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THE ROLE OF THE WT1 GENE IN CANCER AND DEVELOPMENT

N. Hastie, MRC Human Genetics Unit, Western General Hospital, Edinburgh, U.K.

Wilms' tumour has always been regarded as an excellent model for the relationship between cancer and abnormal development. These childhood kidney cancers arise from mesenchymal stem cells which should normally differentiate into the epithelial components of the nephron. Nephron components are made in some of the tumours, but aberrantly. In a small percent of Wilms tumours, ectopic tissues such as muscle, bone and cartilage can be observed. Recently, a Wilms tumour gene (WT1) mapping to chromosome 11p13 has been isolated. Loss of function of this gene can lead to both Wilms tumours and developmental abnormalities of the kidney and gonad. WT1 encodes a protein with 4 zinc fingers which is likely to be a transcription factor. The gene is expressed during fetal development transiently in the condensed mesenchyme and certain epithelial cells of the nephron. In addition, WT1 is expressed in several other tissues which, like the kidney, experience a mesenchyme to epithelial transition. I will describe experiments which address the function of WT1 in the development of these different tissues. Candidate target genes regulated by WT1 will be discussed.

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WIEDEMANN-BECKWITH SYNDROME

Henry L., Junien C.
INSERM U73 Paris France

The Wiedemann-Beckwith syndrome (WBS) is a malformation syndrome associated with predisposition to different types of pediatric tumors such as Wilms'tumor (WT) and adrenocortical carcinoma (ADCC) for the most frequent but also rhabdomyosarcoma (RMS) and hepatoblastoma (HEP). The WBS segregates like an autosomal dominant disease with sex dependence penetrance and highly variable expressivity.
Cytogenetic and molecular experiments have demonstrated : 1) paternal duplications and maternal translocations involving region 11p15 ; 2) linkage with 11p15 markers ; 3) somatic mosaicism with uniparental paternal isodisomy of 11p15.5 ; 4) a loss of 11p15.5 maternal alleles in the pediatric tumors (WT,ADCC,RMS and HEP) ; 5) an increased risk (10% to 60%) of developping a tumor associated with a disomy of the same region.
These five points strongly suggest that the WBS maps to the 11p15 region and that imprinting may account for the expressivity of the syndrome and the occurence of the associated tumors.
Interstingly, two genes in 11p15.5, namely IGF2 and H19, have been shown to be maternally and paternally imprinted respectively. Recently, it has been demonstrated that this imprint may be relaxed in the tumors presenting or not a loss of the maternal allele. These two genes may account for the pattern of inheritance observed, the variable expressivity, the specific loss of alleles and the loss of imprint. However these genes map 400 kb away from a cluster of breakpoints observed in the cytogenetic cases of WBS suggesting that other genes could be involved.

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STUDIES ON RETINOBLASTOMA CELL DIFFERENTIATION USING A PHOTORECEPTOR SPECIFIC GENE

Albini, A. Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy.

Retinoblastoma (Rb) tumors occur in childhood in two principal forms: hereditary and sporadic. Rb tumors are probably derived from retinal "precursor cells", which cannot terminally differentiate due to inactivation of the Rb oncosuppressor gene. The interphotoreceptor retinoid binding protein (IRBP) is a protein specific for photoreceptor cells of the retina, being expressed only by these cells and some pinealocytes. We have cloned the complete human gene for IRBP (1). This gene is expressed in Rb cell lines (2), and immunohistochemical studies demonstrate that IRBP is expressed in Rb tumors. IRBP expression can be modulated in vitro by differentiating agents, and we have shown that laminin (3) and cAMP (4) can influence morphology and IRBP levels in Rb cells. Studies on the IRBP promoter have revealed the existence of retinal specific DNA-binding factors, which may be involved in the transcription of this gene and other retinal specific genes. We have developed a nude mouse model for transplantation of Rb cell lines (5) which yields tumors with Rb morphology and IRBP expression. This model can allow us to develop new Rb cell lines. Elucidation of molecular genetics of IRBP and the use of its promoter to generate retinoblastoma-like tumors can help in understanding the biology of this childhood tumor. *Financed by AIRC.* 1) Albini, A. et al. N.A.R., 18: 5181-5187, 1990 2) Inouye, L. et al. Exper. Eye Res., 49: 171-180, 1989 3) Albini, A. et al. PNAS, 89: 2257-2261,1992 4) Fassina, G. et al. Int. J. Oncol. 2: 745-751, 1993. 5) Albini, A. et al. Int. J. Cancer. 52: 234-240, 1992.

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GERM-LINE P53 MUTATIONS AND LI-FRAUMENI SYNDROME.

