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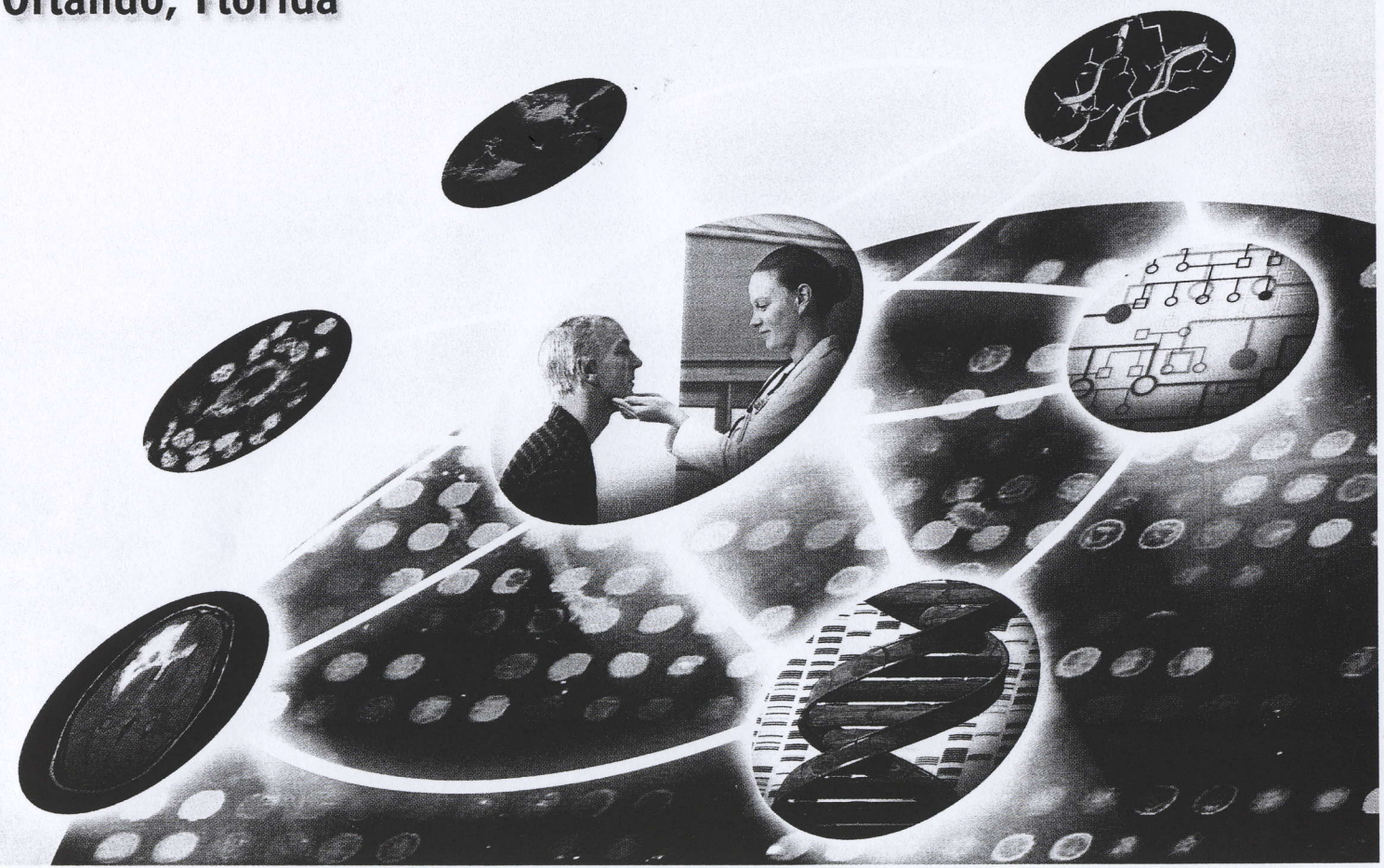

compared their expression. Of particular interest, there were 57 genes that were uniquely changed in the pre-leukemia to leukemia comparison. In addition, there were 31 genes altered in both PRE-LEUKEMIA vs. NORMAL and in PRE-LEUKEMIA vs. LEUKEMIA datasets as well as 60 common genes between LEUKEMIA vs. NORMAL and PRE-LEUKEMIA vs. LEUKEMIA datasets. Among discovered genes were revealed transcription factors (NKX6-1, GTF3A, ZFP95, SOX11, HOXA4 and more), translation factors (EIF3S6, EIF5A, EIF4A1 and more), differentiation factors (RQCD1, NEUROD2, CREBBP/EP300 inhibitory protein 1 and more), oncogenes (Ski, COUP-TF1 and more) and others. We are currently seeking to identify which of these changes may contribute to AML pathogenesis. Gene profiling using such a comparative approach for analysis will allow better understanding of leukemogenesis, and can be also applied for studies of tumor progression in other malignancies.

