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DETECTION OF WILD AND MUTANT TYPE P53 IN HUMAN GERM CELL TUMORS BY HISTO-CHEMISTRY STAINING

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P53, first detected in a complex with SV 40 large T-antigen, was subsequently found to complex to the E1B 58 kd product of adenovirus 5 and 12, and to mammalian heat shock protein HSP 70.

A study of brain, breast, lung and colon tumors showed, that in the majority of the cases, where p53 was deleted, there was a detectable mutation in the remaining p53 allele, which causes tumor progression by loss of growth control by functional inactivation of p53 gene. Frozen testicular specimens from 20 patients with testicular cancer were analyzed. The following monoclonal antibodies were used:

1. Clone PAb 1801, derived by fusion of BALB/c splenocytes with NS-1 mouse myeloma cells. 2. Clone PAb 240 was derived by immunization of BALB/c mice with p53-b-galactosi-dase fusion protein and fusion of splenocytes with SP2 mouse myeloma cells. 3. Clone 1620 derived by immunizing BALB/c mice with VLM tumor cells and fusion of splenocytes with SP2/0-Ag 14 mouse myeloma cells.

The p53 protein in the mutant conformation was localized in the cell cytoplasm, whereas the wild type in the cell nucleus was found in numerous but not in all tumor cells. The wild and the mutant type could be found in the same tumors (e.g. in 3 embryonal carcinomas, 8 seminomas, in the benign Leydig cell tumor, in one immature teratoma, in 3 embryonal carcinomas with seminomas, in one teratoma with choriocarcinoma, in one immature teratoma with seminoma, in one embryonal carcinoma with teratoma). A small number of atypical cells in various stages of spermatogenesis in seminiferous tubules (carcinoma in situ) showed in their cytoblasma the mutant type p53 and the wild-type in their nucleus.

Neither the wild nor the mutant type could be detected in the mature teratoma. In the same of these tumors we also found EBV-DNA. In some of these tumors we could detect the oncogenes: c-myc, N-myc, c-Ha-ras 1, c-fos, and c-jun. The discovery of wild and mutant p53 in human testicular cancer is consistent with the view, that alterations of tumor-suppressor genes play a role in the pathogenesis of this tumor type in cooperation with the Epstein-Barr virus and other oncogenes.

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COMPARISONS AND SIGNIFICANCE OF IMMUNOHISTOCHEMISTRY AND CYTOSOL p53 ASSAY IN MATCHED BREAST CANCER SAMPLES

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p53 alterations are considered as the most frequent molecular abnormalities associated with almost all human neoplasias.The present study deals with the analysis of p53 protein level as determined in breast cancer sections by means of immunohistochemistry and in cytosol derived from matched tumour biopsy specimen using two site ELISA. Over 70 paired samples were tested employing high affinity monoclonal antibodies of DO and BP53 series and polyclonal serum CM-1 to human p53 protein.

The results showed a significant correlation between the degree of staining and the amount of p53 protein measured by ELISA.It appears that the combined p53 assay may increase the test reliability in reducing factors which may account for missinterpretation of the results,e.g.fixation,storage,representative sampling etc.

The correlation of our findings with histological type and biological behaviour of the individual tumour are further discussed.

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SOMATIC POINT MUTATION IN THE HER-2 GENE SEQUENCE CODING FOR THE TRANSMEMBRANE REGION ASSOCIATED WITH HIGH SERUM CONCENTRATIONS OF P185 FRAGMENTS IN HUMAN BREAST CANCER

