Conclusions and Suggestions: Breast cancer occurred at an earlier age than ovarian cancer in both BRCA 1 and BRCA2 carriers, suggesting that breast cancer surveillance should start early in this group. Bilateral oophorectomy should be considered at the time of breast cancer diagnosis or at completion of childbearing.

Timing of prophylactic measures should take into account the lower risk and later age of onset for the BRCA2 6174delT mutation.

38 ORAL

A common Scottish *BRCA1* mutation

A.A. Renwick, F. Harris, R. Davidson, T.G. Cooke, P.D. Stanton, D.M. Black. *C.R.C. Beatson Laboratories, Garscube Estate, Switchback Road, Bearsden, Glasgow; University Department of Surgery, Glasgow Royal Infirmary; Dept. Of Medical Genetics, Yorkhill Hospital for Sick Children, Scotland*

Aims: To assess which mutations are present in breast cancer families in Scotland.

Methods: Families identified as "at risk" by the Dept. Of Medical Genetics had lymphocyte DNA from index cases in breast and ovarian cancer families screened for germline *BRCA1* and *BRCA2* mutations. The screening was carried out by Protein Truncation Testing (PTT) and Single Stranded Conformation Polymorphism (SSCP) on 40 families. Any mutations found were also screened for in a total of 276 patients.

Results: Individual families were found to have the 185 AG deletion and a C insertion at position 5382 in *BRCA1* a 5445 del 7, a 5574 del AA, and an 8525 del C mutations in *BRCA2*. All resulting in truncated protein products Six separate families however showed the same *BRCA1* mutation, an AA deletion in exon 11 of *BRCA1* at position 2800 (2800 del AA). One individual was shown to be homozygous for this mutation, thereby giving rise to a naturally occurring human "knockout" for this gene. All six families share a common haplotype around the *BRCA1* gene.

Conclusions: There appears to be a common mutation which has a prevalence rate of 2%. We have now cloned portions of wild-type and mutant *BRCA1* genes into bacterial expression plasmids, and isolated recombinant proteins. Using these antigens, we have developed antibodies to the carboxy terminal of the 2800 del AA *BRCA1*, and to 3 portions of the wild-type protein. These are currently being tested to see if they are of use in identifying mutation carriers and in identifying interacting proteins.

39 POSTER

Effects of hypusinylatin of eIF-5a on cell cycle progression and specific mRNA translation in human tumor cells

I. Koch[1], H.-M. Hanauske-Abel[2], A.-R. Hanauske[1]. [1]*Dep. of Hematology and Oncology, Klinikum rechts der Isar, München, Germany;* [2]*Dep. of Pediatrics, Cornell University Medical College, New York, NY, USA*

Purpose: eIF-5a is very likely involved in the translation of specific mRNA, similar to its function in HIV infected cells (transporting late mRNAs out of the nucleus as a cofactor of HIV-REV). Hypusinylation of eIF-5a is essential for cell division. Inhibition of hypusinylation by mimosine leads to a reversible cell cycle arrest at the G1/S border. We have analysed cell cycle progression and specific mRNA translation in human mammary tumor cells after withdrawal of mimosine.

Methods: Cell cycle progression of mimosine-treated (300 μM/24 h) and released MDA231 cells was monitored by FACS analysis. mRNA translation was analysed by differential display RT-PCR (DD-RT-PCR) of polysomal RNA.

Results: After withdrawal of mimosine, DNA synthesis becomes visible after 3 h and is completed after 8 h. Mitosis occurs after 12 to 15 h. Readministering mimosine 15 minutes after release does not inhibit S-phase entry. However, S-phase is decelerated and mitosis does not occur. DD-RT-PCR analysis indicates that mRNAs can be identified that are bound to ribosomes and thus translated in cycling cells, but not in cells blocked by mimosine and are bound again and translated shortly after mimosine withdrawl (hypusine-dependent mRNAs).

Conclusion: Hypusinylation of eIF-5a influences the generation of structures necessary for S-phase entry and progression. Hypusine-dependent mRNAs that presumably code for proteins involved in S-phase entry and transit can be identified in the beginning of the S-phase. Interference with tumor-specific hypusine-dependent mRNAs may lead to novel strategies to interfere with tumor cell growth. This work was supported by the Wilhelm Sander Stiftung, Grant 96.022.1

40 POSTER

High resolution HLA-DRB1 genotyping in patients with RCC

E. Özdemir[1,2], Y. Kakehi[1], O. Yoshida[1]. [1]*Dept. of Urology, University Hospital of Kyoto;* [2]*Department of Urology, University Hospital of Dicle, Japan*

Purpose: A variety of malignancies have been linked to MHC complex genes, including the *DRB1* alleles. The association of certain DRB1 antigens with renal cell carcinoma (RCC) has been both claimed and disclaimed. To determine whether HLA-DRB1 genotypes are associated with RCC, we for the first time performed HLA-DRB1 genotyping in RCC patients.

Methods: we used the modified PCR-RFLP method for the high-resolution HLA-DRB1 genotyping of 96 Japanese RCC patients.

