H – Cancer genetics

H01 THE FREQUENCY OF FACTOR V, FACTOR II AND MTHFR MUTATIONS IN PULMONARY THROMBOEMBOLISM CASES IN THE WESTERN BLACK SEA REGION

[1]E Tug, [2]T Tug, [1]S Duzenli Gepdiremen, [2]F Talay, [1]H Aydin, B Kurt[2] *[1]Department of Medical Genetics, Abant Izzet Baysal University, Izzet Baysal Medical School; [2]Department of Chest Diseases, Abant Izzet Baysal University, Izzet Baysal Medical School*

Introduction: In this study, frequency and distribution of hereditary risk factors leading to development of venous thromboembolism (VTE) in patients with pulmonary thromboembolism (PE) were evaluated.
Materials and Methods: We investigated the important risk factors for VTE in total 46 patients (58 ± 18.8), referred to our clinic with diagnosis of PE by Real Time PCR. We also evaluated distribution of inherited thrombophilia mutations and the family history of VTE.
Results: We determined the rate of FVL, FH1299R, MTHFR (C677T and A1298C) mutations in our patients as 32.6%, 2.2%, 67.4% and 63%, respectively. FIIG20210A mutation could not be determined. Twenty-six percent of the patients had only one mutation, while 71.7% of them had two or more mutations. In 77% of individuals with family history, there were two or more mutations. Sixty-three percent patients had at least one of MTHFR gene mutations alone. In 34% of the patients, a combination of at least one of MTHFR gene mutations with either FVL or FVH1299R mutation was available. In 21.7% patients, the history of VTE was present in different body part. Inherited thrombophilia with neoplasia was present in 8.7% of patients.
Conclusion: In patients with PE, rate of FVL, and mutations (C677T and A1298C) of MTHFR gene was higher than the previous limited data for Turkey. Determination of the relationship between VTE and inherited abnormalities will be useful for society health and familial risk relations, correctly, in addition to diagnosis and following of patients.

H02 MALDI-TOF MS BASED GENOTYPING OF SINGLE-NUCLEOTIDE VARIATIONS PREDISPOSING TO CARDIOVASCULAR DISEASES: PREVALANCE IN TURKEY

[1,2]S KARACA, [2]T Cesuroglu, [2]E Kocaman *[1]GENAR Institute for Public Health and Genomics Research, Ankara, Turkey; [2]Aksaray University, Sc.H., Aksaray*

Introduction: Cardiovascular disease (CVD) is an emerging public health problem in the worldwide. Numerous polymorphisms have been identified, directly and indirectly involved in CVD etiology. These genetic factors have an affect on blood pressure regulation, blood coagulation, homocysteine and lipid metabolisms, which all leading to cardiovascular problem. Here, we evaluated the distribution of some allelic variants predisposing to CVDs. Participants were healthy individuals (n = 500). Variants involving in lipid metabolisms, APOC3 **c.3175C > G**, LPL **c.1595C > G**, **CEPT c.279G > A**, blood coagulation, factor V **1691G > A** (Leiden), factor II (prothrombin) **20210G > A**, and blood pressure regulation eNOS **c.894G > T**, polymorphisms influencing single carbon metabolism MTHFR **c.677C > T**, MTHFR **c.1298A > C**, **MTRR c.66A > G**, **MTR c.2756A > G** were genotyped using MALDI-TOF based mass spectrometry. Polymorphic sites were analyzed in according to manufacturer's protocol of Sequenom hME platform. The genotype and allele frequencies were calculated and their distributions were compared with those reported for other regions. Deviation from the Hardy–Weinberg equilibrium was not observed. Genetic and non-genetic factors play a great role in the occurrence of the complex disease. Identification of individual's genetic predisposition and proper regulation of the lifestyle in accordance with the requirements of genetic background is important to prevent disease and detect them early. Better knowledge of a common genetic structure of population may prompt targeted preventative healthcare strategies.
Keywords: CVD susceptibility, genetic factors predisposing CVD.

H03 EFFECTS OF FTO (FAT MASS AND OBESITY ASSOCIATED) GENE POLYMORPHISMS ON TURKISH ADULT RISK FACTOR (TARF) STUDY POPULATION

[1]AB Yüzbasiogullari, [1]E Komurcu-Bayrak, [2]A Onat, [3]G Hergenc, [4]N Mononen, [4]R Laaksonen, [5]M Kähönen, [4]T Lehtimäki, [1]N Erginel-Unaltuna *[1]Department of Genetics, Istanbul University, Institute for Experimental Medical Research, Istanbul, Turkey; [2]Turkish Society of Cardiology, Istanbul, Turkey; [3]Department of Biology, Yildiz Technical University, Istanbul, Turkey; [4]Department of Clinical Chemistry Tampere University Hospital and University of Tampere, Medical School, Tampere, Finland; [5]Department of Clinical Physiology Tampere University Hospital and University of Tampere, Medical School, Tampere, Finland*

Introduction: The aim of this study was to determine the associations between the FTO rs9939609T > A and rs1421085T > C polymorphisms and the anthromorphic/biochemical variables of human metabolism. FTO is located on chromosome 16q12.2 region, was originally described in a mouse model and may play a role in lipid tissue metabolism. FTO polymorphisms have been found to be associated with diabetes type 2 and obesity in previous studies.
Methods: Turkish adult risk factor (TARF) is an epidemiologic follow-up study that examines the mortality and morbidity of heart diseases and their relationship with risk factors in Turkey. We genotyped two single nucleotide polymorphisms (FTO rs9939609 and rs1421085) in TARF study population. From EDTA whole blood, genomic DNA was isolated from leukocytes using QIAmpR DNA Maxi KIT. Genotyping was performed using the ABI prism 7900HT Sequence Detection System for both polymerase chain reaction and allelic discrimination with TaqMan technology, Windows SPSS was used for statistical analyses.
Results: Study subjects (n = 1997 and 2006) were genotyped for the rs9939609 and rs1421085 polymorphisms. Genotype frequencies of rs9939609T > A were 0.36(TT), 0.48(TA) and 0.16(AA). Genotype frequencies of rs1421085T > C were

0.36(TT), 0.48(TC) and 0.16(CC). Allele frequencies of rs9939609 were 0.6 (T) and 0.4 (A). Allele frequencies of rs1421085 were 0.575 (T) and 0.425 (C).

Conclusion: After adjustment for the major cardiovascular risk factors, two FTO SNPs were significantly associated with higher BMI, higher waist circumference and metabolic syndrome in all subjects, whereas rs1421085T > C polymorphism was associated with higher waist circumference, higher glucose levels and metabolic syndrome in men. No association was detected in women.

H04 EVALUATION OF ALTERATIONS ON PTEN GENE BY PCR-SSCP-DNA SEQUENCING TECHNIQUES IN PATIENTS WITH GASTRIC CANCER

[1]A Kalaycı, [1]T Cora, [2]H Toy, [1]H Acar, [3]E Kurar *[1]Department of Medical Genetics, Selcuklu Medical Faculty, Selcuk University, Konya, Turkey; [2]Department of Pathology, Meram Medical Faculty, Selcuk University, Konya, Turkey; [3]Department of Zootecni, Veterinary Faculty, Selcuk University, Konya, Turkey*

Although the incidence of gastric cancer (GC) has been declining over the past several decades, it is still second place in cancer related deaths. Concurrently with its etiology, GC can not be clarified accurately; both genetic and environmental factors play roles. It is informed that different pathways set in at carcinogenesis and progression of cancer and several genes take a role at these pathways where regulation is deteriorated. Phosphatase and Tensin homolog deleted on chromosome 10 (PTEN) gene is a tumor suppressor gene which has a role in PI3K/AKT signaling pathway. This gene is localized on 10q23.3, has nine exons and encodes 403 amino acids. In the present study, we evaluated the alterations of PTEN gene (exons 2–6) in the normal (n = 47) and tumor tissues (n = 47) of the cases with gastric adenocarcinoma, respectively by using PCR-SSCP and DNA sequencing techniques. In the end of the sequence analysis a single nucleotide polymorphism (SNP) was found in intron-exon boundary in all of tumor and normal tissues (100%) of the eight patients. This SNP (rs1903858) was in intron-exon boundary region and had T > C genotype. It is believed this SNP is germline in all of our cases since both normal tissue and tumor tissue had this change. We believe that this SNP might predispose the patients to gastric cancer. Expanding our study in matched-control cases is in progress.

H05 ANALYSIS OF COPY NUMBER ALTERATIONS OF EGFR, HER2 AND TOP2A GENES IN GASTRIC CARSINOMAS

H Saygili, M Ozdemir, B Durak Aras, M Oznur, MH Muslumanoglu, O Cilingir, S Artan *Faculty of Medicine, Department of Medical Genetics, Eskisehir Osmangazi University, Eskisehir, Turkey*

Gastric cancer (GC) is the second most common cause of cancer death worldwide and treatment remains a challenge for physicians. The EGFR and HER2 genes are therapeutic targets in various tumors. The TOP2A gene is located close to HER2 and co-amplification of HER2 and TOP2A is associated with sensitivity to anthracycline therapy in several types of cancer. The study was aimed to determine the relation of copy number variations of EGFR, HER2A and TOP2A genes in different clinical stages of GCs and to address the relationship between alterations in EGFR, HER2, and TOP2A and chromosome polysomy. Following direct preparations from 15 fresh gastric samples, FISH analysis was carried out using the LSI EGFR/CEP7 (Vysis), LSI TOP2A/LSI HER-2 /CEP17 probes. The copy numbers of genes and of centromeres 7/17 were evaluated. Of the samples, 66.7% were grade IV whereas 13.4% grade II, 6.7% grade IIIB and 13.4% grade IB. HER2/neu and TOP2A amplification were detected in 46% and 40% of the samples. TOP2A gene amplification was significantly associated with HER2 gene amplification. EGFR gene amplification was seen in 20% and no relation was detected among EGFR, HER2/neu and TOP2A aberrations. Fifty-three percent of the tumors showed chromosome 7 polysomy and it is significantly with EGFR FISH- positivity. The data suggested that chromosome 7 polysomy may be responsible for increased EGFR gene copy number in GCs. TOP2A and HER2 amplicon amplification is frequently seen in GCs and may facilitate the finding an effective chemotherapeutic protocols for GC patients.

