17

EXPRESSION OF P53 IN CIRRHOTIC LIVERS ADJACENT TO HEPATOCELLULAR CARCINOMA

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Mutated p53 acts as a oncogene, whereas wildtype p53 gene product suppresses cells growth. Abnormalities in p53 gene are reported in more then 50% of malignant tumors. Mutations in p53 lead to stabilization of the gene product, thus rendering it detectable by immunohistochemistry. Recently an allelic loss of chromosome 17p, where the p53 gene is located was demonstrated to be more frequent in hepatocellular carcinoma (HCC), both in cell lines and human tumors. Additionally, in half of cases of HCC from an hepatitis B virus and aflatoxin endemic areas, a hot spot point mutation at codon 249 was detected, as we previously reported. Further reports had suggested that mutations in the p53 gene is a late event in the development of HCC. To assess the relation between p53 function at a premalignant state, prior to the development of HCC, we have analyzed by immunohistological techniques, utilizing MoAb against *wt* and mutated P53 (BP 53.12, Zymed, CA USA), the level of *P53* in HCC and in the adjacent non-tumor liver tissue. In a number of cases studied P53 was overexpressed in the nontumor tissue adjacent to HCC tissueas well as in the tumorus tissue. It might be suggested that in part of cases with HCC, p53 gene is mutated or functions abnormally prior to tumor development while supporting hepatocarcinogenesis.

18

A NOVEL *P53* GERMLINE MUTATION ASSOCIATED WITH A FAMILIAL BRAIN TUMOUR SYNDROME

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Mutations of the *p53* tumour suppressor gene are the single most frequent genetic alterations in human neoplasms. Germline mutations are associated with an elevated and inheritable risk to develop early and sometimes multiple cancer, as has recently been demonstrated in the case of the Li-Fraumeni Syndrome.
We now report on a novel *p53* mutation that was detected in the germline of a Swiss family with four brain tumours and an adrenocortical carcinoma in two generations, all presenting before age 25. Molecular genetic analyses were made on genomic DNA extracted from white blood cells and tumour biopsies. Mutations in the *p53* tumour suppressor gene were detected with the SSCP assay and subsequently confirmed with Sanger's direct sequencing technique. We suspect that the uncommon clustering of brain tumours in this particular family is directly linked to the specific and novel *p53* mutation which we have found.

19

GLIOMA POLYPOSIS (TURCOT SYNDROME): A CASE REPORT OF A RARE HEREDITARY DISORDER.

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The "Turcot Syndrome" is a rare hereditary disease. Its characterisics are the association of colonic polyposis with primary neuroepithelial tumors of the central nervous system first described by CRAIL 1949 and TURCOT et al. 1959. We report the case of a 16 years old child suffering from focal cerebral seizures. The history of the disorder began 2 years ago with an anemia and a diarrhoea within some wecks. The diagnosis revealed a colonic polyposis with an adenocarcinoma of the colon. After a hemicolectomy 8 months later a formation of metastases in the liver was seen and a partial resection of a lobe of the liver was done. Half a year later the first onset of cerebral focal seizures was observed with a mild hemiparesis of the left side. CT scan showed a right temporo-parietal cystic lesion. This led to MRI scanning revealing 2 lesions highly suggestive of 2 metastases. The histological examination following surgery was that of a astrocytoma grade III WHO. After surgery with a resection of the brain tumor a tumor of the skin was detected and identified histologically as a cutaneous metastasis of the adenocarcinoma. In contrast to other previous lab studies in glioma polyposis we could show cytogenetic defects with a failure of the supressor gen p 53 and a delation in the primary tumor already. The clinical neuroradiological and genetic findings are discussed summerizing the recent literature.

20

TRANSFORMING POTENTIAL OF DBL ONCOGENE IN TRANSGENIC MICE

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The *dbl* oncogene, initially detected by DNA transfection, results by substitution of the N-terminal portion of proto-*dbl* with unrelated human sequences. The *dbl* oncogene encodes a nucleotide releasing activity for the *Ras* related protein CDC 42HS. Despite the fact that the oncogene displays in vitro a strong transforming activity on the NIH 3T3 cell line, transgenic mice generated by directing the *dbl* oncogene expression in various tissues failed to demonstrate in vivo transforming activity, thus supporting the idea that the activation of this oncogene is not sufficient by itself to initiate a transforming process. On the other hand expression of the protooncogene is mainly confined to tumors or normal tissues of neuroectodermal origin. Thus we have generated transgenic mice by using a molecular construct in which the *dbl* coding sequence has been fused to the rat NSE promoter. The NSE/*dbl* mice have been crossed with a p53 nullisomic mutant to give rise to a line of transgenic mice expressing the NSE/*dbl* construct in a $p53^-$ genetic background.

In addition, by using a Yeast Artificial Chromosome strategy, we have isolated the entire human proto-oncogene and its flanking DNA regions. In order to determine the structural organization of the promoter region, we have isolated and sequenced a 2Kb flanking sequence 5' to the coding region.

21

PROLIFERATIVE ACTIVITY AND p-53 EXPRESSION IN HPV-DNA-POSITIVE URINARY BLADDER CARCINOMA.

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The unpredictability of evolution of urinary bladder carcinoma brought to analyze biologic markers like cell proliferation rate and p-53 expression as predictors of clinical course. Besides, the possible role and significance of HPV infection is still controversial. In this study we have investigated the presence of HPV-DNA by a non-isotopic in situ hybridization technique and cellular proliferation indices by immunohistochemical demonstration of Ki-67, PCNA, p-53 and AgNORs in a series of 101 histologic samples of 43 patients with bladder carcinoma. HPV-DNA was detected in 17 cases (40%), 62.5% of which harbouring HPV types 31/33/35, either alone or in association with other virus types. Virus infection and tumor grade did not show any significant correlation. But, virus-negative cases were mainly in T1 tumors and in disease-free survivors. Proliferation indices and p53 expression did not appear to change in relation to HPV infection, though p-53 was significantly increased in T3 tumors. About AgNOR expression, only AgNOR number was significantly increased in HPV positive cases, but no differences were found in relationship with grade and stage or follow-up. In conclusion, though the presence of HPV-DNA seems to worsen the clinical course of bladder carcinoma, it does not appear to modify cellular proliferative activity and p-53 expression, except for the increase in the number of AgNORs.

22

EFFECTS OF THE ACTIVATED KI-*RAS* EXPRESSION ON THYROID EPITHELIAL CELLS *IN VIVO* AND *IN VITRO*.

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Transgenic mice have been generated bearing a fusion gene consisting of a rat thyroglobulin promoter followed by the complementary DNA of the human activated Ki-*ras*. These mice, which express the transgene at low levels in the thyroid gland, show benign thyroid lesions, although at very low incidence and after a long latency period. However goitrogen treatment of this line of transgenic mice significantly increases the occurrence and severity of lesions. *In situ* hybridization experiments confirmed the presence of Ki-*ras* mRNA in the majority of thyroid cells and moreover every cell of the single carcinoma observed at the end of goitrogen treatment gave a strong positive signal. Activated Ki-*ras* has also been inserted in a different vector (164/7), always under the rat thyroglobulin promoter, to raise the expression levels in the thyroid. Preliminary results of transfection experiments of this construct demonstrated a partial transformation of a rat thyroid epithelial cell line FRTL-5, with a reduced production of the differentiation markers : thyroglobulin, thyroperoxidase and TSH receptor. These results support the hypothesis that ras expression is involved in the early steps of thyroid tumorigenesis.