Division Cytogenetics (H0400)

Head: Prof. Dr. rer.nat. Manfred Schwab

Scientists:
Dr. Hanxiang An (01/98-12/02)
Dr. Patrizia Perri (09/97-05/99)
Dr. Christian Praml (10/92-03/01)
Dr. Larissa Savelyeva
Dr. Frank Westermann (01/00-12/01)
Dr. Jianbing Zhang (06/98-05/00)

Graduate students:
Detlev Bannasch (02/93-04/99)
Anja Bauer (11/95-09/99)
Stefan Brouwers (07/99-07/02)
Andreas Claas (02/94-04/01)
Kai-Oliver Henrich (12/99-11/02)
Min-Kyoung Kim (10/00-09/03)
Britta Mädge (08/96-06/01)
Isabel Matzner (12/97-08/01)
Andrea Pillmann (11/97-04/01)
Isabel Wittke (08/97-07/01)
Ruprecht Wiedemeyer (11/98-10/01)
Sabine Zitzmann (01/99-12/01)

Assistants:
Eva Koziolek (05/98-04/00)
Sonja Ortmann (05/99-05/01)
Young-Gyu Park (08/00-07/02)

Secretary:
Cornelia Kirchner

Genes, chromosomes and cancer.

Genomic lesions are considered to be a central players in the development of human cancers. The research topic of this Division is to identify the contribution that genetic alterations have to three types of human cancer: neuroblastoma, the most frequent extracranial, solid cancer in young children; colorectal cancer; and breast cancer with particular emphasis on defining modifying factors. In pursuit of analyzing the genome of the human cancer cell and of determining the functional consequences of genomic lesions we are employing a combination of cytogenetic, molecular genetic and protein-biochemical approaches.

Genomic Lesions in Neuroblastoma, Colon Cancer and Breast Cancer

M. Schwab

In cooperation with: Prof. Peter Schlag, Robert-Rössle Clinic, Berlin-Buch; Prof. Frank Berthold, Children’s Clinic University, Köln; Prof. Siegfried Schmeck, MDC, Berlin-Buch; Dr. Javed Khan, NCI, Bethesda, USA; Dr. Adi Kimchi, Weizmann Institute, Rehovot, Israel.

1. Neuroblastoma

Neuroblastoma develops from the peripheral nervous system and is the most frequent extracranial solid tumor in young children. Two genomic lesions have been found frequently associated with neuroblastoma: a) amplification of the MYCN oncogene; and b) alterations, mostly deletions and translocations, in chromosome 1p. Amplification is a parameter predicting poor patient outcome, and these patients are subjected to more intensive therapeutic regimens; and patients with stage 1-3 status lacking amplification do not benefit from chemotherapy.

A fascinating feature is the unusually high incidence of spontaneous regression. Up to 10% of the tumors, in spite of metastatic features, regress without any therapeutic interference. It has been suggested that this clinical outcome results from apoptosis. Our aim is to identify cell death relevant genes in neuroblastoma and to analyze if they are related to spontaneous regression.

In pursuit of this we have developed a cellular system in which we can study apoptosis. An important gene in neuroblastoma is the MYCN gene, which is frequently over-expressed, either consequent to amplification, as the result of gene duplication or by deregulation at the level of gene expression or of RNA/protein stabilization. We have introduced MYCN under the control of the bacterial tetracycline repressor into a human neuroblastoma cell line. Programmed cell death can be triggered in this in vitro system by inducing Mycn protein expression and additional treatment with the cytokine IFN-γ or with cytostatic drugs. Obviously the enhanced expression of the MYCN protein results in a sensitization of neuroblastoma cells for drug induced apoptosis. Why then are neuroblastomas expressing enhanced level of MYCN consequent to amplification not more sensitive to therapeutic drugs? The tumor cells appear to be in a state where apoptosis is dysfunctional.

To isolate genes related to drug-associated apoptosis in neuroblastoma cells both a global and a functional approach were initiated: In the global approach we have enriched differentially expressed, pro-apoptotic genes by suppression subtractive hybridization. A cDNA library was the basis for generating a microarray, to which RNA probes extracted at different time points after induction of apoptosis were hybridized. In parallel we are in the process to generate, by using microarrays of differential cDNA libraries, expression profiles of individual tumor samples, both from regressing and progressing tumors.
The functional “Technical Knock Out” (TKO) approach is based on the random inactivation of gene expression with antisense cDNA libraries, followed by selection of those cells that survive in the continuous presence of an apoptotic stimulus. DNA clones in cells of surviving colonies have been isolated and further characterized. The subtractive library of pro-apoptotic genes has been cloned into the episomal EBV-based pTKO vector, transfected into the MYCN expressing neuroblastoma cells and selected in the continuous presence of IFNγ. After 4 weeks of IFNγ selection we isolated 6 different cDNA clones from surviving colonies. Mapping by chromosomal fluorescence in situ hybridization (FISH) will inform us about the chromosomal position of apoptosis related genes. Their analysis in neuroblastomas should reveal if deletion might be a molecular basis for apoptosis dysfunction in tumors and if they are related to spontaneous regression.

