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.

331

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

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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.

332

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

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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.

333

Characteristics of novel non-hydroxamate inhibitors of histone deacetylases

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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.

334

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

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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-

nology to lung preneoplastic lesions.Thus both protein and gene expression profiling can identify molecular classes of non-small cell lung cancers. Profiles of proteins and genes obtained with MALDI-TOF MS and cDNA microarray analysis may lead to a better understanding of human lung cancer development and behavior. The identification of key differentially expressed proteins between individual tumors and between tumors and normal tissues may lead us to identifying novel targets for diagnostic markers and therapeutic intervention.

335

Paired-box containing transcription factors as targets for therapy in solid tumors, melanomas and brain tumors

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Paired-box containing transcription factors are crucial regulators of developmental processes. Interestingly, they also seem to play an important role in tumor development as implied by their involvement in specific chromosomal translocations. PAX3 and PAX7, mostly as chimaeric proteins resulting from the fusion with FKHR, a member of the forkhead family of transcription factors, are found in the pediatric tumor rhabdomyosarcoma (RMS). Expression of PAX5 is upregulated via a translocation in B cell lymphomas and PAX8 is involved as a fusion partner in thyroid carcinomas.Furthermore, screening studies done in our laboratory of additional cancer types by RT-PCR and in situ hybridisation revealed PAX3 expression in about 50% of brain tumors and in about 75% of melanomas. Previous experiments in our laboratory have shown that one of the possible oncogenic functions of PAX3/FKHR in RMS is protection from apoptosis. Surprisingly, a similar function was found for the native PAX3 in these tumor cells. Reporter-gene assays demonstrated that the anti-apoptotic function might be mediated through direct transcriptional stimulation of the bcl-xl gene. Additionally, modulation of both PAX and bcl-xl protein levels in tumor cells through antisense oligonucleotides showed that both proteins are functionally important for cell survival, suggesting that PAX3 and PAX3/FKHR might be important targets for therapy in a range of tumor types.To study the influence of PAX3 and PAX3/FKHR on RMS development in more detail, we are currently analysing gene expression of RMS cells and their normal parallel, myoblasts, by means of cDNA microarray technology. Transcriptome analysis may help classify subtypes of RMS on the basis of gene expression profiles, allowing for patient tailored treatment regimes.

336

Approaches to structure-based drug discovery for the HSP-90 family

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HSP90a, HSP90b, GRP94 and TRAP1 are a family of cellular chaperone proteins responsible for maturation of a variety of key client proteins involved in cell growth [1]. The last step in the refolding process is ATP-dependent dissociation of the client protein from HSP90 and other chaperones. HSP90s contain an unusually shaped ATP binding cleft that can be inhibited with some selectivity by ansamycin antibiotics such as geldanamycin. Recent trials with geldanamycin derivatives suggest that cancer cells are particularly sensitive to HSP90 inhibition, with the mis-folded client proteins inducing apoptosis and subsequent tumour cytostatis. These studies suggest the HSP90 family may be an appropriate target for anti-cancer drug development. [1] We have determined the crystal structure of members of this family of proteins using X-ray crystallography, in complex with known and novel inhibitors. These structures provide the starting point for series of structure-based drug design approaches. We are using our in-house virtual screening program, RiboDock® to search our large library of 4m compounds for novel ligands that may bind. In addition, we have developed a streamlined fragment-based approach combining NMR and crystallography to identify novel structural moieties that will bind to the ATP binding pocket. These structural studies lay the foundations for the design and development of improved compounds with appropriate drug-like properties as inhibitors.

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337

A protein containing the DHHC domain is upregulated in ovarian carcinomas

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Summary: Ovarian Cancer is still the gynecological tumor with the highest mortality. Early diagnosis and biological characterization of the tumors will be crucial to improve prognosis and individualize therapy of the disease. The aim of this study was to identify genes which are differentially expressed in ovarian carcinoma and encode proteins which could serve as marker proteins or targets for novel biological therapies. Amongst others found by differential display analysis, a cDNA encoding a protein with a conspicuous cysteine-rich motif was found to be overexpressed in ovarian tumors.

Methods: Cells were obtained by taking swabs from ovarian carcinomas as well as from normal epithelium of the distal inner part of the fallopian tube from the same patients, or from ovarian surface epithelium. Samples contained > 95% epithelial cells as judged by quantitative PCR with primers for cDNAs specific for hematopoietic and stroma cells. CDNA of cells microdissected from frozen tissue sections was used for quantitative PCR. Differential display was performed using a modification of Liang's and Pardee's method and a different set of primers. Differential gene expression was confirmed by PCR using gene-specific primers and quantitation in the Agilent Bioanalyser system.

Results: By differential display analysis, a 379 bp cDNA fragment was found to be 4-30 fold overexpressed in 4 out of 5 ovarian tumors analyzed (one borderline tumor and 4 serous carcinomas). Databank searches showed it to encode a member of the DHHC-family of proteins featuring a strictly conserved cysteine-rich domain. The overexpression was confirmed by quantitative PCR using cDNA from tumor as well as microdissected ovarian surface epithelium. Data bank searches revealed a protein from the DHHC family containing a strictly conserved cysteine rich domaine. These proteins are highly expressed in developing tissues in the later stages of organogenesis whereas expression in adult tissues is much more limited.

Conclusions: A cDNA found to be upregulated in ovarian tumors encodes a member of the highly conserved DHHC protein family. The degree of conservation indicates its important role in cellular functions. Its involvement in the pathogenesis of ovarian cancer and its possible use as a prognostic marker have to be further investigated.

338

Gene expression profiling of tumor cells with varying levels of stromal involvement: a novel *in vitro* model for studying tumor-stroma interactions

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The importance of stromal cells in tumor progression has been well-recognized but their role in the malignant process has not been fully explored. We have developed a novel, multicellular assay system that utilizes the three primary cell types involved in tumor growth and progression (endothelial, myofibroblasts and tumor cells). Fluorescently-labeled human microvascular endothelial cells and human fibroblasts expressing alpha-smooth muscle actin (myofibroblasts), are cultured in the presence of a tumor cell cylindroid embedded in a layer of Matrigel. After two days, the endothelial cells surround the tumor cell cluster, while the fibroblasts invade the cluster localizing in the center of the malignant cell mass. When endothelial cells are cultured in the presence of fibroblasts, they also invade the tumor cell cluster and co-localize with the fibroblasts. The extent of stromal infiltration into the tumor cell cylindroid depends on the tumor cell type. The breast carcinoma cell line, MDA-MB-231, and melanoma cell lines, MEL624 and A375, have significant stromal invasion, while prostate carcinoma cell lines, PC3 and DU145, fail to demonstrate significant stromal invasion. Serial analysis gene expression (SAGE) profiles of these cell lines identify genes that may be responsible for promoting stromal invasion. These observations support the hypothesis that the interaction between malignant cells, endothelial cells and myofibroblasts is essential in the process of tumor growth and progression. We have also identified potential therapeutic targets for interfering with malignant cell promotion of stromal involvement.