

Conclusions and Suggestions: Breast cancer occurred at an earlier age than ovarian cancer in both BRCA1 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.

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ORAL

A common Scottish BRCA1 mutation

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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.

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POSTER

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

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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. Read-ministering 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 withdrawal (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

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POSTER

High resolution HLA-DRB1 genotyping in patients with RCC

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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.

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POSTER

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

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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.

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POSTER

Unusual distribution of HLA-DRB alleles in tumour patients

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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