H06 DETERMINATION OF MIRNAS' EXPRESSIONS IN BLADDER CANCER BY MICROARRAY TECHNOLOGY

[4]M Canturk[1], S Artan[1], C Can[2], S Öner[3], M Özen, [5]M Ozdemir[1], H Aslan[1], E Ciftci, O Cilingir[1], F Celayir[1] *[1]Faculty of Medicine, Department of Medical Genetics, Eskisehir Osmangazi University, Eskisehir, Turkey;[2]Faculty of Medicine, Department of Urology, Eskisehir Osmangazi University, Eskisehir, Turkey; [3]Faculty of Medicine, Department of Biostatistics, Eskisehir Osmangazi University, Eskisehir, Turkey; [4]Cerrahpasa Faculty of Medicine, Department of Medical Genetics, Istanbul University, Eskisehir, Turkey; [5]Faculty of Medicine, Department of Pathology, Eskisehir Osmangazi University, Eskisehir, Turkey*

Bladder cancer is the second malignancy of genitourinary system in developed countries. Urothelial carcinoma (UC) is the most frequent (90%) histological type and 5 years survival is nearly 20–40% in the advanced stage bladder cancer. Increasing evidence has suggested that dysregulation of certain microRNAs (miRNAs) may contribute to tumorigenesis. To seek specific miRNA expression profiles characterizing the grades and stages of UC, we profiled the expression of 723 unique human miRNAs in 6 normal and 17 bladder tumor samples using oligonucleotide microarrays. Following Hierarchical clustering analysis, several differentially expressed miRNAs between normal and tumor samples and between the different disease stages could be distinguished. The mir-143 (27↓), mir-145 (16↓), mir-125b (11↓), mir-99a (11↓), mir-133b (10↓), mir-100 (6↓), mir-497 (4.9↓), mir-139-5p (3.7↓) were down regulated ($p < 0.01$) and mir-18a (3.1-fold ↑) was the sole unregulated miRNA in cancer compared with normal. In the comparison of results with clinicopathological features of the samples, mir-10a, mir-34a, mir-141, mir-210 expression were significantly decreased in tumors with advanced grade and stage ($p < 0.05$) and they might be used to predict prognosis of UC. The results suggested that the down-regulated miRNAs may be directly involved in pathogenesis of UC as tumor suppressor miRNAs. The expression of mir-99a located on chromosome 21q21, was significantly decreased in tumor samples. The chromosomal region deleted in UC might be a fragile region containing additional tumor suppressor genes. Therefore, the role of 21q21.1 in the pathogenesis of UC should be determined through advanced molecular analysis.

H07 CLINICAL GENETIC SIGNIFICANCE OF TRAIL AND TRAIL RECEPTORS IN PATIENTS WITH HEAD AND NECK CANCER

[1]B Yoldas, [2]C Ozer, [3]O Ozen, [3]T Canpolat, [2]I Dogan, [4]TS Griffith, [1]S Sanlioglu, [2]LN Ozluoglu *[1]Human Gene and Cell Therapy Center of Akdeniz University Hospitals and Clinics, Antalya, Turkey; [2]Department of Otolaryngology – Head and Neck Surgery, Baskent University, Faculty of Medicine, Ankara, Turkey; [3]Department of Pathology, Baskent University, Faculty of Medicine, Ankara, Turkey; [4]Department of Urology and Gene Therapy Center of University of Iowa, Iowa City, IA, USA*

IntroductionSquamous cell carcinoma constitutes 95% of head and neck cancer (SCCHN), and TNF-Related Apoptosis-Inducing Ligand (TRAIL) is a death ligand currently under clinical trials for cancer. The gene encoding TRAIL is located at chromosome 3q26 and several single-nucleotide polymorphisms (SNPs) regarding TRAIL have already been reported in diseased individuals. TRAIL receptors are all located on human chromosome 8p21–22, which is a frequent site of translocations in head and neck cancers. Knowing that the molecular profile of TRAIL and TRAIL receptors has not yet been mapped for patients with laryngeal squamous cell carcinoma (LSCC) or patients with oral cavity squamous cell carcinoma (OCSCC) we aimed to disclose TRAIL and TRAIL receptor protein profile in LSCC and OCSCC patients to illuminate its significance in SCCHN carcinogenesis process.
Materials and Methods: Paraffin embedded tissues of 60 patients with LSCC, 14 patients with OCSCC were analyzed by immunohistochemistry using antibodies developed against TRAIL and its receptors. Forteen patients with benign laryngeal hyperplasia and 12 patients with benign oral cavity lesions were included in the study for comparison purposes
Results: An increase in Decoy-R1 (DcR1) but a decrease in Decoy-R2 (DcR2) expression was observed in both LSCC and OCSCC patients compared to control individuals with benign lesions. Clinical and pathologic grading revealed distinctive TRAIL and TRAIL receptor profile in patients with SSCHN.
Conclusion TNF-Related Apoptosis-Inducing Ligand and TRAIL receptor expression profile might be useful to follow up disease progression by virtue of its connection to clinical staging and pathological grading in LSCC patients.

H08 MICROSATELLITE INSTABILITY ANALYSIS IN NONTUMOR COLON TISSUE AND POLYP

[1]E Tug, [2]YH Balaban, [1]E Kaplan *[1]Faculty of Medicine, Department of Medical Genetics, Abant Izzet Baysal University, Bolu; [2]Faculty of Medicine, Department of Gastroenterology, Abant Izzet Baysal University, Bolu, Turkey*

Colorectal carcinogenesis (CRC) is a multistep process involving a series of mutations. Genomic instability in CRC occurs as either microsatellite instability (MSI) or chromosomal instability. In the present study MSI has been examined in normal colon and polyp tissue if detected, for MLH1 and MSH2 genes. During colonoscopy, the tissues were sampled from the four segments of normal colon. DNA samples extracted from tissue and blood of 102 subjects (mean age 56.3 ± 14.9) were amplified by PCR and analyzed by PAGE for the MSI status according to Bethesda consensus panel (BAT25, BAT26, D2S123, D5S346, and D17S250). Forty-eight percent of 102 individual were microsatellite stable for all 5 makers at all locations, 20% have L-MSI and 32% have H-MSI. The frequencies of MSI markers were statistically significantly different from each other ($p = 0.003$). The most frequently positive marker was D17S250. There was family history of any type of cancer and colon cancer in 44.8% and 9.4% rates, respectively. The presence of MSI was strongly correlated with family history of colon cancer ($p < 0.001$). The early detection of individuals with CRC might be possible the MSI analysis of DNA mismatch repair genes at normal colonic tissue taken from different sides during colonoscopy. MSI might be used to screen individuals with family history of CRC. In the presence of any mutations that cause dysfunction of MLH1 or MSH2 gene in CRCs, we have argued that MSI testing is crucial to describe these events in the very early stages and can be applied easily.

H09 WHOLE GENOME EXPRESSION, CANONICAL PATHWAY AND GENE NETWORK ANALYSIS IN THE CASES OF PAPILLARY THYROID CANCER

[1]N Cine, [2]I Tarkun, [3]Z Canturk, [4]Y Gurbuz, [1]D Sunnetci, [1]H Savli *[1]Medical Genetics Department, Medicine Faculty of Kocaeli University, Turkey; [2]Department of Internal Medicine, Medicine Faculty of Kocaeli University, Turkey; [3]Department of General Surgery, Medicine Faculty of Kocaeli University, Turkey; [4]Pathology Department, Medicine Faculty of Kocaeli University, Turkey*

Introduction: Papillary carcinoma is the most frequent thyroid cancer and constitutes 75–80% of thyroid cancers. Finding of scientific markers for papillary thyroid cancer and its variants can make easier to confirm the results taken from Fine Needle Aspiration cytology. The objective of this study consists in elucidating the role of genetic factors in the mechanisms of the development of papillary thyroid cancer and to screen patterns of whole genome expressions in patients with papillary thyroid cancer.
Materials and Methods: RNA samples were obtained from healthy and cancerous tissues taken from cancer detected nodule from eight patients diagnosed as papillary thyroid cancer. These RNA samples were hybridized with microarray chips (Agilent Human 4×44K Oligo Microarrays). Gene expression, canonical pathway and network analysis were performed using GeneSpring GX 11.0 software.
Results: Forty down regulated and 124 unregulated genes were detected in our study. The canonical pathways significantly regulated were extracellular region, collagen, multicellular organism process, cell adhesion, biological adhesion and multicellular organism development.
Conclusion: Up regulation of HMGA2 gene which was reported before as a novel molecular marker in development of thyroid carcinoma is noteworthy in our study. This gene upregulated in malignant forms of thyroid cancers has been reported. It is suggested that HMGA2 might be used as a molecular marker for classification of thyroid tumors in terms of being malignant or benign forms.

H10 CYTOMORPHOLOGICAL, IMMUNOCYTOCHEMICAL AND MOLECULAR BIOLOGICAL FEATURES OF LEUKEMIAS EXPOSED TO IONIZING RADIATION

[1]H Savli, [1]N Cine, [2]DF Gluzman, [1]D Sunnetci, [2]LM Sklyarenko, [2]VA Nadgorna, [2]MP Zavalevych, [2]SV Koval [1]*Medical Genetics Department, Medicine Faculty of Kocaeli University, Turkey;* [2]*R.E. Kavetsky Institute of Experimental Pathology Oncology and Radiobiology, Ukraine*

Introduction: After Chernobyl accident leukemia ratios are further expected to increase both in Turkey and Ukraine because of the ionizing radiation effects. Although immunoenzyme cytochemical techniques with the broad spectrum of monoclonal antibodies can be used for early and precise diagnosis, there are only a few data about gene expression in the role of the long-term exposure to ionizing radiation in various biological forms of leukemias and malignant lymphomas.

The objective of this research consists in elucidating the role of the long-term exposure to ionizing radiation in the mechanisms of the development of B-CLL.

Materials and Methods: Blood samples were collected from 40 B-CLL patients in Kavetsky Institute in 2008–2009. Cytomorphology, cytochemistry, immunocytochemistry studies were performed for diagnosis according to nine subgroups (Acute Myeloid Leukemia, Chronic Myeloid Leukemia, Acute Lenfositic Leukemia, Chronic Lenfositic Leukemia, Hairy Cell Leukemia, Multiple Myeloma, Hodgkin Lymphoma, Non Hodgkin Lymphoma, rare myeloproliferative diseases) in Ukraine. RNA isolation and gene expression studies of seven genes (BCL2, BAX, FAS1, MYC, P38MAPK, APAF1, P53) using Quantitative Real Time PCR (LightCycler 1.5; Roche Diagnostic GmbH, Germany) were performed in Medical Genetics Department of Kocaeli University.

Results: BCL2, BAX, FAS1 were found upregulated 1.197-, 1.506- and 2.476-folds where as P38MAPK, MYC, APAF1, P53 were found down regulated 1.579-, 3.138-, 1.277- and 1.825-folds, respectively in B-CLL population.

Conclusion: It is claimed that down regulation of APAF1 is an indicator of poor prognosis for CLL. Beside this, radioactive induction related down regulation of P38MAPK is observed specifically in the same group of leukemia.

H11 GENE NETWORK AND CANONICAL PATHWAY ANALYSIS IN EARLY AND LATE PHASE RESPONSE AND NON-RESPONSE TO THERAPY IN CERVICAL CANCER

[1]N Cine, [1]D Sunnetci, [2]T Limpaiboon, [1]H Savli [1]*Medical Genetics Department, Medicine Faculty of Kocaeli University, Turkey;* [2]*Faculty of Associated Medical Sciences, Centre for Research and Development of Medical Diagnostic Laboratories, Khon Kaen University, Thailand*

Introduction: Cervical carcinoma is the second most malignancy in women worldwide. Human papillomavirus infection, particularly high risk types, is the notoriously known cause of this disease. The standard regimen for the treatment of cervical cancer is radiotherapy which is a generally effective therapeutic method. However, individuals show different patterns of radiotherapy response in which one-third of cured patients become resistant during or after complete course of the treatment. To date the molecular mechanism for the development and progression of therapeutic resistance in cervical carcinoma is not well defined.

