Genetic Alterations in Carcinogenesis (C0700)

Head: Dr. Monica Hollstein

Scientists:
Dr. Walter Beerheide (05-12/00)
Dr. Manfred Hergenhahn
Dr. Jun-Li Luo (10/99-)
Dr. Hasan Seker (02/99-11/99)
Dr. Gisela Werle-Schneider (50 %) (-12/00)

Students:
Firouzeh Biramijamal (6/99-10/00)
Boris Zielinski

Technical assistants:
C. Kalla (-12/00)
Karl-Rudolf Muehlbauer
Ute Schmitt (50 %)
Annette Weninger

Over the past decade, a wealth of information on genetic and phenotypic differences between normal and tumour cells has accumulated. A major research objective of the Division of Genetic Alterations in Carcinogenesis, established in the year 2000, is to define pivotal changes characterizing a particular type of cancer, and determine how this knowledge can be used to improve early diagnosis, prognosis, treatment, and primary prevention. Elucidation of quantitative relationships in gene expression networks, and of post-transcriptional and posttranslational regulation of cellular macromolecules governing tissue homeostasis and cell growth will promote our understanding of the essential principles of normal growth and development and how they go awry during carcinogenesis. Precise information on disrupted pathways in tumors is a prerequisite for development of new therapeutic drugs targeting specific anomalies. In addition to characterizing the molecular alterations of potential use in the clinical setting, we are working to find new approaches for determining which and to what extent alterations are induced primarily by exposure to external agents, and on the other hand which are largely inevitable or spontaneous consequences of the carcinogenic process, or of inherited genetic variants responsible for cancer susceptibility syndromes.

1. Gene expression analysis in tumors, cell lines, and primary mammalian cells: implications for diagnosis and chemoprevention

M. Hergenhahn, H. Seker, W. Beerheide, G. Werle-Schneider
In collaborations with H-J Groene, DKFZ; AL Cheng, CH Hsu, Taiwan; in cooperation with BASFLyx AG, Heidelberg

A primary long-term goal of our group is to define the gene expression profiles and protein fingerprints associated with neoplastic progression of human cancers. In addition, we are exploring how these patterns are modulated by tumor promoters on the one hand, and chemopreventive substances on the other. With respect to Epstein-Barr Virus (EBV)-associated malignancies, this work encompasses investigation of molecular mechanisms involved in EBV reactivation. A new B-lymphoid cell line, Raji-DR-LUC carrying the luciferase reporter gene, was used to examine modulation of mRNA and protein synthesis of ZEBRA, a key regulator of viral reactivation, by the chemopreventive compound curcumin. Using commercial DNA microarrays, further experiments were conducted with this cell line to define cellular genes involved in EBV reactivation, gene expression changes induced by the tumor promoter TPA, and the inhibitory effects of curcumin on gene induction.

Within the framework of an external cooperation project to develop microarrays for toxicology, we have defined gene expression patterns in primary rat hepatocytes and rat liver slices following exposure to the barbiturate phenobarbital using membrane and glass chip microarrays.

2. Mutation profiling of tumor suppressor genes in human tumors

F. Biramijamal, Q. Yang
In cooperation with: A. Allameh, Tehran, Iran; A. Mandard, Caen, France; P. Hainaut, IARC, Lyon, France; H-J Groene, H. Wesch, DKFZ

Although overall more than half of all human cancers harbor a p53 mutation compromising gene function, there is great variability in the prevalence of mutation, and in the mutation spectrum in distinct sets of tumors [1]. The fluctuations, in frequency, timing and base change pattern are related to many factors, including the histopathology of the cancer, and risk factors characterizing the patient groups [2]. Specific signature mutations have been linked to specific carcinogens, suggesting a molecular epidemiology approach to investigation of cancers for which the causes have remained elusive. For example, while it is well-established that tobacco and alcohol are major causes of esophageal squamous cell carcinomas (ESCC) in the USA in Europe [3], neither is thought to be of major importance in the etiology of this cancer in Iran, a country that experiences one of the highest ESCC mortality rates in the World. In a recently completed study we showed...
that the prevalence of p53 mutations is over 60% in ESCC from Iran, and that the mutation profile differs significantly from ESCC in other parts of the World [4]. Studies in Tehran on the endogenous/environmental factors associated with the distinctive profile will be pursued.

Point mutational inactivation, gene silencing by methylation, and loss of genetic material have been the focus of research on the mechanisms of tumor suppressor gene inactivation in the development of human tumors. It is conceivable, however, that disruption of suppressor function occurs by additional mechanisms. Human DNA contains mobile elements that remain capable of transposition, which can lead to insertional mutagenesis at the site of integration. We developed a method to monitor retrotransposition in a human breast cancer cell line, and showed that even single copy genes are capable of spontaneous de novo retrotransposition [5]. This assay can be used for exploring endogenous and environmental factors that influence host cell-mediated retrotransposition of unbiased cellular sequences in human cells.

3. Development of humanized rodent models for pre-clinical testing of pharmaceuticals targeting human oncoproteins and mutant tumor suppressor proteins.

JL Luo, M. Hergenhahn, M. Hollstein

In cooperation with: ZQ Wang, IARC, Lyon, France

Although the p53 gene is highly conserved throughout evolution, there are differences between the DNA binding domain (DBD) of the murine p53 tumor suppressor gene and the DBD of the human p53 gene. The precise DNA and amino acid sequences influence mutation spectra and macromolecular structure. We designed and generated a human knock-in mouse (hupki) mouse by gene-targeting technology in which sequences encoding the DNA binding domain of the endogenous mouse p53 gene have been replaced by the homologous segment of the human p53 gene [6,7]. In a series of biochemical studies we showed that the chimeric human/mouse p53 gene is transcribed normally, and that in the hupki mouse, multiple functions of the wild-type protein are preserved [7]. Exposure of mice to UVB, and screening for induced p53 mutations within a population of largely unmutated epidermal cells have yielded the expected base change pattern typical of human skin cancers from sun-exposed individuals. Molecules designed to target the p53 tumor suppressor protein (either to restore DNA binding activity of the common human tumor missense mutations, or conversely to block wild-type protein function temporarily in order to reduce side effects of chemo- or radiation therapy) can be investigated with this strain.

Selected Publications (* = external co-author)