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Genome-scale analysis of gene expression in T-cell lymphoma during malignant progression using a cDNA microarray

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The expression of approximately 10,000 genes was compared between two clonally-related T-cell lines derived at different stages of T-cell lymphoma involving skin using a cDNA microarray. For selected genes, RT-PCR and Northern blot were performed to validate the microarray results. Using a 5-fold difference in gene expression as significant, we found that 180 genes were differentially expressed. Compared with cells from the earlier stage lymphoma, 56 genes were upregulated while 124 genes were downregulated in cells from the advanced lymphoma. Among the differentially expressed genes, approximately 75% are genes with currently unknown function. Most of the other genes are involved in cell growth, differentiation and/or survival. The profile of gene expression correlated well with tumor progression. For example, the expression of the bleomycin hydrolase-related gene was increased in the advanced lymphoma suggesting the development of drug resistance. In contrast, the expression of genes coding for ataxia-telangiectasia and for inhibitor of GDP-dissociation proteins, was down-regulated. The former is involved in DNA damage repair and the latter is a negative regulator of the Rho signal transduction pathway. We conclude that the differences in gene expression between the two cell lines correlates with biological behavior of the T-cell lymphoma and may provide new insights into mechanisms of tumor progression. cDNA microarray analysis of gene expression may lead to improved diagnosis and therapy of T-cell lymphomas and other malignancies.

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DIFFERENTIAL GENE EXPRESSION IN MURINE MESOTHELIOMA CELL LINES. L. Xia, J. Pietruska, N. Messier, R. Smith, C. Vaslet and A. Kane. Brown University School of Medicine, Providence, RI 02912.

Heterozygous *p53*-deficient mice show increased susceptibility to development of malignant mesotheliomas induced by intraperitoneal injection of crocidolite asbestos fibers. 50% of these tumors lost the wild-type allele and showed reduced levels of apoptosis, genetic instability, and accelerated growth with lymphatic invasion. It is hypothesized that inactivation of *p53* alters expression of genes involved in growth and local invasion of malignant mesotheliomas. In order to identify genes that are differentially expressed in *p53*-deficient malignant mesotheliomas, expression microarrays were hybridized with ^{32}P-labeled cDNA prepared from normal and malignant murine mesothelial cell lines. Genes related to apoptosis and DNA repair were underexpressed, while genes related to oxidant stress and signaling pathways were overexpressed in a *p53*-deficient mesothelioma cell line. The role of these specific *p53*-regulated genes in the progression of malignant mesothelioma will be assessed in stable transfectants with inducible expression of the *p53* tumor suppressor gene. Supported by a grant from NIEHS-R01 ES03721.

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EPIGENETIC REGULATION OF TUMOR SUPPRESSOR GENE EXPRESSION IN A RAT LIVER EPITHELIAL TUMOR CELL LINE *IN VITRO*. S. Tepper, S.L. Ricketts, J.W. Grisham, and W.B. Coleman. Department of Pathology and Laboratory Medicine, University of North Carolina School of Medicine, Chapel Hill.

GN6TF is a tumor cell line derived from normal WB-F344 (WB) rat liver epithelial cells. WB cells express abundant levels of BRCA1, WT1, p27/KIP1, and p21/WAF1 tumor suppressor-like genes (TSGs). GN6TF does not express BRCA1 mRNA and displays significantly reduced expression of mRNAs for WT1, p27, and p21. These observations suggest that these TSGs are important in the neoplastic transformation of WB cells. We investigated the possibility that epigenetic mechanisms of gene silencing, including gene methylation and/or alteration of chromatin structure, affecting these TSGs are important in rat hepatocarcinogenesis. GN6TF cells were treated with $1x10^{-3}$M 5-aza-2'-deoxycytidine (5-aza-dC), $1.25x10^{-4}$mM trichostatin A (TSA), or 5-aza-dC + TSA for 7 days, to effect demethylation of CpG dinucleotides/islands and/or relaxation of chromatin structure. Treatment of GN6TF cells with 5-aza-dC altered cell morphology and reduced proliferation. Coordinate with the alterations of cell phenotype, 5-aza-dC treated GN6TF cells re-express BRCA1 at normal levels (to levels expressed by WB cells). The expression of WT1, p27, and p21 were enhanced in 5-aza-dC and 5-aza-dC + TSA treated cells. Treatment with TSA alone induced a modest increase in BRCA1 mRNA levels in GN6TF cells, while WT1 and p27 mRNA levels were unaffected. p21 mRNA levels increased to normal upon treatment with TSA alone, suggesting alteration of chromatin structure plays a significant role in the silencing of this gene. DNA methyltransferase (Mtase) is abundantly expressed in GN6TF cells, but is not expressed in normal WB cells. Expression of Mtase in the tumor cells may account for silencing of TSGs via methylation of their promoters. These observations suggest that coordinate reduction of BRCA1, WT1, p21, and p27 TSGs represents an important step in the neoplastic transformation of WB cells, and that Mtase functions in the epigenetic regulation these genes in rat liver epithelial cells. Support: NIH CA29323 and CA78434

