good activity and selectivity in preclinical models and has now progressed to Phase I clinical trial. During a pre-clinical study to identify pharmacodynamic markers of Hsp90 inhibition and to understand the consequences of Hsp90 inhibition we demonstrated altered gene expression profiles following treatment of colon carcinoma cells with 17AAG. In this study, we have compared mRNA and protein expression following treatment of an ovarian adenocarcinoma cell line with 17AAG using microarray or 2D proteomic analysis. To identify -on- versus off-target- effects of 17AAG, analysis was also performed following treatment with radicicol or an inactive analogue of 17AAG. 23 genes were increased and 12 genes were decreased by >2-fold following 17AAG treatment. Genes included Hsp70-8 and Hsp90-beta that we have previously demonstrated as 17AAG-responsive genes. 2D gel analysis identified 44 protein spots that were altered by 17AAG treatment. Peptide mass mapping by MALDI mass-spectroscopy identified a number of cell cycle regulators and heat shock-regulated genes. Proteins decreased by 17AAG did not exhibit a corresponding decrease in their mRNA. This suggested these proteins could be Hsp90 clients that were depleted following inhibition of Hsp90 by 17AAG. Proteins increased by 17AAG treatment also showed evidence of an increase at their mRNA level. These included components of the Hsp90 chaperone complex, such as Hsp70-8, Hsp90 and a novel Hsp90 co-chaperone required for Hsp90 function. The induction of Hsp70-2, Hsp70-8 and the novel co-chaperone were confirmed by western blotting. We also identified Hsp27-1 as a novel pharmacodynamic marker of Hsp90 inhibition, which was induced by 17AAG and radicicol, but not by the inactive analogue. In conclusion, gene expression profiling provides valuable, complimentary, information that can be used to identify changes induced by Hsp90 inhibition. This can be used to identify pharmacodynamic markers and genes involved in sensitivity and resistance to Hsp90 inhibitors.

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Cloning of tumor metastasis associated cDNA fragments (est)in human lung giant cell carcinoma

Y. Lu, *Pathobiology, Institute of Basic Medical Sciences, Beijing, China*

Identifications of genes involved in metastasis process will cast a new light on our knowledge of molecular regulation mechanism in human cancer. 'Phenotype Cloning' is undoubtedly a very effective way to identify and clone the underlying genes associated closely with the external biological or pathological presentations, while without the detailed background of their localization in the genome and sequence information. Based on this general idea, recent years have seen the new technique - Suppression Subtractive Hybridization (SSH), which is aimed to profile the gene expression changes, or more precisely to find the different expressed genes in tumor metastasis. The emerging technology of cDNA microarry hybridization offers the possibility of providing a rapid, large scale, high throughput method to screen a cDNA library for differential expression in parallel, sensitive, objective way. So combination of SSH and cDNA microarry techniques will improve the screening efficiency and enrich experimental data.
Objective: To clone the metastasis associate genes related to human lung-giant-cell carcinoma cells.
Methods: We applied SSH combined with cDNA microarray technique to identify candidate genes that are expressed differently in two human lung giant cell carcinoma cell strains (PLA-801C / PLA-801D) originated from the same tumor tissue with only metastatic potential difference. In SSH, cells with high metastatic potential (PLA-801D) were treated as driver or tester, while the cells with low metastatic potential (PLA-801C) as tester or driver respectively. The sequence obtained from SSH were further analysed by cDNA microarray.
Results: First of all, we cloned 101 sequences that were expressed two times more in PLA-801D than in PLA-801C in the microarray assay. After sequencing, many were found to be identity with known genes encoding protein of below: (1) cell factors and receptors (2) kinase and related protein (3) hypothetical proteins (4) other proteins including enzymes, heat shock protein, receptor, proteins of cell skeleton, production of oncogenes etc. Besides, there were fifteen sequences only have homology with chromosome sequence, they have ORFs which can encode polypeptides with amino acid residues ranged from 60 to 300 after elongation by the method of electronic-extension, and may be novel genes related to tumor metastasis. Second, we acquired 36 clones were up-regulated expression in low metastatic potential cell strain-PLA-801C, which may be associated with tumor suppression metastasis. After sequencing, electronic PCR extension and homologous analysis, these clones were identified as tomoregulin, gamma-actin, cytochrome C oxidase, and Tl227H, af112208 that were newly discovered genes assumed to be associated with metastasis and differentiation.
Conclusion: A great number genes were involved in the process of tumor metastasis. Many of them may play important role in normal physiological process, such as cell division, signal transmit, cell mobility etc. in this research, we noted that some known genes may have impact on tumor metastasis if expression levels or pattern of those genes were remarkably changed. More *in vitro* and *in vivo* experiments will be taken to evaluate their roles as well as expression regulating mechanisms in tumor metastasis.

