Title	Heterogenous expression of beta-catenin, p16, e-cadherin, and c-myc in multi-stage colorectal carcinogenesis detected by tissue microarray
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414

A conserved domain within Snm1 is required for the repair of DNA interstrand cross-links. *X. Li[1], L. Thrun[1], D. Bruun[1], S. Jones[2], R. Moses[1].* 1) Dept. of Molecular & Medical Genetics, Oregon Health & Science University, Portland, OR; 2) Dept. of Cell Biology, University of Massachusetts Medical Center, Worcester, MA.

DNA interstrand cross-links (ICLs) block transcription, replication and segregation of DNA. *SNM1* of yeast is required for normal ICL repair and homologs exist from yeast to human. The function of Snm1 is unknown. *SNM1* shows some homology to *Artemis*, acting in V(D)J recombination. Mutations in *Artemis* cause human radiosensitive severe combined immune deficiency (RS-SCID). In response to treatment with psoralen plus UVA radiation, *snm1* mutant yeast cells were similar to wild type cells in inducing chromosomal breaks as analyzed by pulsed-field gel electrophoresis (PFGE), but they were defective in the subsequent repair of such breaks, suggesting Snm1 is required for processing of ICL-induced chromosomal breaks. Double-stranded breaks (DSB) are thought to be an intermediate in repair of ICLs, but we found Snm1 is not required for the repair of mating type switch endonuclease HO-induced DSBs. These results suggest that ICL-induced DNA breaks (as repair intermediates) are structurally different from DSBs induced by HO, or that SNM1 is not involved in the DSB repair step. A recent report (Ma et al. Cell 108: 781-94) showed that both Snm1 and Artemis contain β-lactamase motifs and that Artemis possesses a single-strand exonuclease activity, possibly acting in processing intermediates in recombination. We find that a point mutation in motif-2 of the β-lactamase domain abrogates functional complementation for ICL repair in a *snm1* deletion mutant, indicating this motif is important for the action of Snm1. We have made SNM1$^{-/-}$ mice in which the gene was disrupted upstream of all of the lactamase motifs. Results show *snm1*$^{-/-}$ fibroblast cells are sensitive to the ICL agent CDDP. However, FACS studies with T-cell and B-cell-specific markers indicate no abnormality. Our results do not support a role for *SNM1* in immunocompetence, but do indicate the β-lactamase domain is required for function in ICL repair.

415

Heterogenous Expression of beta-Catenin, p16, E-cadherin, and C-myc in Multistage Colorectal Carcinogenesis Detected by Tissue Microarray. *D. Xie[1], W.F. Zeng[2], J.S.T. Sham[1], H.L. Lin[2], L.H. Che[2], Y. Fang[3], J.M. Wen[2], L. Hu[1], X.Y. Guan[1].* 1) Dept. of Clinical Oncology, The University of Hong Kong, Hong Kong, China; 2) Department of Pathology, Sun Yat-Sen University, Guangzhou, China; 3) Cancer Institute, Sun Yat-Sen University, Guangzhou, China.

Most colorectal carcinomas (CRCs) arise from adenomas through an archetypal pathogenic pathway, the adenoma-carcinoma-metastasis sequence. Aberrant expressions of β-catenin, p16, E-cadherin, and c-myc have played important roles in carcinogenesis of CRC, but their distribution pattern and associations in different pathological loci along CRCs tumorgenetic progress is not fully understood. In this study, a tissue microarray (TMA) containing 85 advanced CRCs in different Dukes stages was constructed. In all 85 cases, tissue specimens from normal mucosa, primary carcinomas in different layers of the bowel wall were included in the TMA. Tissue specimens from matched adenoma, lymph node metastases, and distant metastases were recruited from 22, 21, and 21 cases, respectively. Expression pattern of β-catenin, p16, E-cadherin, and c-myc in multistage colorectal carcinogenesis and progression was evaluated by immunohistochemistry. The results revealed 1) Nuclear overexpressions of β-catenin, p16, and c-myc was increased apparently from normal mucosa to adenoma, primary carcinoma, and lymph node metastatic tumor; 2) The frequencies of nuclear overexpression of β-catenin and p16 in lymph node metastases were significantly higher than that in distant metastases ($p<0.05$). This result implies that nuclear overexpression of β-catenin and/or p16 might be associated with CRCs local lymph node metastasis but probably not distant blood-bore metastasis; 3) A well correlation of nuclear expressions of β-catenin and c-myc either in primary carcinomas at subserosa layer or lymph node metastases was observed ($p<0.05$), suggesting overexpression of c-myc could be transcriptionally activated by nuclear β-catenin in both primary CRC and its nodal metastases.