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Lack of SHP-1 phosphatase expression in T-cell lymphomas results from hypermethylation of the SHP-1 promoter

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SHP-1 is an important negative regulator of signaling by several receptors including receptor for IL-2 (IL-2R. SHP-1 acts by dephosphorylating the receptors and receptor-associated kinases such as the IL-2R-associated Jak3 kinase. Lack of expression of the SHP-1 protein was recently identified in a few HTLV-I+ T-cell lines which display constitutive phosphorylation of the IL-2R Jak/STAT pathway. The extent and mechanism of this lack of SHP-1 protein expression remained unknown. We found that SHP-1 protein was not detectable also in 6 HTLV-I- T-cell lines derived from different types of T-cell lymphoma. Most T-cell lines tested (5/8), both HTLV-I+ and HTLV-I-, failed to express SHP-1 mRNA, even after stimulation. They did not, however, carry any mutations in the SHP-1 gene coding, splice junction and promoter regions. Importantly, DNA from CpG-island rich SHP-1 promoter region was resistant to digestion with 3 different methylation-sensitive restriction enzymes. This resistance was reversed by treatment of the cells with a demethylating agent, 5-deoxyazacytidine. The treatment led also to re-expression of SHP-1 mRNA and, less frequently, SHP-1 protein. The re-expression of SHP-1 protein was associated with dephosphorylation of the Jak3 kinase. These results indicate that lack of SHP-1 expression in malignant T cells is frequently due to transcriptional silencing which is secondary to hypermethylation of the SHP-1 gene promoter. Furthermore, they provide evidence that SHP-1 loss may play a role in the pathogenesis of T-cell lymphomas by permitting persistence of the cell activating signals generated by IL-2R and, possibly, other receptor complexes.

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TA1 AND AMINO ACID TRANSPORT RESPOND TO ARGININE AVAILABILITY IN RAT HEPATIC CELLS: LOSS OF RESPONSE IN TUMOR CELLS. W. Campbell, D.E. Sah, W.B. Coleman and N.L. Thompson. Medical Oncology, R.I. Hospital - Brown Univ., Providence, RI 02903 and Dept. Pathology, U.N.C., Chapel Hill, NC 27599

TA1 was cloned in our laboratory on the basis of its differential expression in rat hepatoma cells relative to normal adult liver. TA1 is identical to the light chain of the heterodimeric CD98 complex - a complex consisting of the cell activation antigen 4F2 heavy chain disulfide linked to light chain. CD98 has been ascribed numerous diverse functions in different systems/tissues while the associated light chain has been shown to mediate amino acid transport specificity. We have found TA1 RNA and protein levels elevated in numerous rat and human primary cancers and cell lines. We used a panel of well characterized rat hepatic cell lines differing in transformation and tumorigenicity to study the role of TA1 and 4F2 in hepatocarcinogenesis. We found that arginine, but not glutamine, availability could modulate TA1 RNA levels in an early and persistent response; 4F2 RNA levels did not respond to arginine availability; response to arginine availability was lost in transformed, tumorigenic cells; loss of response correlated with expression of the gamma-glutamyl transpeptidase oncofetal gene; amino acid transport activity corresponded to both TA1 RNA levels and transformation status of the cell. We are also examining the cellular localization and regulation of TA1 and 4F2 peptides in normal versus tumor cells and asking whether altered TA1 expression provides neoplastic cells with a growth or survival advantage particularly in conditions of limited amino acids, i.e. the tumor microenvironment.

324.6

ARYL RADICALS FROM ARENEDIAZONIUM IONS INDUCE AP-1 *IN VITRO* AND *IN VIVO*. Peter M. Gannett, Jeannine Powell, Jonathan Daft, Zianglin Shi, Jianping Ye, and Bela Toth. School of Pharmacy, West Virginia Univ., Morgantown, WV 26506, Pathology & Physiology Research Branch, NIOSH, Morgantown, WV 26505, and Eppley Inst. for Res. in Cancer, Ne. Med. Center, Omaha, NE 68198.

Agaricus bisporus, the mushroom of commerce, contains genotoxic arylhydrazines and arenediazonium ions. *In vitro*, in cellular systems, and *in vivo*, arylhydrazines which are metabolically converted to arenediazonium ions and aryl radicals cause DNA damage that correlates with arylhydrazine or arenediazonium ion genotoxicity. The biochemical processes which connect the arenediazonium ion and genotoxicity has not been elucidated. We show here that arenediazonium ions induce AP-1 (activator protein) in a dose-dependent manner in murine epidermal JB6 P$^+$ cells. Co-treatment with the antioxidant N-acetylcysteine (NAC) inhibits AP-1 induction and ESR spin-trapped aryl radicals. Acetylsalicylic acid (aspirin), however, does not induce AP-1 nor does it quench aryl radical formation. In transgenic mice, selective AP-1 induction was observed in the lung, a target tissue. Thus, activation of AP-1, caused by DNA damage and aryl radicals, may be involved in arylhydrazine genotoxicity. (Supported by NIH Grant CA 31611).