clarified in just 25% of them.

Conclusion: Cancer medical specialists are moderately informed in the cancer genetics problems. There is a demand for a collaboration with genetic counseling clinic.

46 POSTER

Amplification of ERBB-2 (HER-2/NEU), ERBB-1 (HER-1) and C-MYC oncogenes often combines with the deletion of chromosome 17 short arm in human carcinomas

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Purpose: Do tumours possess co-incidence of oncogene activation and suppressor oncogene inactivation?

Methods: Amplifications of ERBB-2 (HER-2/NEU), ERBB-1 (HER-1), and C-MYC oncogenes and losses of heterozygosity (LOH) at chromosomes 11p (probe HRAS-1), 17p (probe YNZ-22) and 17q (probe THH-59) were studied in 165 malignant tumours (60 breast, 22 ovary, 40 colorectal, 23 lung and 20 thyroid carcinomas) by Southern-blot.

Results: A statistically significant correlation ($P < 0.01$) between amplification of these genes and 17p deletions was demonstrated: increased oncogene copy number was observed in 11 of 46 (24%) informative tumours with LOH, but in only 3 of 61 (5%) without LOH. This association was mainly due to a high incidence of ERBB-2 amplifications combined with 17p deletions, whereas the occurrence of extra copies of ERBB-1 and C-MYC was too low for any certain conclusions. On the other hand, the tendency ($P < 0.1$) to negative correlation between ERBB-2 gene extradosage and chromosome 11p losses was revealed in breast tumours.

Conclusions: The data confirm the critical role of non-random combination of oncogene activation and suppressor gene inactivation in malignant growth development.

47 PUBLICATION

Apoptosis corrected proliferation fraction in childhood sarcoma shows binominal distribution suggestive of switch mechanism in proliferation control

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Purpose: Previous studies of tumour proliferation control may have been confounded by variable degrees of apoptos. We studied tumour proliferation

rates, corrected for apoptosis in 3 types of childhood sarcoma for evidence of switch-like control effects.

Methods: Diagnostic tissue samples of 29 rhabdomyosarcomas (RMS), 14 Ewings sarcoma (ES) and 18 Osteosarcomas (OS), were studied using routine immunocytochemistry for S-phase related nuclear antigens (Moab against cDNA defined subsegment of the Ki67 antigen, MM1, Novocastra, UK) and in-situ labelling for apoptosis derived DNA fragments (CalBiochem, USA). Quantitation was based on established image analysis (Quantimet 570 C). Apoptosis corrected proliferation fraction was calculated as: Ki67 labelling % divided by (100 – Apoptosis %).

Results: In the groups studied results were distributed as follows:

Group	N	Low prolif fr.	N	High prolif. fr.	N	
RMS	29	<30%	9	>51%	19	(1 case 41%)
ES	14	<21%	8	>45%	6	
OS	18	<30%	12	>75%	6	

Conclusions: Clear binominal/non-Gaussian distribution was consistently observed in all 3 groups of childhood sarcoma. This suggest the presence/abscence of a cell cycling controlling effect, indicative of controller gene switching mechanism activation/suppression of which may be limited to a subgroup of lesions.

48 PUBLICATION

Expression of p53 protein in endometrial carcinomas: Relationship with ER and PR status

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Purpose: To study the expression of p53 protein in endometrial carcinomas and the correlation of this finding with the hormonal status [oestrogen (ER) and progesterone (PR) receptors] and the histological type of the tumours.

Material and Method: Imprint smears from 50 surgically resected endometrial carcinomas were studied by immunoperoxidase method with the use of monoclonal antibody against p53. Twenty normal endometrial smears, were the control group.

Results: Expression of p53 was found in none of the 20 normal endometrial smears but was identified in 14/50 (22.2%) endometrial carcinoma smears. Nine of 14 p53 positive tumours were serous and clear cell carcinomas. No relationship was found between ER and PR status and the presence of p53 negative tumours ($p < 0.01$).

Conclusions: p53 immunoreactivity may have a prognostic role in patients with endometrial carcinoma.

