418 POSTER

Mismatch repair defects among italian cases of secondary acute leukemia and myelodysplastic syndrome

I. Casorelli[1,3], J. Offman[2], L. Mele[3], L. Pagano[3], S. Sica[3], G. Leone[3], P. Karran[2], M. Bignami[1]. [1] *Istituto Superiore di Sanita', Laboratory of Chemical Carcinogenesis, Rome, Italy;* [2] *Imperial Cancer Research Fund, Clare Hall Laboratories, South Mimms, UK;* [3] *Universita' Cattolica del Sacro Cuore, Istituto di Ematologia, Rome, Italy*

Purpose: The frequency of secondary acute leukemia (sAL) and myelodysplastic syndrome (sMDS) is increasing as a consequence of successful therapy for primary malignancies. Estimates of the proportion of sAL cases displaying microsatellite instability (MSI), the hallmark of DNA mismatch repair deficiency, vary widely. We are examining MSI and hMLH1 promoter methylation in a panel of Italian cases in order to investigate: the frequency of mismatch repair defects, the mechanism by which repair genes are inactivated and which therapeutic regimes are particularly associated with mismatch repair inactivation Methods: DNA was extracted from mononuclear bone marow cells collected at diagnosis from 23 patients (18 sAML, 2 sMDS, 3 sALL) most of whom had received previous therapy for Hodgkin or non-Hodgkin lymphoma or breast carcinoma. Microsatellites BAT26, BAT25, D2S123, D17S250, D18S61 were analyzed by PCR. Samples with alterations at BAT26 were considered to be MSI+. Where normal DNA was available, instability was examined at the other loci. Methylation of the hMLH1 promoter was examined by PCR following digestion with either HpaII or MspI. Results: 14/22 DNA samples from which BAT26 could be amplified were MSI+. In the 8 cases for which normal DNA was available, instability was confirmed at additional loci. One case in which BAT 26 was not amplifiable was unstable at 2 other loci (BAT25 and D2S123). Evidence of hMLH1 promoter methylation was obtained for two samples, both of which were MSI+. The hMLH1 promoter was unmethylated in three other MSI+ cases. All (4/4) secondary malignancies from Hodgkin or Non-Hodgkin lymphoma patients treated with a methylating agent (procarbazine or dacarbazine) were MSI+. Conclusions: Inactivation of MMR is common among sAL and sMDS which may be MSI+ in > 60% of cases. Methylation of the hMLH1 promoter does occur but is not the only mechanism of MMR inactivation. MSI+ sAL is particularly – but not exclusively – associated with the use of methylating agents.

419 POSTER

Cell cycle arrest and induction of apoptosis by novel Cdk inhibitor MCS-5A is associated with p16Ink4A up-regulation in Human promyelocytic leukemia cell

B. Choi[1,2], M. Kim[2], J. Kim[1], H. Kim[1], Y. Cho[2], C. Lee[2]. [1] *C&C Research Laboratory, Molecular biology & phamacology, Kyunggi-do, KOREA;* [2] *HanYang University, Collage of Medicine, Medical Genetics, Seoul, KOREA*

Purpose: MCS-5A, novel Cdk inhibitor, has been reported that it has exerted cell cycle arrest action and apoptotic effect to the human promyelocytic leukemias cell. The purpose of this study is to verify these effects of MCS-5A on HL-60 cells and to clarify the action of mechanism on MCS-5A-inducing apoptosis.

Methods: HL-60 cells were evaluated for antiproliferative effect and apoptosis using cell viability test, protein kinase assay, DNA fragmentation, Flow cytometry assay and electromicroscopic examination. To clarify the action of mechanism, we also performed immunoblot assay for cell cycle and apoptosis proteins. To determine the possible of apoptosis to pcDNA-p16 mediated cytotoxicity, we tansfected pcDNA-p16 transiently and performed TUNEL assay on A549 cell, human lung cancer cell, which has homozygous deletion of p16.