LB-161 Influence of Neodajuvant Therapy in Breast Cancer on Circulating Tumor Cells. Katharina Pachmann, Oumar Camara. University of Jena, Jena, Germany.

Circulating tumor cells (CTC) are detected at a high frequency (90%) in patients with breast cancer before therapy. If these cells can be monitored tightly, they might serve as an early indicator of therapy response. In order to study their response to therapy more closely CTC were analysed during neoadjuvant therapy and their respone compared to the final outcome as evaluated from the original tumor by the pathologist after surgery. During the first cycles of neoadjuvant therapy CTC responded with a less than 10 to 100 fold reduction. Subsequently, however, the number of circulating tumor cells reincreased accompanying the regression of the tumor. Therefore this increase was interpreted as dissemination of tumor cells from disintegrating tumor tissue into circulation. Depending on therapy schedules we observed a following redecrease in the numbers of CTC but only in rare cases below the threshold of detection. The degree of the initial response during the first 3 cycles of therapy correlated very well with the final outcome with a 100 fold or more decrease corresponding to a final complete remission of the tumor and less than tenfold decrease corresponding to only partial remission. Thus analysis of the response of circulating tumor cells may provide not only a valuable tool in early analysis of response to neoadjuvant therapy but also to monitor adjuvant therapy where no other measurable parameter is available.

LB-162 The candidate tumor suppressor protein ING4 regulates brain tumor growth and angiogenesis. Igor Garkavtsev, Sergey V. Kozin, Olga Chernova, Frank Winkler, Lei Xu, Edward Brown, Rakesh K. Jain. Massachusetts General Hospital, Boston, MA, Cleveland Clinic Foundation, Cleveland, OH.

Gliomas are the most common primary tumors of the central nervous system, with nearly 15,000 diagnosed annually in the United States and a lethality approaching 80% within the first year of glioblastoma diagnosis. The dramatic induction of angiogenesis in glioblastomas suggests that it is a necessary part of malignant progression. However, the precise molecular mechanism(s) for the regulation of brain tumor growth and angiogenesis remain unresolved. We report the identification of a novel candidate tumor suppressor gene ING4 that is involved in the regulation of brain tumor growth and angiogenesis. ING4 expression is significantly diminished in gliomas compared to normal human brain tissue, and the extent of reduction correlates with tumor progression from lower to higher grades of tumors. Xenografted in mice, human glioblastoma U87MG with decreased expression of ING4 grew significantly faster than control tumors and had higher vascular volume fractions. We show that ING4 physically interacts with p65 (RelA) subunit of NF-kB and that ING4 regulates brain tumor angiogenesis through transcriptional repression of NF-kB responsive genes. These results indicate an important role of ING4 in brain tumor pathogenesis.

LB-163 DEDD Regulates Distribution of Caspases to Intermediate Filaments during Apoptosis. Justine Lee, Gary Wang, Olaf Schickling, Marcus Peter. University of Chicago, Chicago, IL.

The coordination of cellular events during apoptosis critically depends upon regulated recruitment of caspases to downstream target substrates. DEDD, a highly conserved and ubiquitous death effector domain containing protein, constitutively exists in non, mono, and diubiquitinated forms. Unlike nonubiquitinated DEDD, mono and diubiquitinated DEDD exhibited increased levels during apoptosis and coimmunoprecipitated with keratin 18 (K18), an intermediate filament protein, and procaspase-3. Immunofluorescence and electron microscopy studies detected DEDD in filamentous structures and proteinaceous intracellular inclusions following apoptotic induction that colocalize with K18, active caspase-3 and -9, and ubiquitinated proteins. Overexpression and in vitro assays indicate that DEDD binds ubiquitinated proteins. In addition, siRNA mediated DEDD knockdown cells exhibited inhibition of staurosporine-induced DNA degradation suggesting that DEDD may serve as a novel scaffold protein in the intrinsic apoptosis pathway. Finally, we show that the ubiquitination of DEDD is regulated by the cellular inhibitor of apoptosis proteins 1 and 2 (cIAP-1/2). In addition, the cotransfection of DEDD with cIAP-1 or cIAP-2 results in the relocalization of the IAPs to the nucleoli.