49 PUBLICATION

Expression of novel growth suppresing gene, TOB, in patients with esophageal cancer

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Purpose: Recently, a novel gene, termed TOB has been found to encode a 38-KDa protein homologue to the growth suppressing protein Btg-1. The gene product interacts with erb B2, which plays an important role in the progression of esophgeal cancer. Elevated expression of the TOB protein suppressed growth of NIH 3T3 cells. For the purpose of analysis of TOB's function in the progression of esophageal cancer, we examined TOB mRNA and protein expression in the tissue by immunostainig and RT in situ PCR.

Method: Tissue specimens of esophageal cancer and of noncancerous esophagus were obtained from patients who had undergone subtotal resection of esophagus at Department of Surgery, IMSUT. Paraffin-embbeded specimens were sliced into 4-μm-thick sections, then to which immunostaining and RT in situ PCR were performed.

Results: we found that TOB mRNA transcripts and proteins decreased in the tissue of esophageal cancer as compared with non cancerous tissues.

Conclusion: This study suggested that TOB may play a part in progression of esophageal cancer.

50 PUBLICATION

Genomic instability as assessed by micro satellite analysis in childhood rhabdomyosarcoma (RMS)

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Purpose: Microsatellite instability (MSI) as a reflection of inherent genomic instability, may be of relevance to sensitivity to genome directed therapy. We studied MSI in a representative series of childhood RMS, related to proliferation and apoptosis.

Methods: Diagnostic tissue samples of 30 consecutive, unselected RMS cases were studied. Patients, 18 M/12 F, age 3 m–17 y (mean 6 y4 m)/2 m–16 y (7 y5 m), were diagnosed as Embryonal (20, 8 with mets), Alveolar (6, 3 with mets) and variants (4). After routine extraction, amplifications were carried out at the loci D3S1304 and D3S1537 (both closely distal to the VHL tumour suppressor gene), ELN gene, D7S1870, IFNA, D1S243 (1p36) with isotopic labelling during amplification, non-denaturing gel electrophoresis and autoradiography. Apoptotic (Frag-EL) and proliferation fraction (Ki-67labelling) were determined in all cases.

Results: Limited abnormal amplification products were seen in 5 patients: 1. F, 13 y11 m, embryonal thumb lesion, metastatic disease at presentation, deceased; 2. F, 13 y9 m, alveolar, neck node primary, deceased; 3. M, 2 y4 m, embryonal, thigh lesion, alive; 4. M, 3 m, atypical, axillary mass, deceased; 5 M. 9 y2 m, embryonal, nasopharynx, deceased. Apoptotic corrected proliferation fraction of these lesions was high compared to other lesions.

Conclusion: Microsatellite abnormality was recorded in only 5/30 cases of RMS association with high proliferation rates warrants further studies.

Prevention of therapy-related side effects

51 ORAL

The modification of bleomycin-induced lung toxicity in a S.C. mice model and an I.T. rat model

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Purpose: Bleomycin (BLM) remains an important drug in the treatment of a variety of tumours, e.g. teratocarcinoma, Kaposi's sarcoma, lymphomas. The main toxicity of BLM is a dose-dependent pulmonary fibrosis. This study investigated the potential of the cytoprotective agent amifostine (A), WR-2721, for the prevention or attenuation of BLM induced lung damage.

Methods: Eight week old Swiss NIH mice were injected twice weekly with 5, 10, 20 and 40 mg/kg of BLM for two, four or six consecutive weeks. Routine and trichome stained analysis of lung changes were performed. Two groups six mice each were treated with BLM 20 mg/1 g 2x/week with or without A at 200 mg/kg also twice weekly s.c.

In a third set of experiments adult male Wistar rats were treated with unique intratracheal dose of BLM at 1.5 IU and 2.5 IU. A third group received prior to this I.T. administration a once daily s.c. dose of A.

Results: In the mice experiments BLM s.c. produced a dose-dependent increase in lung damage measured by alveolar wall thickness, intra-alveolar mononuclear cells and pulmonary consolidation. Treatment with amifostine resulted in a decrease in mortality and in an improvement in different pathological parameters. In the rat model with i.t. administration a clear protective effect was again observed with a complete pathological protection in 2/4 animals in the 1.5 IU group.

Conclusion: Both in the chronic s.c. model in mice as in the acute i.t. model in rats, amifostine treatments results in an impressive attenuation of BLM induced lung toxicity.