2. Colorectal cancer

We were the first to demonstrate the significance of distal 1p damage for colorectal cancer, although the relevant region appears to be slightly different from that associated with neuroblastoma. This region is being subjected now to a vigorous analyses of its genomic structure. It is possible that this region contains a modifier gene contributing to the severity of tumor phenotype expression. Independent analyses by other groups had revealed the existence of a modifier gene, MOM1, determining the number of hereditary colon polyps in the mouse. We have mapped the candidate gene, secretary phospholipase A2, to 1p35-36 within the region frequently altered in human colorectal cancers. Further studies should show, if genetic information in this region is involved in modifying human colorectal tumor development. More recently we have cloned the gene Aflatoxin B1-Aldehyde Reductase from 1p35-36 and could also be an important determinant of tumor susceptibility.

3. Breast cancer modifier genes

Inheritance of one defective allele of the BRCA2 gene predisposes humans to familial breast cancer. Tumor cells usually have lost the other, normal allele, often by loss of heterozygosity as if BRCA2 acts as a tumor suppressor gene. Comparison between different families carrying the same BRCA2 mutation has shown that the risk for cancer can widely differ. Some families have high risk (in the order of 80-90%), others have low risk (in the order of 50%). Further, the same BRCA2 mutation can be associated with breast cancer in females in some families, and with breast cancer in males in others. And finally, some families develop other types of cancer while others exclusively show a breast cancer phenotype. Collectively this has suggested that the cancer phenotype, in spite of a strong germline transmitted genetic determinant, can be modified by other, genetic or environmental, factors.

In pursuit of defining genetic modifying factors we have systematically analyzed constitutional karyotypes of BRCA2 carriers and have originally identified conspicuous chromosome 9p alterations in a family in which four males (father and 3 sons) have breast cancer. Chromosomal fluorescence in situ hybridization (FISH) has allowed to map the intrachromosomal rearrangements to 9p23-24, distal to a “multiple tumor suppressor” locus (MTST1), that is frequently deleted in different types of human cancer, including breast cancer. High resolution FISH revealed that the rearrangement consists in a duplication of a roughly 3 Megabase long DNA region. This region is present in multiple copies in a male breast cancer cell line established from an unrelated patient. The three brothers, from which DNA is available, have a mutation in their BRCA2 genes, as expected from studies of other labs that had determined the role of BRCA2 mutation in male breast cancer. Thus, we have identified the first breast cancer family in which two independent genetic alterations, 9p23-24 duplication and BRCA2 mutation, are transmitted through the germ line. More recently we have identified three similar families, one from Philadelphia, one from Berlin, and the other from Iceland. It has been shown that BRCA2 mutations are associated with breast cancer in females as well as in males. Further, the same BRCA2 mutation has been found in the Icelandic population, in some families responsible for breast cancer in females and in others in males.

Future analyses should show, in co-operation with colleagues from Iceland, whether 9p23-24 harbours a modifier gene that targets breast cancer to males.

While this observation could point to the genomic position of modifying factors, another possibility is that the rearrangements signal the biological activity of mutant BRCA2 proteins. It is thought that the BRCA2 protein has a role in DNA repair and recombination. Mutant BRCA2 genes often encode truncated BRCA2 proteins apparently unable to assume a nuclear localization and a range of possibilities exists for how they might contribute to breast cancer. One of the current ideas is that deficient DNA repair results in the stepwise accrual of chromosomal changes with the consequence of the activation of oncogenes and inactivation of tumor suppressor genes. The genomic phenotype of gross chromosomal changes often is a hallmark of advanced cancers, however, this model so far has failed to explain the tissue specificity with which the basic cellular mechanism of DNA repair and recombination might lead to breast cancer development. It is possible that mutant BRCA2 is involved in the distal 9p-rearrangements, and this perspective will be analyzed in more detail.

Publications (* = external co-author)

Original papers and invited reviews