Results: We investigated the involvement of cell cycle regulatory events during MCS-5A mediated apoptosis in HL-60 cells. The treatment of HL-60 cells with MCS-5A(3uM, 12hrs) resulted in inhibition of the phosphorylation of Rb protein, a critical step for G1/S transition. The kinase activities of Cdk4, Cdc2 were inhibited in HL-60 cells treated with MCS-5A(IC50 values of 8.8 and 9.6μM, respectively). Furthermore, MCS-5A increases the level of Cdk inhibitor p16. MCS-5A promoted binding of p16 to Cdk4. The induction of apoptosis by MCS-5A is associated with p16 up-regulation. Transient transfection of A549 cell with pcDNA-p16 resulted in a rightward shift of the mean fluorescene intensity when compared to the baseline fluorescence following tansfection with pcDNA vector. MCS-5A can induce apoptosis through different pathway of caspase activation with caspase-8 and caspase-9 playing a pivotal role. Caspase-8 can also activate the pro-apoptotic Bcl-2 family member Bid through proteolytic cleavage. The activation of caspase-9 in MCS-5A treated HL-60 cells is likely to occur via the caspase-8-Bid-mitochondria pathway which leads to cytochrome c release, followed by cleavage of caspase-9. MCS-5A exerted antiproliferation of HL-60 through the induction of apoptosis which is mediated by p16 and caspase-3 pathway, not by mitochondrial pathway.

Conclusions: These results indicate that MCS-5A exerts cell cycle arrest and apoptosis inducing activity in HL-60 cells and might have a potent activity as a new concept anticancer agent in human leukemias and that p16 is capable of mediating apoptosis in human cancer cells

420 POSTER

Deletion of chromosome 15 represents a rare but recurrent chromosomal abnormality in myeloid malignancies

J. Dierlamm[1], L. Michaux[2], G. Schilling[1], D. Seeger[1], P. Leberecht[1], M. Eggers[1], K. Hinz[1], E.M. Murga Penas[1], A. Hagemeijer[2], D.K. Hossfeld[1]. [1] *University Hospital Hamburg-Eppendorf, Dept. of Oncology and Hematology, Hamburg, Germany;* [2] *University of Leuven, Center for Human Genetics, Leuven, Belgium*

Chromosomal abnormalities characterize biological and prognostic subgroups of acute leukemias and point to genes relevant for malignant transformation and disease progression. We report on three cases with myeloid disorders cytogenetically characterized by a deletion of the long arm of chromosome 15 occurring as the sole cytogenetic aberration. The deletions were defined as del(15)(q12q21) (two cases) and del(15)(q11q21) (one case), respectively. Two cases were diagnosed with an acute myeloid leukemia (AML) with dysplastic features classified as AML-M6 and AML-M4 according to the FAB classification. The third case had a chronic myelomonocytic leukemia. In two cases, the aberration was found at the time of primary diagnosis, whereas the third case showed the del(15) during relapse of the leukemia. Both cases with acute leukemia did not adequately respond to intensive chemotherapeutic treatment and died 13 and 11 months, respectively, after primary diagnosis. Cytogenetic analysis was supplemented by fluorescence in situ hybridization using a chromosome 15 specific whole chromosome painting probe and a cosmid probe hybridizing to the PML gene located on 15q22. Hereby, a cryptic translocation involving chromosome 15 could be excluded. Moreover, we could show that the breakpoint occurred proximal to the PML gene, which was retained in both cases analyzed.

Our finding and the data of the 7 previously published cases with an isolated del(15) indicate that (1) del(15) represents a rare but recurrent abnormality in myeloid hemopathies, (2) del(15) occurs frequently in disorders with myelodysplastic or myeloproliferative features and may therefore affect early hematopoietic progenitor cells and (3) del(15) may occur during disease progression and is often associated with an unfavourable prognosis.

421 POSTER

Genetical alterations occur in the atypical bronchial epithelium accompanying interstitial pulmonary fibrosis (IPF)

K. Inoue, Y. Nakanishi, M. Izumi, T. Harada, N. Inoshima, H. Wataya, T. Minami, R. Ishibashi, Y. Horiuchi, N. Hara. *Kyushu Univ., Reserch Institute Of Diseases Of The Chest, Postgraduate School Of Medical Scien, Fukuoka, Japan*

Background and purpose: High incidence of lung cancer is reported in patients with IPF. We investigated the genetic alterations in the atypical bronchial epithelia (ABE) accompanying benign lung diseases including IPF.

Materials and methods: We performed LOH (loss of heterozygosity) analysis on the short arm of chromosome 3 (3p) and 9 (9p) and evaluated by utilizing polymerase chain reaction(PCR) and direct sequencing analysis of K-ras oncogene. A total of 215 transbronchial biopsy specimens diagnosed as ABE were used for the study. These specimens were obtained from 74 benign lung diseases and 29 concurrent lung cancers.

Results: LOH frequency at 3p21.3 was 32%. The comparison between the frequency of LOH at the 3p14.2 (FHIT) in smokers and that in non-smokers was statistically significant (34% vs. 3.9%, $p < 0.0001$). In the ABE lesions obtained from benign lung diseases, 16 specimens demonstrated LOH at the 9p22 (IFNA) locus. In these 16 specimens, 14 (88%) of them were obtained from IPF. All of these 16 specimens demonstrated LOH at one or two chromosomal 3p loci. Mutations of K-ras codon 12/13 were found two cases of IPF patients.