416

Comparative Expression Profiling of the Mouse Cytochrome P450 Gene Family. *I. Stoilov[1], D. Choudhary[2], I. Jansson[2], J.B. Schenkman[2], M. Sarfarazi[1].* 1) Molecular Ophthalmic Genetics Laboratory, Surgical Research Center, University of Connecticut Health Center, Farmington, CT; 2) Department of Pharmacology, University of Connecticut Health Center, Farmington, CT.

Expression profiles for 48 mouse genes representing all known cytochrome P450 families were developed with the assistance of multiple tissue cDNA panel Mouse I (Clontech). This panel contains normalized cDNA samples from 4 embryonic stages (days 7, 11, 15 and 17) and 8 adult tissues: heart, brain, spleen, lung, liver, skeletal muscle, kidney and testis. Individual gene-specific PCR assays were uniformed with respect to their primer Tm, amplicon size, reaction conditions and number of cycles used. The amplification reactions were analyzed by agarose/EtBr gel electrophoresis. Low-mass DNA ladder (Invitrogen) was used for band sizing and as a quantitative reference. Gel images were digitized and quantitative densitometry analysis was performed with Scion Image software program. The level of expression of each gene was translated into ng of RT-PCR product. Mouse P450s were detected in the gastrulation phase and number of expressed genes steadily increased during somitogenesis and organogenesis phase of development. In adult tissues, the largest number of P450s was detected in liver (n=34) followed by kidney (n=33), lung (n=27) and testis (n=26). Heart had the fewest number (n=12) of P450s. Of special interest was the expression profile of P450 genes belonging to sub-family Cyp1 because the mutant forms of Cyp1b1 ortholog in human cause Primary Congenital Glaucoma. Both Cyp1a1 and Cyp1b1 showed a complex non-overlapping pattern of expression during embryonic development. Cyp1a1 was detected only at embryonic day 7 while Cyp1b1 was detected on days 11, 15 and 17. In adult tissues, the highest Cyp1a1 expression was in lung and liver. Interestingly, Cyp1b1 was not detected in liver but was present in all other tissues. On the contrary, Cyp1a2 was detected only in the adult liver. This data suggests that Cyp1a1 and Cyp1a2 are not able to compensate for Cyp1b1 deficiency during embryonic development. Supported by AHAF-National Glaucoma Research grant to I.S. and NIH (EY-11095) grant to M.S.

417

Loss of heterozygosity in premalignant lesions and invasive tumors of the breast: examination of 26 commonly deleted chromosomal regions. *R.E. Ellsworth[1], C.D. Shriver[2], D.L. Ellsworth[1], B. Deyarmin[1], V. Mittal[1], S. Lubert[1], R.I. Somiari[1].* 1) Windber Research Institute, Windber, PA; 2) Walter Reed Army Medical Center Washington, D.C.

Despite tremendous efforts towards the elucidation of the genetic events involved in breast cancer etiology, the majority of molecular dysfunctions associated with breast cancer are still unknown. The successful sequencing of the Human Genome has revealed that tumor suppressor genes (TSGs) are often found clustered together in small chromosomal regions. We have taken a high-throughput genotyping approach to examine 26 chromosomal regions, including the BRCA1, BRCA2 and TP53 gene regions, frequently deleted in breast cancer tumors to identify TSGs and TSG gene clusters involved in a variety of breast cancers. Approximately 100 tumor samples have been studied, including cases of atypical hyperplasia, in situ carcinoma and infiltrating carcinoma (stages 0 3). DNA was extracted from relatively homogenous cell populations using laser microdissection technology. First-pass genotyping on a 96- capillary electrophoresis sequencer equipped with an ultra-high throughput genotyping softwarc involved the use of two microsatellite primer pairs per chromosomal region for the development of a global LOH map. Fine mapping was then carried out on the subset of samples showing specific chromosomal loss using large, custom panels of microsatellites from each region. Of note, ~60% of cases of DCIS show a loss of heterozygosity on chromosome 8p22 with a commonly deleted region encompassing the deleted in liver cancer 1 (DLC1) gene. Development of this large map, representing 26 loci on 17 chromosomes, will greatly facilitate the identification of novel tumor suppressor genes and/or surrogate markers for breast cancer.