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Signal therapy of RAS cancers by blocking PAK pathways

M. Hiroshi[1], H. Maruta[1], H. He[1], Y. Hirokawa[1], T. Nheu[1], A. Levitzki[2], J. Wood[3]. *[1]Ludwig Institute for Cancer Research, Melbourne Branch, Melbourne, Australia; [2]Hebrew University, Biological Chemistry, Jerusalem, Israel; [3]Yale University, Chemistry, New Haven, USA*

Oncogenic mutations of RAS contribute to the development of more than 30% of all human cancers, notably more than 90% of panceatic cancers and 50% of colon cancers. We found that a RAC/CDC42-dependent Ser/Thr kinase called PAK1 is essential for RAS-induced malignant transformation. Furthermore, using specific inhibitors, we found that both RAS-induced activation of PAK1 and transformation require several proteins such as PIX, ErbB1, ErbB2, a Src family kinase, and ETK, in addition to the GTPases Rac/CDC42. In particular the combination of PP1 (a potent inhibitor specific for Src family kinases, IC_{50}: around 10 nM) and AG 879 (a potent inhibitor specific for ETK, IC_{50}: 5 nM) almost completely suppresses the growth of RAS-induced sarcomas in nude mice. However, these inhibitors do not inhibit PAK1 directly *in vitro*. We have recently developed the first chemical compound, CEP-1347 (3,9-bis methylthioethyl derivative of the ATP antagonist K252a) that directly inhibits PAK1 in a highly selective manner and suppresses the growth of RAS transformants *in vitro* (IC50: around 1 uM). To develop a much more potent PAK1-specific inhibitor, we are currently synthesizing a new indolocarbazole compound, 3-OH 9-methylthioethyl derivative of staurosporine (ST), by modifying the position 9 of a marine compound called ST-2001 (3-OH ST) whose anti-PAK1 activity is a thousand times stronger than CEP-1347 or K252a. For the methylthioethyl chain at position 9 dramatically reduces the inhibitory activity of indolocarbazoles (non-specific kinase inhibitors) such as ST and K252a against most kinases except for PAK family kinases, while the hydroxyl chain at position 3 selectively potentiates their anti-PAK activity.

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Hypermethylation of RASSF1A CpG promoter region and deletion aberrations in 3p21.31 LUCA region in major epithelial tumors

W. Loginov[1], A. Malyukova[1], V. Sentchenko[2], I. Bazov[1], N. Petrash[1], T. Kazubskaya[3], V. Ermilova[3], V. Kashuba[4], E. Zabarovsky[5], E. Braga[1]. *[1]Russian State Genetics Center, Cancer Gene Group, Moscow, Russian Federation; [2]Karolinska Institute, Center for Genomic Research, Stockholm, Sweden; [3]Blokhin Cancer Reserch Center, Oncogenetics, Moscow, Russian Federation; [4]Karolinska Institute, Microbiology and Tumor Biology Center, Center for Genomic Research, Stockholm, Sweden; [5]Blokhin Cancer Research Center, Pathomorphology, Moscow, Russian Federation*

Chromosome 3p is involved in numerous epithelial tumors and it includes critical regions containing cancer specific and multiple tumor suppressor genes (TSGs). Inside of lung cancer candidate gene (LUCA) region marked by homozygous deletions in 3p21.31 ten candidate genes were suggested. Transcription inactivation of LUCA candidate TSG RASSF1A correlated with hypermethylation of CpG promoter islands as was shown recently for lung, breast and renal carcinomas. Here we analyzed methylation status of RASSF1A promoter region in breast, renal and ovarian cancer samples and compared frequency of this inactivating event with frequency of deletion aberrations inside of 3p21.31 LUCA region detected by allelotyping and TaqMan real-time PCR (PE-Applied Biosystems). Up to four polymorphic microsatellite markers (D3S1767, D3S2409, D3S2456 and D3S3667), surrounding RASSF1 gene and located inside of 0.5-1.0 Mbp DNA segment, were used in allelotyping. NL3-001/D3S3874 NotI-linking clone, localized 0.35 Mbp telomeric to RASSF1 gene, was used for design of TaqMan probe and primers. RASSF1A methylation status was analysed using bisulfate treatment of tumor/norma DNA samples followed by methylation specific PCR (MSP) and sequencing of selected samples. As we found, frequencies of RASSF1A promoter region methylation constituted 90%(18/20) for renal (RCC), 70%(14/20) for ovarian (OC) and 57%(12/21) for breast (BC) primary carcinomas. These values were higher than the sum of all deletion aberrations for RCC (constituted 65%(13/20) according to real-time PCR and 70% (26/37) according to allelotyping) and for OC (constituted 61%

