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CONCEPTS OF MULTISTAGE EVOLUTION OF NEOPLASIA

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Concepts of multistage evolution of human cancer have mainly been inferred from histo- and cytopathological observations, the statistical analysis of epidemiological data, and the finding of a variety of genetic changes related to oncogenes and tumor suppressor genes. In laboratory animals, the induction of neoplasia of the skin, liver and other tissues by two- or three-stage protocols suggested a separation of carcinogenesis in the stages of initiation, promotion, and progression. However, an unequivical explanation of these operationally defined stages in biological terms remained elusive. The more recent discovery of characteristic sequential cellular changes during neoplastic development in different organs such as the liver or kidney has opened a new approach for the distinction of stages of neoplastic development by biological rather than operational criteria. In this context preneoplasia has been defined as altered cell populations that precede both benign and malignant neoplasia. As a rule, preneoplasia and benign neoplasia represent successive stages in a biological continuum leading from the normal state to malignant neoplasia. This concept will be exemplified using liver and renal cell tumors.

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TRANSCRIPTION FACTORS ACTIVATED BY CHROMOSOMAL TRANSLOCATIONS IN HUMAN CANCER

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The most common chromosomal abnormalities in leukaemia and lymphoma (and possibly also in the less well characterised group of non-haematological tumours or solid tumours) are chromosomal translocations or inversions. A number of different situations have been studied at the molecular level and the consequences of these abnormalities in tumour development assessed. Two general characteristics of these abnormalities are apparent; either one of the antigen receptor gene loci (immunoglobulin or T-cell receptor genes) is involved, resulting in activation of a gene on the joined chromosome, or breakage occurs within a gene on each of the involved chromosomes resulting in a fusion gene, and in turn a fusion protein. Examples of each of these types of situation will be discussed.

The *HOX11* gene, activated by translocation t(10;14)(q24;q11), and RBTN1 and RBTN2 genes, activated by t(11;14)(p15;q11) and t(11;14)(p13;q11) respectively, are examples of the former. The gene *HOX11* encodes a protein with a homeodomain and an N-terminal region with features of a transcriptional activation domain. A crucial DNA contacting residue in helix 3 of the HOX11 homeodomain is a threonine residue rather than the more usual valine or isoleucine. The role of this residue in DNA recognition is being studied and we have identified a family of related genes with a threonine at this position indicating the importance of this substitution. *RBTN1* and *RBTN2* encode cystein-rich proteins (LIM domain proteins) and transgenic mice with either *rbtn1* and *rbtn2* expressed in thymus cells develop acute lymphoblastic T cell lymphomas. The frequency is variable, with about 20-50% of transgenic animals developing disease, but many of the aspects of the disease are like the childhood ALL from which these genes were first identified. The nature of the protein products and normal expression characteristics of these genes suggests roles in gene regulation, and that abnormal expression after translocation may upset normal cellular transcription networks.

Gene fusions in acute leukaemia translocations of chromosome 11q23 and in t(12;16) of liposarcoma also involve transcription factors. The translocations create fusion proteins with potential chimaeric roles in the transcription machinery of the tumour cells. The nature of these fusion genes and proteins will be discussed in relation to possible mechanism of the tumorigenesis.

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MOLECULAR PATHOLOGY OF HUMAN NEUROBLASTOMA

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Human neuroblastoma cells very often carry amplified DNA derived from the chromosome 2p23-24 region and have lost genetic information from chromosome 1p36. Amplified DNA is usually arranged as head-to-tail tandem repeats 100 to more than 1,500 kilobase pairs long and encompasses the N-*myc* gene. N-*myc* is highly expressed consequent to amplification both on the level of RNA and on that of protein. The N-MYC proteins have relative molecular masses of 60 and 62 kilodaltons (kd), are located in the nucleus of the cell, and are phosphorylated by casein kinase II. The amino-terminus has activities in transcriptional regulation, whereas the carboxy-terminus can become associated with two phospho-polypeptides of molecular masses of 20 and 22 kd (Max 20/22).