Materials and Methods: In the current study, we performed microarray analysis using high-density Agilent G2556 GeneChip arrays to assess the gene expression changes in early and late phase response to therapy compared with non-response in cervical carcinoma. Further, the genes identified were analyzed for network and gene ontology by ingenuity pathway analysis software to identify networks of interacting genes, other functional groups, and corresponding canonical pathways. In addition, other selected genes confirmed the microarray data by real-time quantitative reverse transcriptase-PCR.

Results: Differentially expressed genes compared with therapeutic non-response were detected; 2400 genes in early phase and 2371 genes in late phase treatment responses to validate microarray results, we randomly selected genes for quantitative RT-PCR analysis. Results were in a good agreement with the microarray data.

Conclusion: The differential gene expression profiling derived from these patients will illustrate an insight to the progression of therapeutic resistance in cervical carcinoma leading to improved strategic treatment and efficacy and prolonged patient survival.

H12 EFFECTS OF CURCUMIN ON GLOBAL GENE EXPRESSION PROFILE IN THE HIGHLY INVASIVE HUMAN BREAST CARCINOMA CELL LINE MDA-MB 231

[1]N Cine, [1]D Sunnetci, [2]P Limtrakul, [1]H Savli [1]*Medical Genetics Department, Medicine Faculty of Kocaeli University, Turkey;* [2]*Biochemistry Department, Chiang Mai University, Thailand*

IntroductionCurcumin, or diferuloylmethane, is a major chemical component of turmeric (*Curcuma longa* Linn) that has been consumed as a dietary spice for ages. This yellow colored polyphenol has a surprisingly wide range of beneficial properties, including anti-inflammatory, antioxidant, anti-tumoral, anti-invasive and anti-metastatic activity.

Materials and Methods: In this study, microarray gene expression analysis was applied to identify the curcumin-regulated genes in a highly invasive human breast-carcinoma cell line (MDA-MB-231). Cells were cultured (20 μM) with curcumin for 24 hours; total RNA was isolated and hybridized to Agilent Whole Human Genome microarray slides. Gene Set Enrichment Analyses on our whole genome expression data revealed the down regulation of the EGF pathway elements followed by curcumin treatment.

Results: Furthermore, gene network analysis identified a significantly relevant network among the differentially expressed genes centered on the EGR-1 and FOS genes. The members of these pathway and network play an essential role in the regulation of cancer cell growth and development, most of them showed decreased expression level after the treatment of curcumin.

Conclusion: We have demonstrated the decreased expression of pathway elements of the EGF signaling cascade and the decreased expression of *EGR-1* gene on mRNA level in MDA-MB 231 human invasive breast carcinoma cell line. According to our knowledge we were the first who applied comprehensive GSEA and network analysis to analyze the gene expression profile of curcumin treatment on breast carcinoma cell line. These observations suggest that the use of curcumin may be an excellent candidate for prevention and treatment of breast cancer.

H13 RET ONCOGENE GENOTYPES IN MULTIPLE ENDOCRINE NEOPLASIA TYPE 2: STUDIES IN FOUR TURKISH FAMILIES

[1]Y Erbilgin, [2]Y Tutuncu, [2]H Boztepe, [2]S Yarman, [2]N Colak, [2]F Aral, [2]R Tanakol, [2]MF Alagöl, [1]U Ozbek [1]*Medical Faculty of Istanbul, Institute of Experimental Medicine, Istanbul University, Istanbul, Turkey*[2]*Medical Faculty of Istanbul, Department of Adult Endocrinology and Metabolism, Istanbul University, Istanbul, Turkey*

Introduction: Multiple endocrine neoplasia type 2 (MEN2) is an inherited, autosomal- dominant disorder cause by deleterious mutations within the RET proto-oncogene. RET mutations are mainly heterozygous, missense sequence change found in RET exons 10, 11, 13–16. The RET proto-oncogene can also have rare as well as common polymorphisms present within hot spot side of the gene. In the present study, we screened four MEN2 family members for RET proto-oncogene and consult approach for managing the disorder.
Material and Methods: Peripheral blood samples were obtained from four patients who were diagnosed in department of adult endocrinology and metabolism, Istanbul medical faculty, Istanbul University. Total DNA was isolated and PCR analysis performed for exons 10, 11, 13–16. All PCR products were screened by direct sequencing.
Results: One patient had p.C618R and one patient had p.C618S disease causing point mutations within exon 10. One patient showed p.C634Y and one patient had p.C634S disease causing missense mutations within exon 11. Also three of the patients had p.G691S (rs1799939), p.L769L (rs1800861) and p.S904S (rs1800863) single nucleotide polymorphisms.
Conclusion: Specific RET proto oncogene mutations predict phenotypic expression of the disease. It has been reported that mutations at 618 are related with MEN2, FMTC and Hirschsprung disease and MEN 2A with cutaneous lichen amyloidosis is associated with mutations in codon 634. In screening family members the result of genetic testing allows for early treatment and intervention. This offers the chance of prophylactic thyroidectomy and cure of medullary thyroid carcinoma in persons with a positive family history.

H14 DETECTION OF TYROSINE KINASE MUTATIONS OF THE EGFR FROM THE SERUM OF TURKISH NON-SMALL CELL LUNG CANCER PATIENTS

[1]H Akca, [1]A Demiray, [2]A Yaren, [1]O Tokgun, [3]J Yokota [1]*Pamukkale University School of Medicine Department of Medical Biology, Denizli, Turkey;* [2]*Department of Medical Oncology, Denizli, Turkey;* [3]*National Cancer Center, Tokyo, Japan*

Introduction: In non-small cell lung cancer (NSCLC), somatic mutations of epidermal growth factor receptor (EGFR) are associated with dramatic responses to the EGFR tyrosine kinase inhibitors, such as gefitinib or erlotinib. EGFR mutations are generally detected in tumor tissue, should be adequate for pathologic review to evaluate cellular heterogeneity. If EGFR mutations can be observed in serum DNA, this could serve as a noninvasive source of information on the genotype of the original tumor cells that could influence treatment and the ability to predict patient response to tyrosine kinase inhibitors.
Materials and Methods: For this purpose; serum genomic DNA will obtain from 33 Turkish patients with NSCLC and EGFR exon 18–21 were amplified by nested PCR and specific mutations were detected by restriction fragment length polymorphism and results were confirmed by direct sequencing.
Results: We detected G719X mutation in exon 18, frame deletion in exon 19, T790M mutation in exon 20, L858R mutation in exon 21. EGFR mutations were detected in nine patients (27%).
Conclusion: Our results indicate that EGFR mutations can be detected from serum of the NSCLC patients, and this is a noninvasive and fast method for detection of EGFR mutations.

H15 DETECTION OF MITOCHONDRIAL DNA MUTATIONS IN BLADDER TUMOURS

[1]D Ergeç, [2]HH Tavukçu, [3]G Koç, [4]D Kıraç, [3]K Ulucan, [3]D Javadova, [2]L Türkeri, [3]Aİ Güney [1]*Faculty of Art and Sciences, Department of Molecular Biology and Genetics, Yeni Yüzyıl Üniversitesi;* [2]*Faculty of Medicine, Department of Urology, Marmara University;* [3]*Faculty of Medicine, Department of Medical Genetics, Marmara University;* [4]*Faculty of Medicine, Department of Biochemistry, Yeditepe University*

Introduction: Mitochondrial DNA (mtDNA) mutations have been described recently in various tumors; however, data focusing on bladder cancer are scarce. It has been suggested that analysis of mtDNA mutations might be useful to monitor the prognosis of bladder cancer and its response to certain therapies. To understand the significance of mtDNA mutations in bladder cancer development, we investigated mtDNA alterations in bladder cancer cases.
Materials and Methods: We studied mtDNA in 38 bladder tumors and 21 microdissected normal bladder tissue samples. Bladder cancer tissues were obtained by radical cystectomy or transurethral resection and genomic DNA was extracted from the samples. Mitochondrial genes *ATPASE6, CYTB, ND1,* and D310 region were amplified by PCR and then sequenced. We examined the relationship between mtDNA mutations and clinical parameters.
Results: We detected 40 mutations in our patient population. Our findings indicate that G8697A, G14905A, C15452A, and A15607G mutations are frequent in bladder cancers ($p < 0.05$) which belong to mtDNA haplogroup T. In addition, the incidence of A3480G, T4216C, T14798C, and G9055A mutations were higher in patients with bladder tumors. mtDNA mutations were not associated of with gender, tumor stage or tumor grade, recurrence and/or progression ($p > 0.05$).
Conclusion: In conclusion, the high incidence of A3480G, T4216C, G8697A, G14905A, T14798C, C15452A, A15607G, and G9055A mtDNA mutations in bladder cancer suggests that mitochondria could play an important role in carcinogenesis and mtDNA could be a valuable marker for early bladder cancer diagnosis. Especially, mtDNA haplogroup T may be important in terms of cancer risk. More extensive biochemical and molecular studies, especially in larger cohorts, are necessary to determine the pathological significance of these mutations.

H16 THE ROLE OF GLUTATHIONE S-TRANSFERASE M1, T1 AND P1 GENE POLYMORPHISMS IN SUSCEPTIBILITY TO CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

[1,2]B Batar, [1]M Güven, [3]T Celkan, [3]N Özdemir, [2]S Salman, [2]E Yosunkaya, [2]M Özen *[1]Department of Medical Biology, Cerrahpasa Faculty of Medicine, University of Istanbul, Istanbul, Turkey; [2]Department of Medical Genetic, Cerrahpasa Faculty of Medicine, University of Istanbul, Istanbul, Turkey; [3]Department of Pediatric Hematology-Oncology, Cerrahpasa Faculty of Medicine, University of Istanbul, Istanbul, Turkey*

Introduction: Interindividual differences in susceptibility to hematologic malignancies may be mediated in part through polymorphic variability in the bioactivation and detoxification. The glutathione S-transferases (GSTs) have been implicated as susceptibility genes in this context for a number of cancers. Polymorphisms within the phase II metabolizer enzymes GSTM1, GSTT1 and GSTP1 affect the body's ability to detoxify a range of potential leukaemogens encountered in the environment. The present investigation was conducted to determine the effects of GSTM1, GSTT1 and GSTP1 genetic polymorphisms on the risk of childhood acute lymphoblastic leukemia (ALL).
Materials and Methods: Our case-control study consisted of 95 patients with childhood ALL and 101 unrelated healthy individuals. Genotyping of polymorphisms in the GSTM1 and GSTT1 genes was performed by multiplex polymerase chain reaction (PCR), while the GSTP1 gene polymorphism was detected using Real Time-PCR (RT-PCR).
Results: There were no significant differences in the distributions of GSTM1, GSTT1 and GSTP1 genotypes between childhood ALL patients and the controls, either alone or in specific combinations.
Conclusion: Our results suggest that GSTM1, GSTT1, and GSTP1 genotypes do not influence susceptibility to childhood ALL. Larger studies will be needed to confirm these results.
Keywords: Childhood ALL, GST, PCR, polymorphism.