(27/44) according to allelotyping). In case of BC RASSF1A promoter region methylation was observed with the same frequency as allele alterations (57%, 12/21) according to LOH, but the sum of all aberrations in NL3-001 locus determined by TaqMan real-time PCR was even higher, constituted up to 81% (17/21). Althow duplication and multiplication (25% for BC and even more for RCC) contributed in these values considerably. Among inactivating events homozygous deletions (HD) also contributed a large portion, equal to 19%(4/21) for BC and 20%(4/20) for RCC. Due to the highest frequency, methylation of RASSF1A CpG promoter region can be considered as an event of earliest carcinogenesis. This TSG appeared to be useful for oncology patient treatment using genotherapy approach.

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Implications of N-terminal truncated p73 for cancer

B.M. Putzer, T. Stiewe. *Institute of Molecular Biology, Center for Cancer Research and Cancer Therapy, Essen, Germany*

Most human cancers harbour aberrations of cell cycle control in the RB/p16-pathway resulting in deregulated E2F activity, cell cycle progression or apoptosis. Apoptosis by E2F1 has recently been linked to activation of p73 which displays significant structural and functional homology to the tumor suppressor p53. However, instead of mutational inactivation, overexpression of wild-type p73 has been reported in various tumor types compared to normal tissues arguing against a classical tumor suppressor function. Recently, N-terminally truncated, transactivation-deficient p73-isoforms (deltaTA-p73) have been identified as a second class of p73 proteins. Since overexpression of p73 in tumors includes deltaTA-p73, we further characterized these novel p73-isoforms. We show that deltaTA-p73 retains DNA-binding competence but lacks transactivation functions resulting in an inability to induce growth arrest and apoptosis. Importantly, deltaTA-p73 acts as a dominant-negative inhibitor of p53 and full-length p73 (TA-p73). Inhibition of p53 involves competition for DNA-binding, whereas TA-p73 can be inhibited by direct protein-protein interaction. Moreover, we show that deltaTA-p73 overexpression results in malignant transformation of NIH3T3 fibroblasts and tumor growth in nude mice, thereby providing evidence for an oncogenic function of deltaTA-p73. Thus, in analogy to cancer associated conversion of the cellular tumor suppressor gene p53 into oncogenic p53 mutants, increased expression of N-terminally truncated p73 isoforms apparently conveys the TP73 gene with oncogenic activity that appears to be actively selected for during tumor development. Thus, the newly identified p73-Isoforms represent attractive targets for molecular anticancer therapy. This work was supported by the Deutsche Krebshilfe.

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Hypoxia hypersensitizes the slow-growing cells of solid tumors to glycolytic inhibitors

T.J. Lampidis[1], H.P. Liu[1], N. Savaraj[1], W.P. Priebe[2]. *[1]University of Miami, School of Medicine, Cell Biology & Anatomy; [2]UT M.D. Anderson Cancer Center, Bioimmunotherapy, Houston, USA*

Since current cancer chemotherapy selectively kills rapidly dividing vs slow or non-dividing cells, the slow-growing cells of solid tumors represent a particularly difficult form of multidrug resistance to overcome. However, due to the hypoxic conditions which many of these slow growing populations of tumor cells are under, a window of selectivity opens for treatment with glycolytic inhibitors. We demonstrate in three distinct *in vitro* models of "hypoxia" (A, B and C) that tumor cells are hypersensitive to inhibitors of different steps of the glycolytic pathway i.e. 2-deoxy-D-glucose and oxamate. Model A are osteosarcoma cells (143B) treated with agents which interfere with mitochondrial oxidative phosphorylation; Model B are Rho 0 cells, a variant derived from 143B cells, which due to their deficiency in mitochondrial DNA cannot perform oxidative phosphorylation and Model C are 143B cells grown under varying levels of reduced external oxygen i.e. 10, 5, 1, 0.5, 0.1 & 0%. In all three models increased levels of lactic acid correlates with hypersensitivity to inhibitors of glycolysis. Overall, the data indicate that as a consequence of "hypoxia", cells switch from aerobic to anaerobic metabolism and by relying on glycolysis as a sole source of ATP synthesis, become hypersensitive to glycolytic inhibitors. Thus, the *in vitro* data suggest that addition of inhibitors of glycolysis to conventional chemotherapeutic protocols should increase treatment efficacy by targeting the slow-growing cells found in most, if not all, solid tumors.