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Introduction: Gene amplification and overexpression of its translational product p185 were previously described as activating mechanism of the HER-2 proto-oncogene. In the homologous neu gene of the rat a point mutation in the transmembrane region leads to malign transformation. Since now a point mutation in the transmembrane region of the human HER-2 has not been detected.Recent findings suggests, shedding of the p185 receptor may be involved in regulation of p185 expression. Material and methods: 100 human breast carcinomas were investigated concerning gene amplification (southern blot), p185 overexpression (KIA,ICA) and in part mRNA overexpression (northern blot) and point mutation of the transmembrane region (direct sequencing) and serum concentrations of the p185 shedding fragment. The detection of a somatic point mutation was confirmed by RFLP with the BSMI restriction enzyme and by sequencing of leukocyte DNA of the same patient. Results : In 6 out of 89 evaluable mamma carcinomas we have found a p185 overexpression without HER-2 gene amplification. Since now, in one of these we could detect a somatic heterozygous point mutation at codon 661 wiht a T to A transition from ATT to AAT and a consecutive change of the amino acid sequence from Ile to Asn. There was no point mutation in the leukocyte DNA of the same patient.In this case we could measure a very high serum concentration of the p185 shedding fragment. The clinical course indicates a very aggressive variant of breast cancer. Conclusion: A point mutation in the transmembrane region of the HER-2 gene could be discussed activating the proto-oncogene in case of p185 overexpression without HER-2 gene amplification and leading to an increased shedding of the p185 receptor.

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BHRF1 - AN EPSTEIN BARR VIRUS GENE PRODUCT WHICH HAS HOMOLOGY WITH BCL2 - IS SIMILARLY LOCALISED.

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BHRF1 is an Epstein-Barr virus (EBV) encoded protein expressed at the interphase between the latent and lytic phases of virus replication. The BHRF1 product has a 40% homology with the bcl2 proto-oncogene which has been localised to the mitochondria and appears to extend cell survival by blocking programmed cell death by apoptosis. There is emerging evidence for a role for EBV in lymphoma.

To explore the function of BHRF1 we have examined an EBV-genome positive cell line, B95.8, using low temperature embedding immunoelectron microscopy. SU-DHL4, a cell line which expresses bcl2 was also studied. B95.8 cells in log phase growth were cultured in either standard (10% foetal calf serum absent) conditions for three days. SU-DHL4 cells in log phase growth were cultured in standard conditions. Cells were then pelleted, washed, embedded in resin and 0.1μ sections prepared for electron microscopy. Expression of BHRF1 and bcl2 were determined using the 5B11* and 100* antibody respectively.

Only B95.8 cells in the lytic cycle expressed BHRF1 and this was localised to the periphery of the mitochondria and to the endocytoplasmic reticulum in a manner similar to that found in bcl2. Mitochondria of lytic cycle B95.8 cells did not cross with 100.

The homology shared by BHRF1 with bcl2 and the common localisation to the mitochondria suggests a functional equivalence.

(*5B11 was kindly provided by Dr G Pearson and 100 was a gift from Dr D Mason)

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ALLELIC DISTRIBUTION OF DRB LOCUS OF II CLASS MAJOR HISTOCOMPATIBILITY COMPLEX GENES IS ASSOCIATED WITH TUMOUR SIZE, NODAL INVOLVEMENT, STAGE OF DISEASE AND AGE OF PATIENTS IN BREAST CANCER GROUP.

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Southern-blot study of 41 breast cancer (BC) patients and 120 healthy donors revealed the drastic increase of the occurrence of DRB-homozygous genotypes (32% vs. 7,5%) and DRB-5-1(DRBw11) allele (25% vs. 12%) in BC group. Moreover, the frequency of DRB-4 allele was significantly higher in BC patients with large tumour size (>5cm: 25%; <5cm: 8%), node positiveness (15% vs. 8%), advanced stage (III-IV: 23%; I-II: 7%) and elderly age (>55 years: 23%; <55: 4%). The occurrence of DRB-homozygotes was increased also in BC group with poor prognostic parameters. This results imply the role of immunity in BC development and prognosis.

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THE USE OF ATOMIC FORCE MICROSCOPY (AFM) TO SCRUTINISE CHROMOSOME STRUCTURE.

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The emerging technology of AFM enables the production of images with resolution at the molecular level. This is achievable without the molecular disruption that occurs during sample preparation characteristic of electron microscopy. Hence biological molecules can be imaged under conditions close to their natural state and furthermore images can be generated in real-time so that molecular process can be followed.

We have used a Nanoscope™ AFM instrument to scrutinise human chromosomes arrested in mitotic division after culture with colcemid.

Features of the chromosomal surface structure were clearly discernable.

This technology has the potential to provide insight into the molecular events involved in chromosome activity. A detailed topographic map of the chromosomes surface may yield information as to the nature of chromosomal fragile sites.