matrix metalloproteinase-2 (MMP-2), microvessels and with enhanced tumor cell apoptosis. Furthermore, 4 of 10 mice bearing well established HEY xenografts had no histological evidence of HEY tumors after treatment withdrawal. The antitumor, antiangiogenic, and apoptotic activities obtained with ABT 627 and the enhanced cytotoxic effect of paclitaxel provide a rationale for its clinical evaluation in ovarian carcinoma. Supported by AIRC, FIRC, CNR and Ministero della Salute.

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Ras and Rho GTPases: targets of FTase and GGTase I inhibitors for cancer treatment?

A. Cox[1,2], J. Fiordalisi[1,2], P. Joyce[1,2], A. Karnoub[2,3], A. Perou[2,3], P. Solski[2,3], C. Der[2,3]. [1]*Radiation Oncology,* [2]*Pharmacology,* [3]*Lineberger Comprehensive Cancer Center, Chapel Hill, University of North Carolina at Chapel Hill, USA*

Ras and Rho family proteins are modified posttranslationally by isoprenylation, which is critically required for correct localization and function. With the advent of inhibitors of prenyltransferases (farnesyltransferase inhibitors, FTIs, and geranylgeranyltransferase inhibitors, GGTIs), it was demonstrated that proteins normally modified by farnesylation could become alternatively prenylated by geranylgeranylation in the presence of FTIs. This has triggered renewed interest in understanding whether there are differential consequences of specific isoprenoid modification of these proteins. We have undertaken direct comparisons of the subcellular localization and biological activity of farnesylated and geranylgeranylated forms of Ras and Rho family proteins, using structural mutants with altered CAAX and hypervariable domains. Results to be discussed were generated by imaging living cells expressing GFP-tagged proteins, transcriptional transactivation assays of reporter genes, transformation assays for morphological transformation and anchorage-independent growth, and microarray analysis of genes whose transcription is altered by a change in isoprenoid modification on the Ras and Rho family proteins. In general, oncogenic forms of Ras (normally F) and RhoA (normally GG) are similarly functional regardless of lipid modification, whereas the function of R-Ras and Rac (both normally GG) is more isoprenoid-dependent. Finally, we also determined whether alternatively prenylated forms of these GTPases could protect cells from growth inhibition by FTIs and GGTIs. We find that farnesylated Rac and R-Ras, but not farnesylated RhoA, protect cells from growth inhibition by GGTIs and are therefore candidate targets for mediating the anti-tumor effects of GGTIs. Taken together, these studies further elucidate the antitumor mechanisms of FTIs and GGTIs.

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A strategy for identification of gene targets by integrating genome and transcriptome data in cancer

M. Wolf[1], S. Mousses[1], P. Kauraniemi[2], E. Hyman[1], S. Hautaniemi[3], P. Huusko[1], M. Ringner[1], A. Elkahloun[1], A. Kallioniemi[2], O. Kallioniemi[1]. [1]*National Human Genome Research Institute, NIH, Cancer Genetics Branch;* [2]*Institute of Medical Technology, University of Tampere, Laboratory of Cancer Genetics;* [3]*Institute of Signal Processing, Tampere University of Technology, Tampere, Finland*

Accumulation of genetic alterations is thought to underlie tumor development and progression. Genes that are altered as a result of somatic genetic alterations are attractive drug targets, since the growth and survival of cancer cells is likely to be dependent on these critical genes. Also, the absence or low-level expression of such genes in normal tissues provides a basis for specific tumor targeting, as indicated by recent clinical success of therapies directed against the BCR-ABL and Her-2 genes. Here, we developed a strategy for a genome-wide search for genes involved in genomic alterations in cancer. Cancer cell lines were first screened by comparative genomic hybridization (CGH) on a microarray with ~14,000 mapped cDNA clones. Expression levels of the corresponding genes were determined in a parallel experiment. A bioinformatic analysis was performed involving integration of genomic and transcriptomic data, and aligning and displaying the results using the 3.2 Bbp human genome sequence as a backbone. Our analyses of breast cancer revealed 270 genes that were highly overexpressed as a result of their location in regions of the genome undergoing amplification. These 270 genes included virtually all previously discovered oncogenes and amplification target genes in breast cancer, including HER-2, EGFR and MYC, and a large number of candidate novel oncogenes with a similar amplification and expression pattern as the known oncogenes. For example, a novel amplicon was found at 17q21.3, corresponding to a base pair position ~48.6-52.2 Mbp from 17pter. This amplification led to the overexpression of HOXB7 gene, a powerful embryonic regulator, whose amplification was subsequently validated in a set of 10.2% of 363 primary breast tumors. In summary, our analyses point to the substantial importance of genetic alterations as a mechanism for deregulating gene expression in breast cancer. Integration of transcriptomic and genomic copy number data is a powerful method for highlighting specific novel genes that may have potential for therapy development.