Deletion of DNA is revealed in more than 70 percent of the tumors by cytogenetically visible loss of material from the short arm of chromosome 1. Upon closer cytogenetic examination, we mapped the smallest visible deletion of neuroblastomas to the distal region 1p36.1-1pter. For a more detailed analysis of this particular region we generated a regional DNA library by microdissection and microcloning, and the DNA probes were mapped both by *in situ* hybridization on normal chromosomes and by Southern blot ananlysis on a mouse x human somatic cell hybrid panel carrying different portions of human chromosome 1. A microclone (p1-56) located in 1p36.3 detected loss of DNA fragments in 9 of 10 neuroblastomas investigated. With microclones located more proximal than p1-56, we molecularly mapped the deletion breakpoints in 5 of the 10 tumors to band 1p36.1 or distal of it. Additionally we found bands 1p36.1-2 as the site of reciprocal translocation in two cases. The same region has also been found involved in constitutional alterations (translocations, deletions) in patients with neuroblastomas. Therefore, we suggest that a putative neuroblastoma suppressor is located at the distal portion of chromosome 1p.

Supported by Deutsche Forschungsgemeinschaft, Dr. Mildred Scheel Stiftung and NCRD (Israel)-DKFZ Cooperation Program.

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PATHOBIOLOGY OF PRENEOPLASIA AND INCIPIENT NEOPLASIA OF THE LIVER

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The multistep process of experimental hepatocarcinogenesis is well studied, however few relevant data are available to describe the pathobiological characteristics of human benign and malignant liver lesions and the possible link between them. Human focal liver lesions such as 17 focal nodular hyperplasias (FNH), 6 hepatocellular adenomas (HCA), and 17 hepatocellular carcinomas (HCC) were studied for detection of "marker" enzyme alterations, for the amount and composition of extracellular matrix components, for the expression of growth factors (TGF-α and -ß), hepatitis B virus (HBV) and tumor suppressor gen p53 by enzyme and immunohistochemical and biochemical methods.

The development of an increasing tendency for tumor heterogeneity was observed in HCC cases, resulting in the lack of marker enzyme alterations in the great majority of HCC samples. Altered glycosaminoglycan composition has been detected in HCC, FNH and HCC. While in the normal liver heparan sulfate, in FNH and HCA dermatan sulfate, in HCC, chondroitin sulfate was the dominating type of glycosaminoglycan. TGF-α expression was detected in the majority of HCC and FNH cases and only in 1 with HCA. Extracellular form of TGF-ß was detected in all FNHs, in no HCA cases and in 7 of 15 cases of HCC. HBV antigens were localized in 3 HCC cases associated with cirrhosis in the same cytoplasmic compartment as TGF-α by double immunohistochemical staining. Overexpression of p53 was demonstrated in 8 of 16 HCC and in none of the benign liver lesions.

Data suggest that the pathobiological characteristics of the studied human benign and malignant liver tumors are very different from each other, which might be because of different etiology or pathogenesis of these tumors.

The work has been partly supported by the National Science Foundation (1133) and Ministry of Welfare of Hungary

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MOLECULAR AND BIOLOGICAL CHARACTERIZATION OF ACUTE PROMYELOCYTIC LEUKAEMIA

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Acute promyelocytic leukaemia (AMP) is characterized by the 15; 17 chromosomal translocation. By cloning experiments we and others have established that the chromosome 17 breakpoints (bp) consistently occur within an approximately 16-Kb DNA fragment of the RAR-α intron 2. Chromosome 15 breakpoints cluster within three regions of the PML gene; intron 6 (breakpoint cluster region 1 or bcr 1), exon 6 (breakpoint cluster region 2 or bcr 2) and intron 3 (breakpoint cluster region 3 or bcr 3). Two fusion genes, PML/RAR-α on the $15q^+$ derivative and RAR-α/PML on the 17q- derivative are formed as a result of the translocation. PML/RAR-α genes generate fusion mRNAs which encode chimeric PML/RAR-α proteins. Nucleotide sequence of PML/RAR-α and PML cDNAs were obtained. Primers have been identified that allow the detection of the chimeric PML/RAR-α mRNA in all 35 APL patients analyzed by reverse PCR and nested primer approach of two rounds of amplification. The method represents the easiest and fastest way to identify the t(15;17) even in the cases where conventional cytogenetics failed to do it. Data will be presented on the clinical relevance to monitor the APL clone by the PCR technique. Moreover preliminary findings on the mechanisms of action of the PML/RAR-α fusion protein on the regulation of differentiation and survival of myeloid cells will be discussed.

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DEVELOPMENT OF COLON CANCER