418

A common haplotype of CYP1B1 is associated with prostate cancer risk in Caucasians. *B. Chang[1], S.L. Zheng[1], S.D. Isaacs[2], K.E. Wiley[2], A.R. Turner[1], G.A. Hawkins[1], E.R. Bleecker[1], P.C. Walsh[2], D.A. Meyers[1], W.B. Isaacs[2], J. Xu[1].* 1) Center for Human Genomics, Wake Forest Univ Sch Med, Winston-Salem, NC; 2) Department of Urology, Johns Hopkins Medical Institutions, Baltimore, MD.

Cytochrome P450 1B1 has been hypothesized to play an important role in carcinogenesis because it catalyzes the conversion of 17-b-estradiol (E2) to the catechol estrogen metabolite 4-OH-E2, the most carcinogenic estrogen which induces DNA single-strand breaks. As recent epidemiological and animal studies suggest that estrogen metabolism is involved in prostate carcinogenesis, we hypothesize sequence variants of CYP1B1 affect estrogen metabolism and in turn affect prostate cancer risk. To test this hypothesis, we systematically genotyped 14 single nucleotide polymorphisms (SNPs) of CYP1B1 in 159 probands of hereditary prostate cancer (HPC) families, 245 sporadic prostate cancer cases, and 211 unaffected controls. These SNPs well cover the gene and include two SNPs in the promoter region (-1549G/A and -1001T/C), five nonsynonymous changes (R48G, A119S, V432L, D449E, and N453S), four in introns (IVS1-13T/C, IVS1-263A/G, IVS2+3015G/A, and IVS2+3653C/A), and three in the 3UTR (+5359T/A, +5639G/A, +7072A/T). Because the majority of study subjects are Caucasians, all the hypothesis tests were limited to Caucasians. Significantly different allele frequencies between sporadic cases and unaffected controls were observed for five consecutive SNPs in the promoter region, intron 1, and exon 2 ($P<0.05$). Due to strong linkage disequilibrium (LD) within these five SNPs, only two major haplotypes were observed (all other possible haplotypes were either not observed or observed in <1%). The haplotype of C-C-G-C-G of SNPs -1001T/C, IVS1-13T/C, IVS1-263A/G, R48G, and A119S were observed in 75% of sporadic cases, compared with 68% of controls ($P=0.038$). These results suggest that sequence variants in CYP1B1 may be associated with prostate cancer risk.

419

Isolation of expressed sequences from a genomic segment carrying senescence gene (SEN16) for breast tumor cells. *G.P. Kaur, R. Athwal.* Fels Cancer Research Inst, Temple Univ Sch Medicine, Philadelphia, PA.

Escape from replicative senescence has been postulated to be a prerequisite for progressive tumor growth. Cells cultured from many tumors exhibit immortal cell growth in culture while all normal human cell types that can be grown in culture undergo cellular senescence after a definite life span. Cellular senescence is expressed as dominant phenotype over immortal cell growth, which indicates that immortal phenotype arises due to recessive genetic mutations in the senescence pathways(s). More than ten human chromosomes have been identified to carry senescence genes by complementation of immortal phenotype of a variety of cultured tumor cells. However, cloning of senescence genes has been a challenging task so far. We have applied an approach that combines functional complementation with traditional positional cloning to isolate human genes that induce senescence in immortal tumor cell lines. Using this functional-positional approach, we have identified a cell senescence gene, SEN16, located at chromosome 16q24 to a 85 kb genomic segment (Reddy et al.1999, Oncogene 18:5100, Reddy et al. 2000, Oncogene19: 217). A high-resolution map of the candidate region was constructed with YAC and BAC clones. Functional testing of these clones identified a genomic segment of 85 kb that could induce replicative senescence in human and rat mammary tumor cells. In following experiments, we isolated expressed sequences from the complementing BAC clone by exon trapping. Twelve exons were identified and their sequence comparison with databases identified five partial cDNA clones. Two of these cDNAs have been characterized in detail with respect to their transcript size and expression in different tissues. Both of these cover 55 kb of the complementing genomic region. One of these cDNA is rearranged in two breast tumor cell lines, SKBR3 and T47D, thus promises to be a more likely candidate. This cDNA has been cloned into LACII inducible expression system. Cloned cDNA will be expressed in tumor cells and resulting phenotype studied.