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Characteristics of novel non-hydroxamate inhibitors of histone deacetylases

K. Glaser, J. Li, L. Pease, M. Staver, P. Marcotte, J. Guo, D. Albert, P. Tapang, T. Magoc, M. Curtin, R.R. Frey, R.B. Garland, H.R. Frey, C.K. Wada, A. Vasudevan, M.R. Michaelides, S.K. Davidsen. *Abbott Laboratories, Cancer Research, Abbott Park, USA*

Gene expression is in part regulated by differential acetylation of nucleosomal histones resulting in either transcriptional activation (hyperacetylation) or repression (hypoacetylation). This phenomenon is tightly regulated by the balance of histone acetyltransferase (HAT) and histone deacetylase (HDAC) activities. HDAC inhibitors induce the hyperacetylation of nucleosomal histones in cells resulting in the expression of repressed genes that cause growth arrest, terminal differentiation and/or apoptosis in carcinoma cells. These pharmacological properties of HDAC inhibitors have generated significant interest in HDACs as targets for anti-cancer therapy. A-161906, a biaryl hydroxamate, was discovered as a TGFb mimetic and is an inhibitor of HDAC, IC_{50} = 9 nM. However, cellular activity in the 3-15 uM range and minimal efficacy *in vivo* necessitated synthetic efforts to increase cellular potency. The succinimide macrocyclic hydroxamate series maintained sufficient HDAC activity, IC_{50} values in the 30 nM range, and improved cellular activity into the submicromolar (i.e. 100-600 nM) range. This series is exemplified by A-173, with an HDAC IC_{50} value of 38 nM and antiproliferative IC_{50} values of 250 and 150 nM for HT1080 and MDA435 cells, respectively. These compounds demonstrated robust hyperacetylation of histones and induction of p21 and gelsolin in carcinoma cells. *In vivo* activity of this series was significantly improved from the original A-161906. The hydroxamate moiety was replaced by an electrophilic ketone. The a-ketoamides were more potent inhibitors of the HDAC enzymes with IC_{50} values in the 5 nM range and maintained cellular activity, IC_{50} values in the submicromolar range. This series is exemplified by A-378 with an IC_{50} value of 1.8 nM for nuclear HDACs and antiproliferative IC_{50} values of 170 and 120 nM for MDA435 and HT1080 cells, respectively. The a-ketoamides produced similar effect as compared to TSA in cellular models of hyperacetylation and induction of p21. This compound also demonstrated anti-tumor activity in the flank tumor growth model at 30 mg/kg, q.d.x2d, i.p. Synthetic efforts have led to the significant improvement in enzymatic, cellular and *in vivo* activity of Abbott HDAC inhibitors in both the hydroxamate and non-hydroxamate series. Compounds with nanomolar potency against nuclear HDACs and submicromolar activity in cell proliferation assays are described.

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Lung cancer molecular fingerprinting: expression profiling of gene and protein with cDNA microarray and MALDI-TOF MS

D. Carbone, K. Yanagisawa, N. Yamagata, Y. Shyr, P. Massion, S. Nadaf, J. Roberts, A. Gonzalez, J. Moore, R. Caprioli. *Vanderbilt-Ingram Cancer Center, Experimental Therapeutics, Nashville, USA*

To develop comprehensive molecular fingerprints of lung cancer, we applied both protein mass spec and cDNA printed array technologies to resected non-small cell lung tumors. For gene expression profiling, we initially analyzed 24 surgically resected human non-small cell lung cancers (NSCLC) and 7 other types of tumors by 5k cDNA microarrays. Using gene expression profiles from these samples, groups of genes were identified that were able to differentiate tumor from normal and lung metastases from lung primary tumors as well as the known histological subgroups of NSCLC. The class-prediction model based on the initial cohort successfully classified most of the samples from an independent test cohort of 14 samples. We then applied Matrix-Assisted Laser Desorption/Ionization-Time of flight Mass Spectrometry (MALDI-TOF MS) to the proteomic profiling of 80 surgically resected human lung tumor and normal lung tissues. Hundreds of protein signals were obtained from both tumor and normal tissues and groups of proteins were specifically expressed in one or the other. Hierarchical clustering analysis successfully distinguished tumor from normal and clustered tumors into related groups by histology. We have begun to apply this tech-