L.Brugières[1], B.Bressac[2], C.Bonaïti-Pellié[3], J.Lemerle[1] and J.Feunteun[4],
1) Département d'Oncologie Pédiatrique, Institut Gustave Roussy, Villejuif, France 2) Laboratoire de Diagnostic Moléculaire, Institut Gustave Roussy, Villejuif, France 3) Unité de Recherches d'Epidémiologie Génétique INSERM U155, Paris, France 4) Laboratoire d'Oncologie Moléculaire, Institut Gustave Roussy, Villejuif, France.

The occurrence of organ-specific malignancies affecting several relatives within a family is an indication of an underlying genetic susceptibility. The Li-Fraumeni syndrome (LFS) is a well documented example of such a condition in which affected relatives develop diverse malignancies including breast cancers, sarcomas, brain tumors, leukemias and adrenocortical carcinomas. Families affected by this syndrome display a proband diagnosed with sarcoma before the age of 45, a first degree relative with cancer before the age of 45 and another first or second degree relative with either a sarcoma diagnosed at any age or any cancer diagnosed under the age of 45. Germ-line mutations of the p53 gene have been reported in affected individuals belonging to LFS or other familial aggregations. In order to evaluate the contribution of a genetic predisposition in childhood cancer, we are reconstituting the family history of all the children treated for any malignant tumor in the Department of Pediatric Oncology at the Institut Gustave Roussy in Villejuif. This study is being conducted with three stepwise objectives: first, to identify LFS families and possibly reveal other types of familial cancer aggregations. Second, to identify the genetic events underlying the observed predisposition to cancer. Third, to evaluate the penetrance of these genetic events, which represents the risk for mutation carriers of developing cancer. Here, we report the results of screening for p53 germ-line mutations on a first set of sixty families selected less stringently than LFS in which, in addition to the proband, at least one second degree or less relative is affected by cancer before age 45. p53 exons 2 to 11 have been sequenced following PCR amplification performed on total blood extracted DNA.

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CLL IN THREE SISTERS: PREFERRED USAGE OF SPECIFIC IG GENE SEGMENTS.

Hakim I., Brok-Simoni F., Grossman Z., Amariglio N., Mor O., Gokel E., Kneller A., Hulu., Ramot B., Ben-Bassat I., Rechavi G. Institute of Hematology, The Chaim Sheba Medical Center, Tel Hashomer 52621, Israel

Familial Chronic Lymphocytic Leukemia (CLL), although rare, is the most common familial leukemia. The study of such families may increase our understanding of CLL in general. We studied a family where three sisters, two of them identical twins as proven by microsatellite analysis, developed CLL. Immunophenotyping of the leukemic cells revealed surface IgM,k in all three cases. Subgroup specific PCR demonstrated that the rearranged Ig heavy chain genes of the malignant cells of all three contained V_HIII gene segments. PCR cloning enabled the detailed analysis of both light and heavy chain gene sequences. The heavy chain gene of all three contained highly homologous V_HIII gene segments. The D segments of the twins were identical and differed from that of the third sister. One of the twins shared J_H4 with the non-twin sister while the second twin used a J_H3 segment. The malignant cells of all three had a rearranged V_KIII with either J_K1 or J_K3 which are almost identical. It can be speculated that on a specific genetic background, the proliferation of CD5+ cells trigered by common antigens encountered in early development (possibly in utero), created an expanded cell population at risk for the development of additional genetic changes resulting in malignant transformation. Familial CLL may therefore represent the combination of a suitable genetic milieu with environmental trigers. We searched for relevant mutations in the p53 gene, which has recently been implicated in some CLL cases, but so far none have been identified.

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TUMOUR PREVENTING GENES IN NEUROBLASTOMA

Weith, A.
Research Institute of Molecular Pathology (I.M.P.), Vienna, Austria

Consistent chromosomal deletions and/or translocations in tumour cells often reflect deletions of tumour preventing genetic elements. Our interest is focused on the distal region of the human chromosome 1 short arm, which is rearranged in a significant number of neuroblastomas. Analyses of allelic deletions using a panel of microcloned probes led to the identification of a consensus deletion at 1p36.2-p36.1. To more specifically approach this consensus deletion we perform both, physical linkage analysis by pulsed field gel electrophoresis (PFGE) and saturation cloning using YAC and P1 clones. - Upon the identification of CpG islands, conserved DNA sequences and exons we attempt to characterise the associated genes with respect to their possible involvement in tumorigenesis. Using this approach, we have cloned a putative "negative regulator" gene whose expression is both, specifically reduced in most tumours and inversely correlated with neuroblastoma-specific overexpression of the N-myc oncogene. Functional analyses will aim at defining a possible function of this gene in the transformation process.