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Protein phosphatase 2A, a novel and unexplored anticancer target

J.A. Sakoff[1], S.P. Ackland[1], M.B. Garg[1], C.C. Walkom[2], A. McCluskey[2]. *[1]Newcastle Mater Misericordiae Hospital, Department of Medical Oncology, Newcastle; [2]The University of Newcastle, Discipline of Chemistry, Newcastle, United Kingdom*

Protein phosphorylation is a major control mechanism intricately coordinated by kinases and phosphatases. While various anticancer drugs target kinases the potential of targeting protein phosphatases (PP) has largely been ignored. Nonetheless, the serine/threonine protein phosphatases 1 and 2A (PP1 & PP2A) play key roles in the cell cycle and apoptosis, and are a valid anticancer target. PP1 and PP2A are known as negative regulators of the cell cycle as they inactivate cdk's and stimulate pRb binding to E2F. Non replicating cells such as neuronal tissue have high PP activity. However, PP's are also crucial for successful mitosis via MAP kinase, histone, condensin, lamin, and vimentin dephosphorylation. PP2A also controls bcl-2 phosphorylation. Inhibition of PP's is counter-intuitive for the treatment of cancer, however, PP inhibition is lethal via aberrant cell cycle movement and mitotic failure. We propose that rapidly dividing cancer cells will be more susceptible to PP inhibition than non-dividing cells. Our lead compound in the development of PP inhibitors is cantharidin (2,3-dimethyl-7-oxobicyclo[2.2.1]heptane-2,3-dicarboxylic-acid-anhydride), a toxin found in blister beetles. It is a potent inhibitor of PP1 (IC_{50}=0.56μM) and PP2A (IC_{50}=0.51μM), small molecule, membrane permeable, not a substrate for p-glycoprotein, stimulates haemopoiesis, and amenable to analogue development. We have shown cantharidin to be cytotoxic in cancer cell lines (HT29, G401, H460, WiDr, A2780, SW480, HCT116, L1210) producing GI50 values of 3.6-16μM; to induce a transient acceleration of cells within in 4h from G1 into S-phase involving a 4-fold increase in 3H-thymidine uptake culminating in G2+M cell cycle arrest within 24h and subsequent apoptotic cell death; and to induce synergistic cytotoxic interactions with the thymidylate synthase inhibitor, Thymitaq. Recently we have shown that PP2A is a better target for drug development than PP1, as the cytotoxicity of cantharidin in cell lines correlated (r=0.87, P<0.01) with PP2A content but not PP1. We have also synthesised two cantharidin analogues that selectively target PP1 (IC_{50}=12.5, 50μM) versus PP2A (IC_{50}=426, >2000μM) which induce no cytotoxicity. Binding and docking studies have led us to synthesise cantharimides which show equipotent PP inhibition and cytotoxicity to cantharidin but which bind to unique grooves in the active site providing the foundation for the development of more selective PP2A inhibitors.

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An integrated approach to the pharmacogenomics and pharmacoproteomics of cancer

J.N. Weinstein[1], W.C. Reinhold[1], K. Bussey[1], S. Nishizuka[1], D. Kane[2], J. Gray[3], E. Petricoin[4], L. Liotta[1], I. Kirsch[1], K. Buetow[1]. *[1]U.S. National Cancer Institute, Bethesda, USA; [2]SRA International, Fairfax, USA; [3]Univ. of Calif. S.F., San Francisco, USA; [4]Food and Drug Administration, Bethesda, USA*

Application of mRNA and protein expression profiling to cancer drug discovery has not proved as straightforward as many expected, in part because clinical tumors are heterogeneous, in part because cancer patients have complex, fragmentary treatment histories. In contrast, the 60 cell lines (the NCI-60) [1-3] used in the NCI drug discovery program have been treated with >70,000 compounds one at a time and independently over a 12-year period. Cell lines in culture do not fully reflect cells *in vivo*, but, historically, most of our knowledge of molecular pharmacology and targets has come from cultured cells, not clinical material. We and our colleagues assessed expression patterns in the NCI-60 using 2-D protein gel electrophoresis [3], high density "reverse-phase" protein arrays, cDNA microarrays [4,5], and oligonucleotide chips [6]. To find patterns in the data, we then developed new data visualizations, including the familiar Clustered Image Map [7], and a tool (MedMiner) that streamlines literature searches on genes and drugs [8]. We and our collaborators next characterized the cells at the DNA level by comparative genomic hybridization (CGH), spectral karyotyping, array-CGH, and SNP chip and then developed algorithms and a program package called LeadScope/LeadMiner [9]. This package makes it possible to predict which molecular substructures will be found in drugs that are active against cells expressing large amounts of a selected gene – and vice versa. Finally, we have also developed the program package GEEVS (GEnome Exploration and Visualization System) to integrate all of the disparate types of data at the DNA, RNA, protein, functional, and pharmacological levels.