H17 MICROARRAY ANALYSES OF DIFFERENTIAL GENE EXPRESSION IN RNA INTERFERENCE-MEDIATED URG4 GENE SILENCED HEPG2 CELLS

[1]NL Satiroglu-Tufan, [2]Y Dodurga *[1]Department of Medical Genetics, School of Medicine, Pamukkale University, Denizli, Turkey; [2]Department of Medical Biology, School of Medicine, Pamukkale University, Denizli, Turkey*

Upregulated gene 4 (URG4, GenBank GeneID: 55665), a novel gene stimulated by HBxAg, is identified by PCR select cDNA subtraction, using HBxAg-positive and HBxAg – negative HepG2 cells. URG4 is located on chromosome 7 (7p13) and the full-length URG4 clone is 3.607 kb. It encodes a polypeptide of 922 amino acids with a molecular weight of 104 kDa. URG4 is strongly expressed in hepatitis B-infected liver and in HCC cells, where it costained with HBxAg, and weakly expressed in uninfected liver, suggesting that URG4 is an effector of HBxAg in vivo. Over-expression of URG4 in HepG2 and GES-1 cells promotes cell growth and survival in tissue culture and soft agar, and accelerates tumor development in nude mice, suggesting that URG4 may be associated with the onset of tumorigenesis. Over-expression of URG4 in osteosarcoma tissues is well correlated with tumor recurrence and metastasis, as well as with the proliferative activity of osteosarcoma cells. Patients with high expression of URG4 had shorter survival time, suggesting that URG4 may be a valuable prognostic marker in osteosarcoma patients. In the present study, differential gene expression analyses in RNA interference-mediated URG4 gene silenced HepG2 cells have been carried out by Affymetrix Human Genome U133 2.0 Plus chip- microarray platform. (This study has been supported by The Scientific and Technological Research Council of Turkey [TUBITAK], SBAG-104S259).

H18 NO ASSOCIATION BETWEEN INTERLEUKIN-1B C-31T POLYMORPHISM AND BREAST CANCER

[1]E Akbas, [1]N Eras-Erdogan, [1]B Arslan-Mamur, [2]O Turkmenoglu, [3]MA Sungur, [4]AA Ozcimen *[1]Faculty of Medicine, Department of Medical Biology and Genetics, Mersin University, Mersin, Turkey; [2]Faculty of Medicine, Department of General Surgery, Mersin University, Mersin, Turkey; [3]Faculty of Medicine, Department of Biostatistics, Mersin University, Mersin, Turkey; [4]Faculty of Science, Department of Biology, Mersin University, Mersin, Turkey*

Introduction: Interleukin-1β (IL-1β) is a potent proinflammatory cytokine that has been suggested to play a role in breast cancer development. In this study, we evaluated the association between the IL-1β C-31T polymorphism and breast cancer.
Material and Methods: Genotypes and allele frequencies were determined in 122 cases and 178 controls by using the polymerase chain reaction–fragment length polymorphism method.
Results: The frequency of the polymorphic T allele in the study population was similar for the cases and the controls: 49.2% and 51.1%, respectively ($p = 0.64$). Among the cases, the prevalence of the CC, CT and TT genotypes were 32.8%, 36.1% and 31.1%, while it was 20.2%, 57.3% and 22.5% among the controls, respectively. These differences were not significant ($p = 0.09$). Elevated risk was observed for women with late age at menopause (mean age at menopause: cases = 49.7 years, controls = 46.8 years ($p = 0.001$)).The presence of the IL-1β *T* allele was not associated with putative risk factors for breast cancer, such as early age at menarche, family history of breast cancer (first-degree relatives), a higher level of education, late age at first pregnancy, premenopausal status, and smoking status ($p > 0.05$).
Conclusion: Our study does not support an association between the IL-1β C-31T polymorphism and breast cancer risk.
Keywords: Breast cancer, Polymorphism, IL-1β.

H19 EXPRESSION AND AMPLIFICATION OF TOPOISOMERASE-2A IN TYPE 1 AND TYPE 2 PAPILLARY RENAL CELL CARCINOMAS AND ITS CORRELATION WITH HER2/NEU AMPLIFICATION

F Düzcan, SE Düzcan, S Şen, K Yörükoğlu, V Caner, N Şen Türk, GO Çetin, C Kelten, B Tuna, B Sarsık, E Tepeli *Department of Medical Genetics, Medical Pathology and Medical Biology, Pamukkale University, Denizli, Turkey; Department of Medical Pathology, Ege University, İzmir, Turkey; Department of Medical Pathology, Dokuz Eylül University, İzmir, Turkey*

The current study was undertaken to investigate chromosomal and genetic aberrations leading to over expression of TOP2α

and to reveal the possible association of these aberrations with HER2/neu over expression and gene amplification, and to search for the relationship between TOP2α and HER2/neu status with prognostic biomarkers such as nuclear grade and tumor stage in papillary RCC. Archival cases of papillary RCC obtained from Departments of Pathology of Pamukkale, Ege and Dokuz Eylul Universities were studied in two groups (type 1 and type 2) each containing 20 cases. The level of TOP2α and HER2/neu expression by tumor cells were determined immunohistochemically. A multicolor FISH probe was used to define both genes, and polysomy 17. The ratio of cells expressing TOP2α in type 1 and type 2 papillary RCC were 24.29% and 6.89%, respectively. The difference was statistically significant comparing the average or median values of groups separately ($p = 0.002$). FISH analysis did not reveal statistically significant difference between type 1 and type 2 tumors in terms of polysomy and amplification. TOP2α was synchronously amplified with Her2/neu in both groups. The most frequent finding detected by FISH method was polysomy of chromosome 17. Consequently, we had contradictory results compared with the findings reported in the limited numbers of literature. It showed us that papillary RCCs constitute a heterogeneous group of tumors with various cytogenetic features and morphological classification of these tumors may not be compatible with their molecular characteristics. (Present research has been supported by TUBITAK Health Sciences Research Group [SBAG] with Project number; 08S298 in 17/03/2009)

H20 PRESENCE OF 3'-*CBFB* GENE DELETION AND HOMOZYGOTE 9P21 DELETION TOGETHER IN A PATIENT WITH T-ALL

[1]O Görükmez, [1]Ö Görükmez, [1]T Yakut, [1]Ş Sağ, [2]B Baytan *[1]Medical Faculty, Department of Medical Genetics, Uludag University, Bursa, Turkey; [2]Medical Faculty, Department of Pediatric Haematology, Uludag University, Bursa, Turkey*

Acute lymphoblastic leukemias (ALL) account for 25% of childhood cancers and are classified as either B cell or T cell in origin. T-ALL, which occurs mainly in children and adolescents, accounts for 15% of all newly diagnosed pediatric ALL cases and is regarded as a high-risk disease with a relapse rate of 30% within 2 years after diagnosis. Cytogenetic abnormalities are found in bone marrow at diagnosis in approximately half of all T-ALL cases. Band p21 on chromosome 9 is one of the most common sites for chromosomal deletions and translocations in pediatric ALL cases (10%). Deletion of 9p21 has been associated with poor prognostic features, which include T-cell phenotype, high white blood cell counts, and the presence of a mediastinal mass at presentation. We present a 5-year-old girl diagnosed with T-ALL on the basis of bone marrow morphology and immunophenotyping. We detected homozygote deletion on 9p21 (with VYSİS LSI p16 [9p21]/CEP 9 probe) and deletion 3' CBFB Gene locus (with VYSİS LSI CBFB Dual Color Probe) by fluorescence in situ hybridization (FISH) in interphase cells. Molecular studies with reverse-transcriptase polymerase chain reaction (RT-PCR) analysis using specific primers for inversion 16 detected no CBFB-MYH11 fusion transcript. 16p and 16q subtelomere probes (Vysis) showed intact and normal terminal signals for chromosome 16 interphase spread. In this case FISH analysis was important for detection of major pathologies, because there was no metaphase at bone marrow culture. The case was contributed to the literature by having rare molecular cytogenetic pathologies of T-ALL

H21 JAK-2 (V617F) MUTATION IN MYELOPROLIFERATIVE DISORDERS IN TRAKYA REGION OF TURKEY

[1]H Tozkır, [2]O Kırkızlar, [3]H Gürkan, [2]S Akpınar, [1]E Göncü, [2]M Demir *[1]Department of Medical Biology, Trakya University School of Medicine Edirne, Turkey; [2]Department of Medicine, Division of Hematology, Trakya University School of Medicine, Edirne, Turkey; [3]Department of Medical Biology, Medical Faculty of the Istanbul University, Istanbul, Turkey*

Introduction: JAK2 kinase is a member of a family of tyrosine kinases involved in cytokine receptor signaling and activating somatic mutation involving the JH2 pseudokinase domain of Janus kinase 2 (*JAK*2 [V617F]) has been associated with myeloproliferative disorders.

In the current study, missense mutation in JAK2 gene (V617F) was performed in DNA samples from 87 patients (52 male [mean age: 53.3], 35 female [mean age: 61.4]) with myeloproliferative disorders with essential thrombocythemia (43), with polycythemia vera (33), with idiopathic myelofibrosis (11) and detected by Real Time polymerase chain reaction.

Results: Of the 87 patients, 49 (56.3%) had a JAK2 V617F missense mutation. Approximately 48.4% (16/33) of polycythemia vera, 60.5% (26/43) of essential thrombocythemia and 63.6% (7/11) of idiopathic myelofibrosis showed JAK2 V617F missense mutation (Table 1).

Conclusion: Our result shows that the prevalence of JAK2 V617F missense mutation in essential thrombocythemia was in correlation with other studies. However, the prevalence of JAK2 V617F missense mutation in polycythemia vera and idiopathic myelofibrosis were not comparable to studies of other groups from Worldwide. This state of clinical diagnostic criteria differs between other studies and / or could be due to small sample group. In addition, genetic screening and counseling should be offered to patients with myeloproliferative disorders routinely.

Table 1. JAK-2 (V617F) Mutation frequency in myeloproliferative disorders

	Polycythemia vera		Essential thrombocythemia		Idiopathic myelofibrosis		Total
	Male	Female	Male	Female	Male	Female	
JAK2 V617F (+)	8	8	13	13	3	4	49 (56.3%)
JAK2 V617F (-)	15	2	9	8	4	–	38 (43.7%)
TOTAL	23	10	22	21	7	4	87

H22 INVESTIGATION OF GST-P1 (IIE105VAL) GENE POLYMORPHISM IN CHRONIC MYELOID LEUKAEMIA

[1]M Karkucak, [1]T Yakut, [1]T Gulten, [2]R Ali *[1]Faculty of Medicine, Department of Medical Genetics, Uludag University, Bursa, Turkey; [2]Faculty of Medicine, Department of Internal Medicine, Uludag University, Bursa, Turkey*

Introduction: Associations between polymorphisms for genes encoding Glutathione S-transferases (GST) enzymes involved in Phase II detoxification reactions and susceptibility to several cancers have been shown in several studies. The aim of the present study is to investigate the influence of GST P1 IIe105-Val gene polymorphism in susceptibility to chronic myeloid leukaemia (CML).
Material and Methods: Seventy-one CML patients and 67 control subjects with no cancer history were enrolled in our study. PCR-restriction fragment length polymorphism method was used for GSTP1 lle105Val gene polymorphism. Genotype was determined according to the bands that formed in agarose electrophoresis gels. In statistical analysis, the level of significance was set at $p < 0.05$.
Results: The frequency of GSTP1 Val allele was found to be 22% in CML patients and 31% for controls**.** There was no significant difference for gene polymorphism of GSTP1 between patient group and control group ($p > 0.05$).
Conclusion: Our results showed that there was not any association between GSTP1 IIe105Val gene polymorphism and CML. However, these findings should be confirmed in studies with larger population because our patient numbers were limited
Keywords: Chronic Myeloid Leukaemia, GSTP1 gene, polymorphism.

