300

Tumor suppressor genes in chromosome 3p21.31

A. Malyukova[1], G. Kilosanidze[2], V. Loginov[1], I. Bazov[2], V. Kashuba[2], T. Kazubskaya[3], R. Garkavtseva[3], V. Ermilova[3], E. Zaborovsky[4], E. Braga[1]. [1]*Russian State Genetics Center, Cancer gene group, Moscow, Russian Federation;* [2]*Karolinska Institute, Microbiology and Tumor Biology Center, Center for Genomic Reseach, Stockholm, Sweden;* [3]*Blokhin Cancer Research Center, Oncogenetics, Moscow, Russian Federation;* [4]*Blokhin Cancer Research Center, Pathomorphology, Moscow, Russian Federation*

Genetic alterations in the short arm of chromosome 3 in different epithelial tumors suggested that 3p contains several tumor suppressor genes (TSGs). It was established that 3p21.3 LUCA region (lung cancer TSG region, 600kb) is one of the most frequently rearranged site of 3p and intrinsically involved in development of major forms of human carcinomas (Lerman et al., 2000). In this region twenty candidate tumor suppressor genes were identified. Epigenetic methylation and inactivation of RASSF1A promoter was shown with high frequency in lung, ovary and renal cancer. For another candidate TSG SEMA 3B decrease or loss of expression was found. It was suggested, SEMA3B action in tumorigenesis involve inhibition of tumor angiogenesis through interference with VEGF function (Keith et al., 2000;Tomizawa et al., 2001). Here we analyzed methylation status of SEMA3B CpG-sites in promoter region by sodium bisulfite treatment with followed sequencing. For lung cell lines and primary tumors with silencing SEMA3B Hypermethylation of promoter region was shown. Detailed 3p allelotyping in four epithelial malignancies (approx. 400 T/N DNA samples) with 23 polymorphic markers (including tri- and tetramers) allowed us to describe additional important DNA segment (between D3S2409-D3S2456, 3p21.31) closely telomeric to LUCA site (Braga et al., 2002). Computational analysis of gene sequences mapped in this D3S2409 region was done using GDB, NCBI and other databases.32 UniGene clusters including 22 unique genes and 10 ESTs clusters were identified in this 600kbp DNA segment. Therefore, gene density in D3S2409 region is even higher than in LUCA site (19 genes in 600kb). Using local versions of SAGE database (tag libraries),Tag in UniGene Mapping database and comparative Count Display program from SAGEmap server expression of all these genes was compared in tumor cell and their normal counterpart for breast and ovary cell lines libraries.Four new gene-candidates (MST1,DAG1, USP4, RON) were selected for subsequent investigations.Potential high expression of DAG1 and USP4 in breast and lung cancers is consistent with allele amplifications observed in surrounding D3S2409 and D3S2456 markers.MST1 receptor (RON) and its ligand MST1 gene are located in 3p21 and their overproduction can result in autocrine stimulation and uncontrolled proliferation (Angeloni and Lerman, 2001).

301

The transcription factor Egr-1 promotes prostate carcinoma and systemic treatment of TRAMP mice with antisense Egr-1 inhibits tumour formation

D. Mercola[1,3], G. Gregorio[1,2], S. Duss[1], R. Urcis[1], A. Calogero[2], G. Ragona[2], A. Krones-Herzig[1]. [1]*Sidney Kimmel Cancer Center, Molecular Patholgy, San Diego, USA;* [2]*Instituto di Ricovero e Cura a Caratttere Scientifi, Neuomed, Pozzilli, Italia;* [3]*Cancer Center, University of Califnornia at San Diego, La Jolla, USA*

The transcription factor Egr1 or immediate early growth response-1 gene, is rapidly induced by growth factors to transduce proliferation signals. Induction of Egr1 is usually transient but appears to be sustained in some prostate tumor cell lines and tumors, suggesting that Egr1 stimulates tumor growth. In contradiction, in breast, lung, and brain tumors, Egr1 expression is often absent or reduced. When re-expressed, Egr-1 causes growth suppression via induction of TGF β(1), fibronectin(2), and PTEN(3). Several groups(4-5) have observed that Egr-1 is over-expressed in human prostate cancer and likely plays an oncogenic role. To test this, we re-expressed Egr-1 in immortalized normal human 267B prostate epithelial cells and observed accelerated growth, increased focus formation, and growth in soft agar. In contrast, Egr-1 is expressed in mouse TRAMP tumor cells. We developed a high affinity (ED50 = 0.15 μM) antisense Egr-1 reagent targeted against both human and mouse Egr-1 that substantially reduces Egr-1 mRNA and protein expression but not that of other family members (Egr-2 to Egr-4 and WT1). Antisense treatment inhibits proliferation by 50% ($p < 0.001$), reduces focus-forming frequency by 40 - 60% ($p < 0.005$), and eliminates growth in soft agar. When male TRAMP mice were treated systemically by I.P. injection 3 d/week for 10 weeks from an age of 22 weeks, a marked reduction prostate carcinoma incidence (3/7) as judged by staging necropsy was observed whereas 3-bp mismatch control oligonucleotide treated mice and vehicle alone treated mice exhibited frequencies of 6/8 and 7/7 yielding a significant overall decrease ($p = 0.045$). Mechanistic studies of TRAMP C-cells indicate that Egr-1 induces TGF β1, a potential growth factor but does not regulate PTEN in contrast to the role of Egr-1 in normal cells3. Taken together, these observations strongly indicate that Egr-1 is a functional growth and transformation promoting agent in human prostate carcinoma cells and in the TRAMP model and is a potential new target of therapy. Antisense Egr-1 maybe a useful reagent in the analysis and treatment of prostate cancer.

