Abstracts: Session II

links to other related online resources. The MTB database is designed to facilitate the selection of experimental models for cancer research, the evaluation of mouse genetic models of human disease, the review of patterns of mutations in specific cancers and the identification of genes that are commonly mutated across a spectrum of cancers. Recent enhancements to MTB include a redesigned Web interface for more powerful querying and improved views that simplify and better organize data presentations. A significantly expanded archive of histopathological images also is available. The MTB prototype database is accessible at http://tumor.informatics.jax.org. User support is available by e-mail to mgi-help@informatics.jax.org.

Kuzumaki, Noboru [32]

Epigenetic silencing of the tumor-suppressing gelsolin gene involves histone deacetylation of its promoter region in human urinary bladder cancer

Kazunori Haga[1,2], Hisakazu Fujita[1], Ataru Sazawa[1,2], Masato Takimoto[1], Nobuo Shinohara[2], Tomohiko Koyanagi[2] & Noboru Kuzumaki[1]

[1]Division of Cancer Gene Regulation, Institute for Genetic Medicine, Hokkaido University, Hokkaido, Japan
[2]Department of Urology, School of Medicine, Hokkaido University, Hokkaido, Japan

Alterations in histone acetylation seem to play a central role in the repression of several important genes, such as tumor suppressor genes. Previously we reported that gelsolin expression was reduced in human urinary bladder cancers and that ectopically expressed gelsolin acted as a tumor suppressor[1]. We have examined the effects of trichostatin A (TSA), a specific histone deacetylase inhibitor, to examine the histone acetylation status of the gelsolin promoter region in bladder cancers. We measured gelsolin protein expression by western blotting in bladder cancer cell lines treated with various doses of TSA. We assessed promoter activities of the gelsolin gene by luciferase assays in both nontreated and TSA-treated bladder cancer cell lines. The acetylation status of histones linked to the gelsolin promoter was checked using chromatin immunoprecipitation and a dot blot assay. Gelsolin protein production was much reduced in cancer cell lines. Both re-expression and the promoter activity of gelsolin induced by TSA were dose- and time-dependent. An antibody that reacts with acetylated histones H3 and H4 immunoprecipitated chromatin containing the gelsolin promoter in the TSA-treated bladder cancer cells, but not in nontreated cells. These results suggest that TSA activated the gelsolin gene promoter through histone acetylation. In bladder cancer, the repression of the gelsolin gene is related to the deacetylation of histones to its promoter region. Together with the reported epigenetic changes in histone acetylation in breast cancer, acetylation-mediated gene silencing could be a common mechanism of gelsolin downregulation in several cancers.

1. Haga, K. *et al. Cancer Res.* **55**, 3228 (1995).

Lacher, Markus [33]

Wnt-Frizzled signaling in cell proliferation and survival

Markus Lacher & Robert Friis

Department of Clinical Research, University of Berne, Berne, Switzerland

We have used the differential display method as a coincidence analysis technique to isolate genes expressed in different rat tissues undergoing physiological apoptosis, such as involuting mammary gland or prostate after castration. One isolate, *DDC-4,* encodes a secreted Frizzled-Related Protein of 39.7 kD in an open reading frame of 1,044 nucleotides. This protein family is thought to antagonize the signaling of Frizzled transmembrane receptors. Inappropriate expression of *DDC-4* in lactating mammary glands of transgenic mice induces apoptosis during late pregnancy and early lactation. *DDC-4* presumably acts by blocking a survival pathway. Classical Wnt-Frizzled signaling leads to the stabilization of cytosolic β-catenin and activation of Tcf/Lef transcription factors. Increased activity of this pathway owing to mutations in such genes as *tcf-4* or those for adenomatous polyposis coli or β-catenin results in the formation of tumors. In many colorectal carcinomas and tumor models Tcf/Lef-responsive genes are upregulated. Based on the hypothesis that the genes targeted by Tcf/Lef might be at risk for producing tumors when activated by a second, alternative signaling pathway, we analyzed their promoter regions for further, putative, tumor-associated *cis*-acting elements. The kind of analysis performed is based on the assumption that biologically relevant elements are statistically overrepresented compared with random elements. Since such elements might unravel transcription factor–binding sites active in transformed but not in normal cells, they are candidates for artificial cancer-specific promoters, for which there is an urgent need in several gene-therapy projects.

Larson, Garry P. [34]

Identification of disease gene variants based on gene–gene interactions

Garry P. Larson, Louis Geller & Theodore Krontiris

Beckman Research Institute, City of Hope National Medical Center, Duarte, California, USA

New methods are needed for the identification of pathogenic alleles of candidate genes that may increase cancer susceptibility. Such risk alleles are expected to be of low penetrance and may act alone or modify the effects of other genes. We have developed a method that enriches for pathogenetic disease variants contingent on gene–gene interactions. Candidate gene pairs are chosen based on previous evidence demonstrating genetic or biochemical interaction. Utilizing a cohort of sibling pairs affected with breast cancer, we tested this paradigm by identifying risk variants of cell cycle control gene *CDKN1A* by means of interactions with *TP53* and *BRCA1*. This approach, which we call disease association by locus stratification, first stratified affected pairs based on sharing of both alleles at a microsatellite marker linked to *CDKN1A*. The second stratification was based on microsatellite marker sharing at *TP53* or *BRCA1*. We identified subsets of affected pairs sharing both alleles at both loci as screening targets. Utilizing this approach, we were able to enrich for two noncoding disease haplotypes of *CDKN1A* by virtue of *BRCA1* interactions. We defined each haplotype by single-nucleotide polymorphisms at two positions potentially important in *CDKN1A* transcriptional activation by both p53 and BRCA1p. Our results indicated that an approach based on allele sharing and gene–gene interactions will be valuable not only in identifying risk alleles but also in elucidating their mechanism of action.