Conclusion: These results suggest that genetical alterations occasionally occur in the lesions with ABE particularly in IPF patients. Since high incidence of lung cancer is reported in patients with IPF, the presence

of multiple genetical alterations in IPF specimen might be an indicator of higher risk for lung carcinogenesis.

422 POSTER

Acetylation genotypes and susceptibility to hormonal cancer: breast and prostate cancer

S. Costa[1], R. Medeiros[1], D. Pereira[2], A. Morais[3], H. Rodrigues[2], J. Oliveira[3], C. Lopes[1]. [1] *Instituto Português de Oncologia, Lab. Patologia Molecular - Serviço de Anatomia Patologica, Porto, Portugal;* [2] *Instituto Português de Oncologia, Serviço de Oncologia Médica III, Porto, Portugal;* [3] *Instituto Português de Oncologia, Serviço de Urologia, Porto, Portugal*

Purpose: Increasing exposure to environmental pollutants and intake of dietary fat and proteins seem to have a important role during both breast and prostate carcinogenesis. The N-acetyltransferase 2 (NAT2) comprise one of the major enzyme systems catalysing carcinogens metabolism, like that finding in dietary food and others, and it is therefore reasonable to assume that NAT2 play a role in development of breast and prostate cancer. We analysed NAT2 polymorphism in breast cancer patients, in men with prostate carcinoma, a group of healthy women and healthy men.

Methods: We analysed NAT2 polymorphism in 638 genomic DNA blood samples: 134 women with breast cancer and 162 men with prostate cancer and 151 healthy men and 181 healthy women. We used PCR-RFLP to analyze two common mutants alleles at NAT2 loci.

Results: We analyzed NAT2*5 and NAT2*6 mutants alleles in NAT2 gene. From the possible combinations genotypes we did not find any statistically significant differences between the breast cancer patients and healthy women. However, the NAT2*6/NAT2*6 genotype was found in 24% of patients with prostate cancer and in 14% healthy men, presenting a statistically significant difference (OR= 1.96; 95%CI: 1.05-3.67;p= 0.0224). This association was also found when considering the group of patients with a Gleason score higher than seven.

Conclusion: Our results suggest that acetylation genotypes do not directly affect the susceptibility to breast cancer. However, we found that NAT2*6/NAT2*6 genotype (a slow genotype) represent a higher risk to prostate cancer. Furthermore, this genotype seems to be associated with development of prostate tumors of higher aggressiveness.

423 POSTER

Predictive testing for BRCA1 and 2 mutations among men: cherchez l'homme and beware the phenocopy

P.A. Daly[1], C. Nolan[1], W. Ormiston[1], D. Byrne[1], T. McDevitt[2], N. Miller[2], A. Green[2], D. Barton[2]. [1] *St. James's Hospital and Trinity College, Department of Medical Oncology, Dublin 8, Ireland;* [2] *Our Lady's Hospital, National Centre for Medical Genetics, Dublin 12, Ireland*

Purpose: The implications for women carrying mutations in BRCA 1 and 2 are generally clear and, with options such as screening, prophylactic surgery and chemoprevention, the role of predictive testing is accepted where a disease-associated mutation is identified within a family. The personal implications for males who carry such mutations remain unclear and men generally opt for testing in the interests of other family members, especiallly daughters.We have analysed the outcome and issues arising from testing of men in this setting.

Methods: Between 1996 and 2000, 13 men from 5 families had predictive testing in the context of research studies into BRCA1 and 2 mutations in the Irish context. Ages ranged from 28 to 82 years (median 57). Three brothers had developed cancer, one ureteric and prostate, one prostate and one squamous cell skin cancer at 2 sites. These 3 and another brother sought testing for personal information. Overall 11 cited concerns regarding transmission of genetic risk as their reason for testing. Two had no offspring while 11 had 39 children including 21 daughters.

Results: 11 of thirteen had positive tests and two were negative. The negative results meant that inheritance risk was removed from 9 offspring (4 male, 5 female). The man with prostate cancer only proved to be a phenocopy. In another family, despite dying from breast cancer (age of onset 53 years) the mother of two daughters with bilateral breast cancer and ovarian cancer was also a phenocopy and the 82 year old father carried the mutation in BRCA1. Despite full pre-test counselling three brothers steadfastly refused to communicate their positive results to their 10 offspring (3 male, 7 female) thereby raising major ethical issues. All other outcomes were fully transmitted.