H23 DETERMINATION OF EXPRESSION PROFILES OF ERCC1 STATUS AT PROTEIN, MRNA AND DNA LEVEL IN DIFFERENT STAGES OF NON-SMALL CELL LUNG CANCER

[1]E Tepeli, [2]V Caner, [3]N Büyükpınarbaşılı, [1]GO Çetin, [1]F Düzcan, [2]L Elmas, [2]G Bağcı *[1]Department of Genetics, Pamukkale University, School of Medicine, Denizli, Turkey; [2]Department of Medical Biology, Pamukkale University, School of Medicine, Denizli, Turkey; [3]Department of Pathology, Yedikule Chest Diseases Hospital, Istanbul, Turkey*

ERCC1 is an important DNA repair gene, playing critical role in nucleotide excision repair pathway and having a significant influence on genomic instability. Some studies support that ERCC1 might be a potential predictive and prognostic marker in non-small cell lung cancer (NSCLC). ERCC1 has also been shown to be a promising biomarker in NSCLC treated with a cisplatin-based regimen. Therefore, the determination of ERCC1 expression at protein, mRNA, and DNA level in different stages is important topics in the cancer. Ninety-one archival tissue samples, which were histopathologically diagnosed as NSCLC were examined in the study. ERCC1 expression at protein level was scored by immunohistochemistry. The gene amplification and mRNA expression levels for ERCC1 were determined by real-time quantitative PCR. There was complete concordance among the three methods in 39 tumor samples (42.9%). A strong correlation was found between DNA amplification and mRNA expression ($r = 0.662$) while there was no correlation between mRNA and protein assessment for ERCC1 expression ($r = -0.013$). ERCC1 expression at mRNA and DNA level (63.1% and 84.2%, respectively) in tumors at stage III was higher than at the other stage. In contrast, the protein expression at stage II and III (56.6% and 52.6%, respectively) of NSCLC was lower than that of tumors with stage I NSCLC. These results show that the mechanism by which ERCC1 expression might play a role in tumor behavior. This study was also confirmed that the appropriate validation and qualification in methods used for ERCC1 status were needed before its clinical application and implementation.
Keywords: ERCC1, Non-small cell lung cancer, IHC, Real-time quantitative PCR.

H24 DEREGULATED WNT SIGNALING IN CHILDHOOD T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

[1]M Sayitoglu, [1]O Hatirnaz, [1]Y Erbilgin, [1]S Ozdemirli, [1]N Mavi, [2]JJM van Dongen, [2,3]FJT Staal, [1]U Özbek *[1]Department of Genetics, Institute of Experimental Medicine Research, Istanbul University, Turkey; [2]Department of Immunology, Erasmus Medical Center, Rotterdam, The Netherlands; [3]Department of Immunohematology and Blood transfusion, Leiden University Medical Center, Leiden, The Netherlands*

Wnt signaling is essential for normal T-cell development in thymus. Deregulated Wnt signaling in the thymus causes development of tumors in murine models of T lineage acute lymphoblastic leukaemia (T-ALL). To investigate a potential role of Wnt signaling in T-ALL development we studied expression of several Wnt signaling pathway components and their targets in 71 childhood T-ALL by genome-wide expression analysis and quantitative real-time PCR. Increased levels of *CTNNB1* were found in the majority of T-ALL (89%) patients, which was also confirmed by western blot and immunofluoresence staining. Of note, *AXIN2*, the universal target gene of WNT pathway, was upregulated both in mRNA and protein levels around 40% of the patients compared to normal thymocytes. In cases with high *AXIN2* levels, microarray data revealed down regulation of genes expressed in more mature stages of T-cell development and this T-ALLs showed an immature phenotype. In addition, *CTNNB1, APC* and *AXIN1* gene mutations were found in 11% of the patients with increased Wnt signaling levels. However, such somatic mutations only explain a small part of the deregulated Wnt signaling, which is largely caused by high expression of *WNT3A* by T-ALL cells, likely functioning as an autocrine factor via *FZ-6* expression on leukemic cells. Together, these results demonstrate that Wnt activation occurs in a significant fraction of human T-ALL cases independent of known T-ALL risk factors.

H25 THE PREVALENCE OF JAK 2 (V617F) MUTATION IN PATIENTS WITH MYELOPROLIFERATIVE DISEASES

[1]GG Ceylan, [2]T Hacibekiroglu, [2]I Dilek *[1]Department of Medical Genetics, Ataturk Education and Training Hospital, Ankara, Turkey; [2]Department of Hematology, Ataturk Education and Training Hospital, Ankara, Turkey*

Introduction: The JAK2 (V617F) mutation is recurrent in polycythemia vera and essential thrombocythemia, which are

myeloproliferative neoplasms frequently associated with arterial and venous thromboembolism. This study was conducted to determine the prevalence of the JAK2 mutation in a small group of Turkish patients with myeloproliferative diseases (MPD) and to examine their disease profile.
Materials and Methods: Genomic DNAs from peripheral blood cells were extracted from 140 patients. JAK2V617F gene point mutation and its impact on peripheral blood cells were analyzed by using real-time PCR.
Results: The JAK2 mutation was detected in 27/140 (19.2%) patients. Males (51%) were more likely than females to have JAK2 mutation.
Conclusion: The prevalence of JAK2 mutations in the patients with myeloproliferative diseases was 19.2%. Patients with the JAK2 mutation were significantly more likely to have high-risk disease. Further studies are required to assess the role of JAK2 mutations in risk estimation in myeloproliferative diseases. The significance of screening for this mutation in myeloproliferative diseases like polycythemia vera and essential thrombocythemia cases needs to be analyzed in larger series.

H26 THE DETERMINATION OF RELATIONSHIP BETWEEN "EXCISION REPAIR CROSS-COMPLEMENTING GROUP 1" (*ERCC1*) GENE T19007C AND C8092A SINGLE NUCLEOTIDE POLYMORPHISMS AND CLINICOPATHOLOGICAL PARAMETERS IN NON-SMALL CELL LUNG CANCER

[1]E Koç, [1]V Caner, [2]N Büyükpınarbaşılı, [3]E Tepeli, [4]N Şen Türk, [3]G Çetin, [1]G Bağcı *[1]Department of Medical Biology, Pamukkale University, School of Medicine, Denizli, Turkey; [2]Department of Pathology, Yedikule Chest Diseases Hospital, Istanbul, Turkey; [3]Department of Genetics, Pamukkale University, School of Medicine, Denizli, Turkey; [4]Department of Pathology, Pamukkale University, School of Medicine, Denizli, Turkey*

The development of cancer is a multistep process and it has been reported that the different types of genes such as tumor suppressor genes, oncogenes and DNA repair genes including "Excision Repair Cross-Complementing Group 1" (*ERCC1*) are involved in human lung carcinogenesis. In this study, the determination of T19007C (rs11615) and C8092A (rs3212986) single nucleotide polymorphisms (SNPs) thought to affect mRNA stability of *ERCC1* and the assessment of any correlation between the genotypes and clinopathological parameters including age, sex, the history of smoking, TNM stage and histological subtypes were aimed. In this study, the archival formalin-fixed, paraffin-embedded tissues of 80 cases which were histopathologically diagnosed as non-small cell lung cancer (NSCLC) were used and T19007C and C8092A SNPs were analyzed using real-time PCR. Regarding T19007A SNP, the distribution of TT, TC, and CC genotypes was 40%, 44% and 16%, respectively. As for C8092A SNP, the distribution of CC, CA, and AA genotypes was 38%, 51% and 11%, respectively. No relationship was observed between C8092A SNP and clinicopathological parameters, but T19007A SNP was significantly associated with TNM stage ($p = 0.046$). This study indicated that T19007A SNP genotypes in this study were similar to those in Europe. It was also supported that the SNP may be associated with tumor progression in NSCLC.
Keywords: *ERCC1*, C8092A and T19007C SNP, Non-small cell lung cancer (NSCLC), real-time PCR.

H27 DETERMINATION OF O^6- METHYLGUANINE DNA METHYLTRANSFERASE PROMOTER METHYLATION PATTERN IN NON-SMALL CELL LUNG CANCER

[1]M Ekim, [1]V Caner, [2]N Büyükpınarbaşılı, [3]E Tepeli, [1]L Elmas, [1]G Bağcı *[1]Department of Medical Biology, Pamukkale University, School of Medicine, Denizli, Turkey; [2]Department of Pathology, Yedikule Chest Diseases Hospital, Istanbul, Turkey; [3]Department of Genetics, Pamukkale University, School of Medicine, Denizli, Turkey*

Aberrant methylation of promoter CpG islands of human genes including tumor suppressor genes, and DNA repair genes has been known as an alternative mechanism of gene inactivation and contributes to the carcinogenesis in many human tumors. Some published studies suggest that a relationship to exist between the methylation status of several genes and the prognosis in non-small cell lung cancer (NSCLC). The aim of this study was to determine the relationship between the methylation status of O^6- methylguanine DNA methyltransferase (MGMT) gene as a DNA repair gene and the clinicopathological characteristics including the age, gender, histological subtype, stage and smoking in patients with NSCLC. Eighty patients with NSCLC were included in this study. The analysis of DNA methylation was performed on formalin fixed paraffin embedded lung tissues samples. Following genomic DNA isolation and bisulfate-treatment, the DNA methylation of MGMT gene promoter region was analyzed by methylation-specific real-time PCR. DNA methylation was detectable with a frequency of 64% in our group. The percentage of the methylation was very high within all examined subgroups. These results were in concordance with other studies conducted in solid tumors including NSCLC. The analysis of DNA methylation in paraffin-embedded tissue samples of patients with NSCLC by methylation-specific real-time PCR is technically feasible. The higher methylation percentage of MGMT promoter region was a common result in patients with NSCLC. Further studies are needed to determine the prognostic potential of the DNA methylation status of the gene.
Keywords: MGMT, Non-small cell lung cancer (NSCLC), Promoter methylation, Methylation-specific real-time PCR.