Results: There were no significantly frequent *HLA-DRB1* alleles, whereas the *DRB1*0101* and **0405* alleles had significantly lower frequencies (P = 0.004, RR = 0.2 and P = 0.002, RR = 0.4) in the RCC patients than in the healthy Japanese controls (n = 1216). Moreover, patients with the *HLA-DRB1*0101* or **0405* allele tended to be in earlier stages and to have less aggressive tumors than patients with neither of these alleles. The corresponding serotyping subclassification, however, showed a significantly lower frequency only for DRB1-DR1 (P = 0.01, RR = 0.3).

Conclusion: High-resolution genotyping is essential because the polymorphism of the peptide-binding domain of MHC class II molecules is more precisely determined by genotypes than serotypes. In addition, inherent technical difficulties and potential typing errors render serotyping inefficient. Our data suggest that *HLA-DRB1*0101* and **0405* are protective alleles for both RCC development and tumor progression.

41 POSTER

Differential transcriptional regulation of the human gene for the M1 subunit of ribonucleotide reductase by p53

A.A. Baba[1,2], N. Parker[2], R.M. Fox[2]. [1]*Hospital USM, Kota Bharu, Malaysia;* [2]*Dept. of Medical Oncology, Royal Melbourne Hospital, Melbourne, Australia*

Purpose: Wild-type p53 is implicated in the regulation of genes involved in cellular proliferation, differentiation and DNA repair. Ribonucleotide reductase is a potential target for p53 dependent regulation as it is an essential enzyme in the production of deoxyribonucleotides required for DNA synthesis and repair. Using transfection studies we have analysed the effects of p53 on the human gene for the M1 subunit of ribonucleotide reductase *(RRM1)*.

Methods: pSGp53, a vector which drives expression of human wt-p53 via the SV40 promoter or pCMVp53, a vector which drives expression of p53 via the CMV promoter was transfected into p53-null K562 cells together with a reporter plasmid containing the *RRM1* promoter sequence.

Results: Expression of p53 by pSGp53 resulted in 3–11 fold transactivation of the *RRM1* promoter. Using stepwise deletions of the *RRM1* promoter, we have identified a region close to the transcription start site which confers p53 responsiveness on the *RRM1* promoter. Expression of p53 by pCMVp53 resulted in repression rather than transactivation of *RRM1* transcription. Quantitation of p53 suggests that this differential effect of p53 on *RRM1* transcription may be related to the amount of p53 protein expressed in the transfected cells.

Conclusion: p53 differentially regulates *RRM1* transcription. This has important implication regarding the role of p53 in DNA repair, growth arrest and apoptosis.

42 POSTER

Unusual distribution of HLA-DRB alleles in tumour patients

Anton A. Lyshchov, Evgeny N. Imyanitov, Alexandr V. Togo, Igor V. Komochkov, Oleg I. Chernitsa, Boris V. Afanasiev, Peter G. Knyazev, Kaido P. Hanson, N.N. Petrov. *Research Institute of Oncology, Pesochny-2, St-Petersburg, Russia*

Purpose: Distribution of alleles of DRB locus of HLA class II genes was compared in 212 tumour patients (44 breast, 17 ovarian, 53 colorectal, 23 lung, 10 thyroid, 3 melanoma, 3 soft tissue neoplastic processes and 59 haematological malignancies) and 120 healthy donors.

Methods: Restriction fragment length polymorphism (RFLP) of DRB locus was analyzed by Southern-blot procedure.

Results: The frequency of DRB homozygous patients in both solid tumour group (42 of 153; 27.4%) and leukemia cohort (10 of 59; 16.9%) was significantly higher than in control (9 of 120; 7.5%) (p = 0.000001 and

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

J. Habermann[1], H. Schimmelpenning[1], D. Ludwig[2], S. Krüger[3], M.W. Strick[1], R. Broll[1], P. Kujaht[1], G. Auer[4], H.-P. Bruch[1]. [1]*Dept. of Surgery, Med. Univ. Lübeck;* [2]*Dept. of Internal Medicine, Gastroenterology, Med. Univ. Lübeck;* [3]*Dept. of Pathology, Med. Univ. Lübeck;* [4]*Dept. of Tumor Pathology, Karolinska Hospital, Stockholm, Germany*

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

C. Vahrenholz[1], W. Popp[1], T. Wüst[1], F. Rietschel[1], P. Bauer[2], N. Konietzko[2], K. Norpoth[1]. [1]*Institute of Hygiene and Occupational Medicine, University Medical Center Essen (GSH), Hufelandstr. 55, D-45122 Essen;* [2]*Ruhrlandklinik, Tüschener Weg 40, 45239 Essen Essen-Heidhausen, Germany*

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

M. Stefanova. *Department of Medical Genetics, Medical University, Plovdiv, Bulgaria*

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

K.P. Hanson, E.N. Imyanitov, O.I. Chernitsa, I.F. Nikiforova, O.Yu. Laur, P.G. Knyazev, A.V. Togo. *N.N. Petrov Institute of Oncology, 189646, St.-Petersburg, Russia*

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

L.M. Ball, C. Riddell, L. Resch, K. Laybolt, M. Henry, C L. Lannon, J. Tam, D. van Velzen. *Department of Haemato-Oncology IWK/Grace Health Centre, Halifax, Nova Scotia; Department of Pathology IWK/Grace Health Centre, Halifax, Nova Scotia, Canada*

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