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

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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

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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

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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%