LB-164 Increased lung metastasis incidence in transgenic mice lacking the nm23-M1/NDPK A gene and spontaneously developing hepatocellular carcinoma. Mathieu Boissan, Dominique Wendum, Sandrine Arnaud-Dabernat, Arnaud Bruneel, Annie Munier, Bruno Baudin, Jean-Yves Daniel, Marie-Lise Lacombe. Faculte de Medecine Saint Antoine, Paris, France, Anatomopathologie St-Antoine, Paris, France, Univ. Bordeaux 2, Bordeaux, France, Biochimie St-Antoine, Paris, France.

nm23-H1 and nm23-H2 genes encode A and B NDP kinases. Nm23-H1 was proposed as a metastasis suppressor gene based on 1) an inverse correlation between its expression and the metastatic potential of several types of human tumors including hepatocellular carcinoma (HCC) and 2) a decrease in the metastatic potential of highly aggressive cell lines after its enforced expression. Hepatic tumoral progression was studied in transgenic mice lacking this gene (KO mice) using two models. One was chemically induced by a single injection of diethylnitrosamine (DEN). The other was obtained by crossing the KO mice with ASV mice specifically expressing in the liver the SV 40 virus T antigen and spontaneously developing HCC (gift of Dr. P. Briand, ICGM, Paris).For the two models, the time course of appearance and the number and size of preneoplastic nodules and HCC were similar between the KO and control mice. The expression of nm23-H1 and nm23-H2 was increased in HCC as compared to normal liver. Only the ASV model developed pulmonary metastases which are positive for the Hep Par-1 hepatocyte marker. A highly significant almost two-fold increase in the incidence of pulmonary metastases (37,5 % versus 69,2 % of mice presenting metastases ; $p<0,001$) was observed in the ASV KO mice. Upon analysis of the expression of several markers of proliferation and tumor progression, we have observed a decreased expression of cyclin A in tumors of ASV KO mice. Differential proteomic analysis of the livers from KO and control mice showed a decreased level of several proteins which will be further identified. In conclusion, this work is the first demonstration of a role of NDPK A as a metastatic suppressor in an in vivo model of spontaneous carcinogenesis.

LB-165 A requirement for thioredoxin reductase in electrophile-mediated p53 inactivation. Pamela Cassidy, Kornelia Edes, Frank Fitzpatrick and Philip Moos, University of Utah, Salt Lake City, UT.

A requirement for thioredoxin reductase in electrophile-mediated p53 inactivation. Pamela Cassidy, Chronic inflammation leads to an increased, localized cancer risk at the site of inflammation. One proposed molecular mechanism for this phenomenon is the production of reactive oxygen species (ROS.) However this hypothesis needs to be modified in light of the recent results from vitamin antioxidant clinical trials wherein no preventative effects were observed. We have reported that wild-type p53 in cells treated with certain lipid mediators of inflammation, specifically electrophilic eicosanoids, is conformationally deranged, looses its DNA binding capacity and is therefore unable to activate transcription. The consequences include inhibition of p53-mediated apoptosis. This inhibition of p53 activity could contribute to the inflammation-associated increase in cancer risk. We determined that the mechanism by which electrophilic eicosanoids inactive p53 was indirect, through the impairment of the selenoprotein thioredoxin reductase (TrxR) [1]. Here we show that eicosanoids are not the only class of endogenous metabolites that have these activities; estrogen-derived quinone metabolites are inhibitors of TrxR as well. We have evaluated the inhibition of purified TrxR by estrogen quinones and electrophilic eicosinoids and have found both classes of compounds to be potent irreversible inhibitors of the enzyme. Therefore, the mechanism described for electrophilic eicosanoids could be also be involved in breast cancers where p53 is wild-type but is non-functional [2]. Paradoxically, when we reduce the levels of TrxR in cells using siRNA, p53 is less susceptible to conformational derangement. We show that the inactivation of p53 by endogenous electrophiles is dependent on the presence of TrxR, and is a result of the partial inactivation of TrxR by alkylation the C-terminal active site. Therefore, we propose a model wherein TrxR modified by endogenous (or exogenous) electrophiles, while unable to reduce its cognate substrate thioredoxin, can still be reduced by electrons