H28 INVESTIGATION OF XRCC1 AND CCND1 POLYMORPHISMS IN TURKISH PATIENTS WITH PRIMARY GLIOBLASTOMA MULTIFORME

[1]T Yakut, [1,2]H Kocaeli, [1]M Karkucak, [1]T Gulten, [2]S Işık, [1]Ö Gorukmez, [2]E Korfalı *[1]Faculty of Medicine, Department of Medical Genetics, Uludag University, Bursa, Turkey; [2]Faculty of Medicine, Department of Neurosurgery, Uludag University, Bursa, Turkey*

Introduction: Polymorphisms of DNA repair gene XRCC1 and cell cycle checkpoint regulator gene CCDN1 have been found to be associated with susceptibility to various types of cancer. We examined the association between XRCC1 and CCDN1 genotype and the susceptibility to GBM in a Turkish population.
Material and Methods: To determine the association of the XRCC1 and CCND1 polymorphism on the risk of glioblastoma multiforme (GBM) in a Turkish population, a hospital-based case-control. The study was designed consisting of 51 subjects with GBM and 50 cancer free control subjects

matched on age and gender. XRRC1 genotypes (Arg/Arg, Arg/Gln,Gln/Gln) and cyclin D1 genotypes (AA, AG, GG) were determined using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis on genomic DNA.
Results: For XRCC1 gene the frequency of Arg allele was 74% and 78% in the study group and the control group, respectively ($p > 0.05$). The frequency of Gln allele was 26% and 22% in the study group and the control group, respectively ($p > 0.05$). For CCDN1 gene the frequency of A allele was 58% and 51% in the study group and the control group, respectively ($p > 0.05$). The frequency of G allele was 42% and 49% in the study group and the control group, respectively ($p > 0.05$). No statistical variation was determined between the GBM group and the control in terms of XRCC1 and CCDN1 polymorphisms.
Conclusion: Our results suggest that XRCC1 and CCND1 gene polymorphisms are not associated with an increased risk for the development of GBM in Turkish population.

H29 EVALUATION OF GLUTATHIONE-S-TRANSFERASES (GSTM1, GSTT1 AND GSTP1) AND TOLL LIKE RECEPTOR-9 GENE POLYMORPHISMS IN CERVIX CANCER

[1]B Kiran, [1,2]T Yakut, [1]H Ozan, [2]M Karkucak, [1]K Ozerkan, [2]S Sag *[1]Faculty of Medicine, Department of Obstetrics and Gynecology, Uludag University, Bursa, Turkey; [2]Faculty of Medicine, Department of Medical Genetics, Uludag University, Bursa, Turkey*

Introduction: In this study we aimed to evaluate the role of gene polymorphisms of glutathione-S-transferase M1 (GSTM1), glutathione-S-transferase T1 (GSTT1) and glutathione-S-transferase P1 (GSTP1) enzymes involved in the phase II detoxification reactions and 1237 timin/citosin (T/C) gene polymorphism of toll like receptor (TLR)-9 involved in immune response, at developing cervix cancer.
Material and Method: Forty-six patients and 52 control subjects with no cancer history were enrolled in our study. Multiplex polymerase chain reaction (PCR) was used to evaluate for gene polymorphisms of GSTM1 and GSTT1. PCR-restriction fragment length polymorphism (PCR-RFLP) method was applied for TLR 9 gene 1237 thymine/cytosine (T/C) polymorphism and GSTP1 gene polymorphism. Statistical analyses were performed at Uludag University, Biostatistics Department using SPSS version 13.0 program.
Results: No significant difference was present for gene polymorphisms of GSTM1, GSTT1, GSTP1 and TLR-9 gene 1237 T/C between patients group and controls ($p > 0.05$). In addition, it was not also found any statistical differences between gene polymorphisms of GSTM1 null, GSTT1 null, GSTP1 Ile/Val and GSTP1 Val/Val genotypes, and patient's pathological findings. For only vaginal involvement it was found higher risk ($p = .042$) in patients with TT allele than those of C allele when the patients pathological findings were compared with TLR-9 gene 1237 T/C polymorphism. No other statistical findings were detected regarding other parameters.
Conclusion: Our results showed that there was no any association between GSTM1, GSTT1, GSTP1 and TLR gene polymorphisms and cervix cancer. However, these findings should be confirmed in studies with larger population because our patient numbers were limited.

H30 DETECTION OF CRAB FUSION GENE IN PATIENTS WITH CML, AML, ALL AND CHRONIC MYELOPROLIFERATIVE DISEASES

[1]GG Ceylan, [2]T Hacibekiroglu, [2]I Dilek *[1]Department of Medical Genetics, Ataturk Education and Training Hospital, Ankara, Turkey; [2]Department of Hematology, Ataturk Education and Training Hospital, Ankara, Turkey*

Introduction: Chronic myeloid leukemia (CML) is characterized by the expression of the BCR/ABL1 fusion gene, a constitutively activated tyrosine kinase that commonly results from the formation of the Philadelphia (Ph) chromosome after a t(9;22)(q34;q11) or variant rearrangement. BCR ABL fusion protein comes in three forms: P190, P210 and P230 depending on the breakpoint on the BCR fragment. The P190 is form can be produced by alternative splicing from the P210 is form. It is generally associated with CML but it can also be associated with acute lymphoblastic leukemia (ALL). Quantization of BCR/ABL transcripts can monitor tumor load and the outcome of therapy.
Materials and Methods: A total of 39 patients came to our laboratory to detect the BCR-ABL fusion gene from hematology clinics. Twelve of them were CML, 20 were chronic myeloproliferative diseases (CMP), five were ALL and two were Acute Myeloid Leukemia (AML). Quantitative Real-Time PCR was performed for the detection of the fusion gene.
Results: BCR-ABL was positive in 9 (23%) patients. Eight of them were CML patients and one of them was CMP patient. It was negative in AML and ALL patients.
Conclusion: These data confirm that the intra-chromosomal genomic amplification of BCR/ABL1 that occurs in some CML patients is important for the treatment of the disease and at the follow-up time. The prevalence of this rearrangement identified in this small group demonstrates that the Ph chromosome may help in making early therapeutic decisions in CML, especially after molecular relapse.

H31 INVESTIGATING PARAOXONASE-1 GENE Q192R AND L55M POLYMORPHISM IN PATIENTS WITH RENAL CELL CANCER

[1]OU Ali, [2]M Kara, [2]D Erol, [1]A Ardıcoglu, [2]H Yuce *[1]Department of Urology, School of Medicine, Firat University, Elazig, Turkey; [2]Department of Medical Genetics, School of Medicine, Firat University, Elazig, Turkey*

It is already known that increased oxidative stress has an effect on carcinogenesis. Oxidative stress has also an effect on the development of renal cell carcinoma (RCC). One of these systems is paraoxonase enzyme which protects low density lipoproteins against oxidation. PON1 Q192R and L55M gene polymorphisms were researched in RCC patients in this study. Sixty patients with RCC and sixty healthy control groups were included in this study. Genotypes were found by PCR and Alw I restriction enzyme were used for Q192R polymorphism and Hsp92II restriction enzymes were used L55M polymorphism. Statistical significantly difference was found in patients with RCC and control group in PON1 Q192R polymorphism. There was significantly difference in patients and control group in Q and R allele frequency. Q allele was higher in patients group more than in the control group. R allele was higher in control group more than in the patients group. Between control and patients group no statistical difference was found in

L55M polymorphism. Additionally there was no statistical difference in L and M allele frequency. At the end of this study Q allele is higher and R allele is lower in patients group than in the control group and the difference in PON1 Q192R polymorphism between patient with RCC and the control group was showed. This result shows that R allele can be a protective factor in RCC. According to our results there was no relation diagnosed between RCC and PON1 L55M polymorphism.

H32 MULTIPLE MYELOMA RELATED WITH CHEMOTHERAPY OF BREAST CANCER: A CASE REPORT

[1]ET Elkiran, [2]M KARA, [3]A Gurel, [4]B Aygen *[1]Department of Internal Medicine and Medical Oncology, Medical Faculty, Firat University, Elazig, Turkey; [2]Department of Medical Genetics, Medical Faculty, Firat University, Elazig, Turkey; [3]Department of Internal Medicine, Medical Faculty, Firat University, Elazig, Turkey; [4]Department of Nephrology, Medical Faculty, Firat University, Elazig, Turkey*

Breast cancer is one of the most prevalent malignancies in the world and the most common cancer of women. In spite of its high prevalence mortality rates due to breast cancer has reduced because of the advances in screening and treatment. Multiple myeloma is a hematologic disorder characterized by monoclonal proliferation of plasma cells in the bone marrow that secrete immunoglobulins. For the diagnosis of myeloma it is necessary to detect > 10% plasma cells in bone marrow or tissue biopsy. Marinopoulos S. et al. reported a multiple myeloma case emerged after the chemotherapy composed of cisplatin, docetaxel, vinorelbine, topotekan for non small cell lung cancer. We report a 50-year-old woman presented with medullary breast carcinoma. She has taken neoadjuvant CEF chemotherapy regimen for three cycles before mastectomy and after the operation, she has taken CEF for three cycles again. She had achieved remission for 8 years and in her routine control we determined anemia and high globulin levels. Bone marrow aspiration and biopsy results were concordant with our clinical suspicion of myeloma and reported as plasma cell infiltration of bone marrow. In serum protein electrophoresis there was a gamma band spike. This patient's karyotype was 46, XX and in the end of cytogenetic analyses by FISH IgH/bcl2, t(11; 14)(q13; q32), t(4; 14)(p16.3; q32) 17p13 (p53), (13q14.3) were not detected. After the diagnosis of multiple myeloma melphalane-prednisolon chemotherapy regimen was performed and she has taken this therapy for four cycles. In addition to this chemotherapy we planned autologous bone marrow transplantation for this patient.

H33 GENOTYPING OF NQO1 IN HLH PATIENTS BY PYROSEQUENCING METHOD

[1]E Coskunpinar, [2]W Du, [2]A Niedehrfuhr, [2]EM Schneider, [1]U Ozbek *[1]Genetics Department, Istanbul University, DETAE, Istanbul, Turkey; [2]Genetics Department, Universitaetsklinikum Ulm, Experimentelle Anaesthesiologie, Ulm, Germany*

Hemophagocytic lymphohistiocytosis (HLH) is a rare, life threatening disease characterized by fever, cytopenias and hepatosplenomegaly, hyperferritinemia, hypertriglyceridemia and hypofibrinogenemia, in which the immune system becomes overactive due to its inability to effectively respond to infections and shut down the immune response to such infections (interferon gamma, IL-1, IL-6, TNF-alpha, GM-CSF), a high level of chain of the soluble interleukin 2 receptor (sCD25), hemophagocytosis in the bone marrow, cerebrospinal fluid or lymph nodes. The symptoms are caused by uncontrolled activation of T cells and macrophages and overproduction of inflammatory cytokines. In the classifications that have been identified until now, the presence of unknown mutations in the factors which are thought to cause HLH in perforin syntax, known as muncD mutations, are represented by a higher percentage value. The C609T gene polymorphisms on NQO1 are known to have major effects on HLH disease. In our study, blood samples from 31 patients and a control group consisting of 297 individuals were used and their DNA were isolated. The pyrosequencing method was used in genotyping after the region of the gene had been amplified using the PCR. Table I shows the frequencies of NQO1 genotypes and their respective alleles among patients and the controls. The CC genotype frequencies were found to be decreased, however, the TC genotype frequencies were increased in the patients when compared with the control group (p : 0.05; X^2 : 5.86). By increasing the number of cases of HLH the formation of the possible effects of these genes will be more conclusive.