References

[1] Liu C. et al. PNAS. U.S.A. 1996;93:11831-6.
[2] Liu, C. et al. J. Biol. Chem., 1999; 274:4400-11.
[3] Thierry V. et al. Nature Cell Biol. 2001;3:1124-28.
[4] Svaren J, J. Biol. Chem. 2000;275:38524-38531.
[5] Thigpen A, et al. J Urol 1996;155(3):975-981.
[6] Eid M. et al. Canc. Res. 1998;58(11):2461-2468.

302

Molecular class prediction of acute myeloid leukemia and myelodysplastic syndromes

W. Trepicchio[1], J. Stover[1], A. Dorner[1], M. Richie[2], L. Leopold[2], M. Berger[2]. [1]*Wyeth Research, Molecular Medicine and Pharmacogenomics, Andover, USA;* [2]*Mylotarg Study Group, Wyeth Research, Clinical Research and Development, Collegeville, USA*

Despite recent advances in the treatment of acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS), mechanisms underlying the disease are still not completely understood. In addition, relevant diagnostic markers and surrogate clinical endpoints of chemotherapeutic efficacy and survival are needed. Treatment approaches for AML and MDS are different and correct diagnosis of the patients is critical. As part of an ongoing pharmacogenomic analysis of AML and MDS patients before and after treatment with the antibody-targeted chemotherapy gemtuzumab ozogamicin (anti-CD33 calicheamicin immunoconjugate, Mylotarg®, we have undertaken a global molecular classification of bone marrow to identify diagnostic and surrogate biomarkers of AML and MDS. Bone marrow samples from normal volunteers, AML and MDS patients prior to Mylotarg treatment (n = 38) were obtained and white blood cells purified using Ficoll gradients. Hematological parameters were measured on all samples to determine variation in cell populations across individuals. Total RNA was isolated from individual samples and analyzed on oligonucleotide arrays containing over 12,000 full-length human genes. Correlation metrics determined a high degree of relationship within the similar bone marrow populations. However, nonsupervised hierarchical cluster analysis demonstrated that the gene expression signatures of AML, MDS, and normal bone marrow were distinct from each other. A supervised class distinction algorithm identified unique gene sets that could accurately distinguish AML from MDS and normal marrow. Interestingly, transcript levels that differentiated AML from MDS included an overabundance of members of the HLH transcription factor. Several of these genes have been shown to map to chromosomal breakpoints in a number of leukemia's and solid tumor types suggesting that the deregulation of these genes may play a fundamental role in the oncogenic process. These data indicate that gene expression profiling will allow diagnostic differentiation of patients with AML and MDS and will ultimately contribute to our understanding of disease development, efficacy and treatment resistance.

303

Gene expression microarray and 2D proteomic profiling of human ovarian adenocarcinoma cells following treatment with 17AAG, an inhibitor of the molecular chaperone Hsp90

P.A. Clarke[1], A. Maloney[1], S. Naaby-Hansen[2,3], R. Claus[2,3], R. Stein[3,4], A. Yang[2,3], A. Akpan[2,3], M.I. Walton[1], M. Waterfield[3], P. Workman[1]. [1]*Institute of Cancer Research, CR UK Centre for Cancer Therapeutics, Sutton, United Kingdom;* [2]*University College London, Dept. Biochemistry and Molecular Biology, London, United Kingdom;* [3]*Ludwig Institute for Cancer Research, London, United Kingdom;* [4]*University College London, Dept. Oncology, London, United Kingdom*

The molecular chaperone Hsp90 is of interest as a therapeutic target because of its importance in maintaining the stability and function of key client proteins required for tumour cell proliferation and survival. The natural product Hsp90 inhibitors geldanamycin and radicicol exert their antitumour effect by inhibiting the intrinsic ATPase activity of Hsp90, resulting in degradation of Hsp90 client proteins. A geldanamycin derivative, 17AAG, has

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.

304

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.

305

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.

306

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%