Conclusion: Predictive testing for BRCA1 and 2 mutations is a complex issue. Testing of men is mainly of value to other family members, especially offspring. Inheritance patterns can be defined and phenocopies identified. Care must be taken that, through rigorous pre and post test counselling, information is transmitted to those who can benefit. This may not always be possible as we have demonstrated.

424 POSTER

Genetic alterations in the pancreatic carcinoma. Prospective study

D. Pantalone[1], E. Pelo[2], B. Minuti[2], E. Mazza[3], G. Nesi[4], M. Falchini[3], F. Torricelli[2]. [1] *University of Florence, dpt.Critical Medicine and Surgery, Florence, Italy;* [2] *A.O.C, U.O.Genetic and Citogenetics, Florence, Italy;* [3] *University of Florence, Dept. of Radiology, Florence, Italy;* [4] *University of Florence, Dept. of Human Pathology and Oncology, Florence, Italy*

Purpose: In this research we presented the results of a study that has been performed from 1998. First we examined retrospectively the most frequent alterated genes in pancreatic cancer paraffin-embedded specimens and then we begun the prospective study (the preliminary results are presented previously). The aim of the study is to evaluate the possible clinical applications, i.e. differential and early diagnosis.

Methods: we studied genetic alterations in the duodenal juice of thirthy patients with pancreatic carcinoma (byopsy and histopathological confirmation), for k-ras mutation, p-16, p53, and DPCC4 alterations. The duodenal juice was obtained by biliary percutaneous drainage. The analysis was performed by quantitative PCR, study of microsatellites instability and sequencing analysis of the genes (ABI PRISM 377 sequencing analysis).

Results: We found RER+ and LOH- detectable in the duodenal juice for k-ras and DPCC4. The percentage for the microsatellites D18s46 and D18s47 is informative with reference to the literature

Conclusion: Molecular genetic study performed on duodenal juice is a feasible approach for differential diagnosis of pancreatic cancer. Our results suggest that the examination could be proposed also in clinical applications for the screening of early neoplasia.

425 POSTER

Analysis of thymidylate synthase (TS) and udp-glucoronosyl transferase 1a1 (UGT1A1) gene polymorphisms in colorectal (CRC), breast (BC) and lung (LC) cancer patients: significant differences in ugt1a1 genotypic distribution in lc patients (pts)

E. Martinez-Balibrea, A. Abad, M. Margeli, A. Font, A. Barnadas, C. Plasencia, R. Rosell, M. Taron. *University Hospital Germans Trias i Pujol, Medical Oncology Service, Badalona, Spain*

Genetic polymorphisms have been linked with increased cancer susceptibility as well as to degree of response to different chemotherapeuthic (CT) agents. Specifically, variable number of tandem repeats (2, 3, 4 and 9) in the promoter region of TS gene, which encodes a rate-limiting enzyme in the synthesis of pyrimidine nucleotides correlates with different levels of gene expression and also with 5FU-based CT response in CRC pts. UGT1A1 plays a major role in the detoxification of a diverse range of molecules, including carcinogens and some CT agents such as SN38, the active form of CPT11. An inverse correlation has been demonstrated between the number of TA repeats (6 or 7 in Caucasian population) in the TATA box of the promoter region and the expression of the gene. The present study analyses the genotypic distribution of these polymorphisms in controls and cancer pts and their potential relationship to cancer susceptibility. Genomic DNA was prepared from whole blood, and polymorphisms were analysed by PCR (TS) and direct sequencing (UGT1A1). Up to date 176 pts and 82 controls are included. The polymorphisms were analysed in 158 pts for TS (60 CRC, 49 BC, 49 LC) and 164 pts for UGT1A1 (52 CRC, 45 BC, 67 LC). Male/female: 97/79; 77% of pts aged 50 or over. No statistical differences in genotypic frequencies (f) were observed in TS when controls (2/2: 22%, 2/3: 42%, 3/3: 36%) were compared to patients (2/2: 19%, 2/3: 49%, 3/3: 32%) or between controls and each subgroup of pts. In addition, no differences were found according to age or sex. In contrast, there was a significant difference (xi square test=12.18 p=0.002) between the genotypic distribution of UGT1A1 polymorphism in LC pts (6/6=39%; 6/7=39%; 7/7=22%) and that in controls (6/6=44%; 6/7=46%; 7/7=10%), but no difference was observed between controls and CRC and BC pts. These differences could be derived from the role of UGT's in the metabolism of tobacco related carcinogens such as aromatic amines and polycyclic aromatic hydrocarbons, suggesting an increased risk for LC development among 7/7 individual. Further analysis of UGT1A1 is warranted in order to elucidate the role of UGT1A1 in LC risk.