Table 1. Distribution of NQO1 genotype frequencies in patients and control group

Genotypes/ alleles	Patient n (%)	Controls n (%)	p-value
NQO1			
CC	14 (45.16%)	190 (63.97%)	0.05, X^2:5.86
TC	16 (51.61%)	90 (30.30%)	
TT	1 (3.2%)	17 (5.7%)	
C	44 (70.96%)	470 (79.12%)	0.13, X^2 : 2.20
T	18 (29.03%)	124 (20.87%)	

H34 SURVIVIN GENE 3′UTR POLYMORHİSMS AND ASSOCIATION WITH LUNG CANCER

[1]N Akkaya, [1]E Coskunpinar, [2]E Aynaci, [2]P Yildiz, [3]A Turna, [1]İ Yaylim Eraltan *[1]Molecular Medicine Department, Istanbul University, DETAE, Istanbul, Turkey; [2]Yedikule Chest Diseases and Thoracic Surgery Training Hospital, Istanbul, Turkey; [3]Thoracic Surgery Department, Istanbul University, Cerrahpasa Medical School, Istanbul, Turkey*

Survivin gene polymorphism may affect the survivin production and activity, thus providing a sensitivity for the development of lung cancer. Survivin is usually expressed in embryonic tissues and observed during early embryonic death of homozygous mutation of this gene family in cell growth, differentiation and homeostasis both play an important role in this process. Survivin, is abundantly expressed in the cell cycle G2 / M phase and in G1 phase has a rapid regulation. This condition is controlled at the transcriptional level and the cell cycle elements (CDE) and cell cycle homology region (CHR) are localized in the proximal region of the survivin promoter. In this study, a possible association with survivin gene 3′UTR polymorphisms in lung cancer will be examined. In this study, 68 diagnosed lung cancer patients and 51 healthy controls were examined at the same clinic and they had no chronic illness or malignancy. For the genotyping studies, the PCR and restriction fragment length polymorphism (PCR-RFLP) meth-

ods were used. 3′UTR genotype distribution was in patient group (n = 68), GG: 21.3% (50), AG: 1.6 (18), AA: 0%, and in control group (n = 51), GG: 39.2% (20), AG: 58.8% (30), AA: 1.9% (1), (p = 0.0006). In the light of these results, in our study group AA genotype of the survivin gene is a risk factor for occurrence of the lung cancer in the Turkish population. This study will continue with a larger series of patients and controls in the next period.

H35 INVESTIGATION OF POSSIBLE RELATIONSHIP BETWEEN EPHX1 GENE POLYMORHİSMS AND COLORECTAL CANCER IN TURKISH SOCIETY

[1]Y Musteri, [1]E Coskunpinar, [2]S Arikan, [1]Z Erkisi, [1]İ Yaylim Eraltan [1]*Molecular Medicine Department, Istanbul University, DETAE, Istanbul, Turkey;* [2]*Thoracic Surgery Department, Istanbul training and research hospital, Istanbul, Turkey*

Colorectal cancer (CRC) is one of the most frequent cancers worldwide. Microsomal epoxide hydrolase (EPHX1) plays an important role in the metabolism of polyaromatic hydrocarbons (PAHs) and detoxification of procarcinomas. The best known polymorphisms are the exon 3 SNP which at position 113 (Tyr113His), and the exon 4 SNP at position 139 (His139Arg). The aim of this study is to investigate the association between CRC development and polymorphisms of these regions. Polymorphisms of EPHX1 exon 3 Tyr113His of 74 CRC patients and 84 controls were studied and polymorphisms of exon 4 His139Arg were studied of 76 CRC patients and 110 controls and PCR and restriction fragment length polymorphism (PCR-RFLP) method was used. EPHX1 exon 3 genotype distribution in the control group (n = 82) TT(46): 56%, TC(28): 34.1%, CC(8): 9.7%, and in the patient group (n = 74); TT(36): 48.6%, TC(27): 36.5%, CC(11): 14.9%were found to be (p = 0.52). EPHX1 exon 4 genotype distribution in the control group (n = 109), AA(68): 62.4%, AG(37): 33.9%, GG(4): 3.7%, and in the patient group (n = 76); AA(62): 81.6%, AG(12): 15.8%, GG(2): 2.6% was found to be (p = 0.01). In control group A: 78.89%, G: 21.10%, in patient group A: 89.47%, G: 10.5% was found (p = 0.007; x^2:7.17). In light of these results A allele and AA genotype in exon 4 region of the EPHX1 gene is a risk factor for occurrence of CRC. Our study continues by increasing the number of the patient group.

H36 KB1A NF-(IKB ALPHA) GENE POLYMORPHISM IN NON-SMALL CELL LUNG CANCER PATIENTS

[1]G Ozkan, [1]E Coskunpinar, [2]E Aynaci, [2]P Yildiz, [3]A Turna, [1]İ Yaylim Eraltan [1]*Istanbul University, DETAE, Molecular Medicine Department, Istanbul, Turkey;* [2]*Yedikule Chest Diseases and Thoracic Surgery Training Hospital, Istanbul, Turkey;* [3]*İstanbul University, Cerrahpaşa Medical School, Thoracic Surgery Department, Istanbul, Turkey*

Lung cancer, the overall cause of death after heart disease and cancer mortality by 28% is a multifactorial disease. Nuclear factor kappa b (NF-κB) proteins containing the Rel domain, which is defined as a protein family. NF-κB, in all cells in the cytoplasm, silent state in the nucleus only be activated when the switch and where the immune system, inflammation, cell growth, differentiation, apoptosis regulation, cytokine production, and neoplastic transformation responsible for the large number of gene expression is controlled. In this study, NF-κB in Turkish society between gene polymorphism and lung cancer, and prognosis of disease development was to investigate the possible relationship. Fifty-five diagnosed patients with lung cancer and 57 healthy controls were included in the study. For genotyping studies, polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) method was used. In patient group genotype distribution was (n = 55) 18.1% AA(10), 30.9% GG(17), 50.9% AG(28); and in control group (n = 57) 14% AA(8), 26.3% GG(15), 59.6% AG(34) (p = 0.64). Allele distribution for A and G allele in the patient group 33.3%, 66.3% and in the control group 27.19%, 72.80% was found to be (p = 0.29). In light of these results the relationship between NFKBIA gene polymorphism and lung cancer disease among the Turkish population is not statistically significant. Expanding the patient number may contribute to the interpretation of the data.

H37 AN IN VITRO INVESTIGATION OF THE APOPTOTIC EFFECT OF PERFLUOROOCTANESULPHONATE (PFOS) AND PERFLUOROOCTANOIC ACID ON AMNION CELL LINES

[1]N Aras-Ates, [2]S Karakas-Celik, [2]A Dursun [1]*Department of Medical Biology, Faculty of Medicine, Mersin University, Mersin, Turkey;* [2]*Department of Medical Biology, Faculty of Medicine, Zonguldak Karaelmas University, Zonguldak, Turkey*

Introduction: Fluorinated organic compounds, such as perfluorooctanesulphonate (PFOS), perfluorooctanoic acid, are stable chemicals with a wide range of industrial applications. PFOS change of inner mitochondrial membrane permeability has been implicated as a potential mechanism of toxicity. But there has been no study about apoptotic molecular mechanisms that underlie toxicity of PFOS and perfluorooctanoic acid. In this study, we research that PFOS and perfluorooctanoic acid effects the expression of apaf-1 and caspase-3 genes in the amnion cell line that initiate the cells to undergo apoptosis.

Materials and Methods: To determine the apoptotic gene expression in amnion cell line, amnion cells that obtained from Ankara Foot-and-Mouth Disease Institute collecting of cell culture (Hükük) was cultured. Total RNAs of amnion cells exposed different concentration PFOS and perfluorooctanoic acid was purified using a kit. The expression of caspase-3 and apaf-1 was determined using RT-PCR.

Results: In the study there is significant increase in expression of caspase-3 in amnion cell line exposed 500 μM perfluorooctanoic acid for 48 hours and 72 hours ($p < 0.05$). Also there is significant increase in expression of apaf-1 in amnion cell line exposed 100 μM PFOS for 48 hours ($p < 0.05$) and no significant increase in the other doses.

Discussion: In conclusion, apoptotic gene expression is increased in cells exposed PFOS and perfluorooctanoic acid by dose-dependent manner. So this work is the first study examining the apoptotic effect of PFOS and it will lead the way to the other topical studies.

H38 APPLICATIONS OF FLUORESCENCE IN SITU HYBRIDIZATION (FISH) FOR DETECTING GENETIC CHANGES IN HEMATOLOGICAL MALIGNANCIES

[1]D Taştemir, [2]O Demirhan, [3]E Gürkan, [2]E Tunç, [2]N İnandıklıoğlu *[1]Vocational School of Health Services, Adıyaman University, Adıyaman, Turkey[2]Department of Medical Biology and Genetics, Faculty of Medicine, Çukurova University, Balcali-Adana, Turkey[3]Department of Hematology, Faculty of Medicine, Çukurova University, Balcali-Adana, Turkey*

Fluorescence in situ hybridization (FISH) has become an important tool both for defining initial chromosomal abnormalities within a disease process, and for monitoring response to therapy as well as minimal residual disease. We report the results of interphase FISH (iFISH) analysis of 92 patients. We have used five different FISH probes to detect common cytogenetic rearrangements associated with CML, AML, ALL, CLL and MDS. A total of 83 patients were screened for BCR/ABL gene rearrangements. The great majority of the patients analyzed were CML patients (52.1%) and other patients were; AML (18.2%), ALL (14.3%) and MDS (16.7%). Displayed iFISH patterns of BCR/ABL gene rearrangements in 37.3% of patients (31/83) ranged between 10% and 98%. Among 48 CML cases, 25 were Ph positive, of which 23 cases (92%) were typical FISH pattern (1F2R1G), the other two cases showed two different types of atypical FISH pattern. In addition, while three patients and one patient with AML showed t(15;17) and inv(16;16) respectively, t(8;21) was not found. Furthermore, secondary chromosomal aberrations (6.5% of all cases) were clearly non random in the present study. The diagnosis of BCR/ABL gene rearrangements are likely become an important tool for the monitoring of therapies in patients with CML. Atypical patterns also may have clinical prognostic implications. Further studies in larger groups of patients are needed in order to elucidate the role of AML1/ETO, PML/RARA, CBFB and p53, and to identify the specific chromosomal regions and interacting genes involved in this process.

Keywords: Fluorescence In Situ Hybridization, Bcr/Abl, Gene Rearrangements.

H39 ABNORMAL SIGNAL PATTERNS INVOLVED IN T(12;21) TEL-AML1 IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS

[1]S Berker Karauzum, [2]C F Ozkaya, [1]S Yakut, [1]Z Cetin, [3]A Kupesiz, [3]G Tezcan, [2]O Altıok Clark, [3]V Hazar, [1]G Luleci *[1]Faculty of Medicine, Department of Medical Biology, Akdeniz Üniversity; [2]Faculty of Medicine, Department of Medical Genetics, Akdeniz Üniversity; [3]Faculty of Medicine, Department of Pediatric hematology, Akdeniz Üniversity*

The TEL (ETV6)-AML1 (RUNX1) chimeric gene fusion resulting from the translocation t(12;21) (p13;q22) constitutes the most common genetic abnormality in childhood B-cell precursor ALL. Although the translocation has been associated with good prognosis, deletion of the second TEL allele, gain of the second AML1 allele and duplication of the derivative chromosome 21 has been suspected to negatively influence outcome. We studied bone marrow samples from 15 patients with B-cell precursor ALL. In conventional cytogenetic analysis, no metaphases elevated in seven cases, karyotypes were found to be normal in 4 cases, numerical and structural abnormalities involved in chromosome of 12 were observed in other four cases. We used FISH to find out the translocation between chromosome 12p13 and 21q22. FISH analysis showed the classical TEL (ETV6)-AML1 (RUNX1) chimeric gene fusion in two cases whereas double chimeric gene fusion was observed in other two cases that one of them had wild type TEL allele deletion and the other one had wild type AML1 allele deletion. Not only the wild-type TEL but also AML1 allele was deleted in one t(12;21) positive patient. We found four cases with second TEL allele deletion without t(12;21) translocation. Loss of 12p, monosomy 12, t(6;12)(q16;p12.3) and t(6;9;12)(q13;p22;p13) translocations were observed in these four cases during cytogenetic analysis. One case died because of infection during remission, and other case died due to relapse. The remaining 13 patients are still in remission. Our data shows that deletion of wild type 12p13 locus with/without t(12;21) translocation in childhood patients seems to be good prognostic marker instead of negative influence of clinical outcome and also occurrence of the deletion of TEL allele in without t(12;21) translocation patients, can contribute the identification of new ETV6 partner chromosomes.

H40 CHARACTERIZATION OF THE MN1 PROMOTER REGION

[1]S Sirma, [2]A Kandilci, [1]U Özbek, [2]G Grosveld *[1]Istanbul University, Institute for Experimental Medical Research, Department of Genetics, Istanbul, Turkey; [2]St. Jude Children Research Hospital Department of Genetics and Tumor Cell Biology, Memphis, TN, USA*

MN1 encodes a transcriptional co activator, which is highly conserved among vertebrates. MN1 has no homology to other proteins and the gene is the target of the reciprocal chromosome translocation (12;22) (p13;q12) in some patients with acute myeloid leukemia (AML). In addition, expression profiling experiments showed that the level of MN1 mRNA was specifically elevated in BM samples of patients with inv (16) AML and in BM of AML patients of the M1 subtype. MN1 is a highly effective hematopoietic oncogene, which suggests that MN1 over expression is an important cooperative event in these human AMLs. An important question that remains to be solved is which molecular mechanism provokes MN1 over expression, as little is known about the transcriptional regulation of the gene. To define the transcription factors involved in regulating MN1 expression, we characterized the MN1 promoter region. We amplified a 2310 bp fragment upstream of the human MN1 gene by PCR. The full-length PCR fragment and its eight different 5′ and three different 3′ truncated fragments were cloned upstream of the Luciferase gene in the pGL3 vector. Luciferase assays of transiently transfected 5′-deleted promoter-reporter constructs demonstrated that the region between -74 and -411 bp is essential for the basal promoter activity of the MN1 gene in HeLa cells. Sequence analysis of this region revealed three consensus binding sites for the transcription factor SP1 as well as four potential GAGA boxes. No TATA box or CAAT box are present in this region. We found a CpG island, which spanned the core promoter and exon1. When the SP1 binding sites were mutated a two-fold reduction in promoter activity was observed. Computer-based motif analysis further recognized multiple putative hematopoietic transcription factor binding sites in the MN1 core promoter region including, WT1, E2F, GATA1, CEBPa, CEBPb, and MZF1. In conclusion our study identified that

sequences between -411 and -74 5′ of the MN1 gene have promoter activity and provides a first step in unraveling the molecular basis of MN1 over expression in AML.

H41 DETECTION OF CHROMOSOMAL ABERRATIONS IN MULTIPLE MYELOMA BY CYTOGENETIC AND FISH; PRELIMINARY STUDIES

[1]F Celep, [1]B Artan, [2]M Sonmez, [2]M Yilmaz, [3]U Cobanoglu, [1]A Karaguzel *[1]Faculty of Medicine, Department of Medical Biology, Karadeniz Technical University, Trabzon, Turkey; [2]Department of Haematology, Karadeniz Technical University, Trabzon, Turkey; [3]Department of Pathology, Karadeniz Technical University, Trabzon, Turkey*

Multiple myeloma (MM) is characterized by the neoplastic proliferation of a single clone of plasma cells producing a monoclonal immunoglobulin. Metaphase karyotyping provides additional prognostic information on tumor kinetics. FISH has been used as a diagnostic method since there are difficulties in obtaining sufficient metaphases with good quality from bone-marrow samples of MM patient using different probes. We have performed 33 bone marrow samples of untreated MM patients by cytogenetic evaluation and FISH. 11 patients (33%) could not be analyzed due to absence of metaphases. Conventional cytogenetic analysis could be performed in 22 patients (67%). Among these, eleven patients (50%) were normal either structurally or numerically. Eleven patients (50%) displayed hypodiploid/pseudodiploid karyotype. Recurrent numerical chromosomal aberrations were mostly observed in these patients. Specifically, monosomies, in many cases co-segregating together of chromosomes 7, 9, 10, 13, 18, 21 and 22 were found in 11 patients (50%) whereas trisomies of chromosomes 3, 8 and 9 were seen in 3 of 11 patients. In contrary, the most common chromosome aberrations found in 5 and 4 of 11 hypodiploid/pseudodiploid patients were monosomy 7 and 13, respectively. FISH analyses performed for chromosome 9, 11, 21 and 22 on cases which detected numerical aberrations by cytogenetic analysis and examined a 13q14.3 region aberration which is one of the prognostic factors in MM. Our results revealed that conventional cytogenetics remains an important tool in elucidating the complex and diverse genetic anomalies of MM and FISH analyses should be performed in conjunction with conventional cytogenetic analyses for prognosis in MM cases.

H42 MUTATION SCREENING OF ING1 GENE AND ANALYSIS OF ING1 MRNA EXPRESSION IN BLADDER CANCERS

[1]M Igci, [1]A Arslan, [2]B Cengiz, [3]S Erturhan, [1]S Oztuzcu, [1]Z Eslik, [1]YZ Igci, [1]B Gogebakan, [4]M Karakok, [1]EA Cakmak, [5]C Camci *[1]Faculty of Medicine, Departments of Medical Biology, Gaziantep University, Gaziantep; [2]Faculty of Medicine, Departments of Physiology, Gaziantep University, Gaziantep; [3]Faculty of Medicine, Departments of Urology, Gaziantep University, Gaziantep; [4]Faculty of Medicine, Departments of Pathology, Gaziantep University, Gaziantep; [5]Faculty of Medicine, Departments of Oncology, Gaziantep University, Gaziantep, Turkey*

Introduction: Members of ING family are discovered as tumor suppressor genes in different cancer types. ING1 has multiple cellular functions such as growth regulation, apoptosis, senescence and DNA repair.

Materials and Methods: In this study, 30 paired normal and tumor tissue samples were used for *ING1* expression analysis and *ING1* mutation screening via nucleotide sequence analysis. The promoter sequence was analyzed via Genomatix-MatInspector and TFSEARCH programs to predict the transcription factors which bind to *ING1* promoter.

Results: Seven of 30 cases showed alterations in expression analysis. However, no mutation was detected in the exons of *ING1*. A significant TSG activity of *ING1* in bladder cancer was not observed while higher activity was reported in different cancer types.

Conclusion: Although infrequently mutated in human cancers, down-regulation of ING proteins has been observed in a variety of tumor types. Because there is no data available about the promoter of *ING1*, the transcription factors and regulatory molecules associated with *ING1* promoter, it is hard to interpret the down-regulation. As a result of promoter analysis, it is suggested that c-Rel, c-Ets, ABL, E2F, HIF1, p53, NRSF, SOX, BPTF were some of the factors associated with the promoter region. Additionally, methylation status of *ING1* promoter has not been determined in bladder tumors yet. Molecular analysis of *ING1* promoter warrants further analysis.

H43 LOSS OF HETEROZYGOSITY AT CHROMOSOME 13Q33-34 REGION IN BLADDER TUMORS

[1]M Igci, [1]A Arslan, [2]B Cengiz, [3]S Erturhan, [1]S Oztuzcu, [1]YZ Igci, [4]M Karakok, [1]EA Cakmak, [5]C Camci *[1]Faculty of Medicine, Departments of Medical Biology, Gaziantep University, Gaziantep; [2]Faculty of Medicine, Departments of Physiology, Gaziantep University, Gaziantep; [3]Faculty of Medicine, Departments of Urology, Gaziantep University, Gaziantep; [4]Faculty of Medicine, Departments of Pathology, Gaziantep University, Gaziantep; [5]Faculty of Medicine, Departments of Oncology, Gaziantep University, Gaziantep, Turkey*

Introduction: In Turkey, the frequency of bladder cancer is the third among other cancer types. Cancer is a result of the accumulation of genetic and epigenetic alterations in the cell which can lead to activation of oncogenes or inactivation of tumor suppressor genes (TSG) loss or inactivation of which can cause formation of tumors. LOH leading to inhibition of TSGs has been shown to be one of the mechanisms of tumor development.

Materials and Methods: In this study, 30 paired normal and tumor tissue samples were used for microdeletion analysis of chromosome 13q33-34 region. DS markers used for 13q33-34 region were D13S285, D13S1315, D13S796, D13S278, D13S158, and D13S779.

Results: Loss of heterozygosity (LOH) results were as 23.3%, 20%, 6.7%, 3.3%, 6.7%, and 0%, respectively. The highest LOH scores were obtained with markers D13S285 and D13S1315.

Conclusion: As for the LOH data 13q33-34 region may contain different candidate TSGs like ING1, COL4A1, COL4A2 and SOX1. ING1 plays a significant role in cellular activities such as growth regulation, apoptosis, senescence and DNA repair which are all characteristics typical of tumor suppressors. COL4A1 and COL4A2 are major components of the vascular basement membrane which constitutes an insoluble

structural wall of newly formed capillaries and is speculated to play an important role in regulating pro-/anti-angiogenic events. Type IV collagen promotes cell adhesion, migration, differentiation, and growth. α1(IV)NC1 was discovered as an antiangiogenic molecule with significant antitumor activity. SOX1 is an important transcription factor in the developmental process. It was reported that expression of SOX1 attenuates the tumorigenic potential of neuronal precursors after neural stem cell transplantation.

H44 CLINICAL APPLICABILITY OF A SCREENING METHOD BASED ON DETERMINING FANCD2 MONOUBIQUITINATION IN FANCONI ANEMIA

BS Ada, T Tuncalı, HI Ruhi, HG Karabulut, NY Kutlay, A Vicdan, A Tükün *Medical Genetics Department, Ankara University Faculty of Medicine, Ankara, Turkey*

Fanconi Anemia (FA) is an autosomal or X linked recessive cancer prone disease that has cellular susceptibility to crosslink agents and ionized radiation. FA originates from mutant Fanconi pathway assigned proteins. Thirteen proteins have been reported in FA pathway which work in three subgroups: core complex, ID complex and FA downstream proteins. Eight of FA proteins (A, B, C, E, F, G, L, M) together with FAAP24 and FAAP100, form FA core complex. In the S phase of cell cycle, FA core complex monoubiqinates FANCD2 and FANCI starting the repair process by accommodating the ID complex together with the FA proteins effective on downstream pathways and DNA repair proteins. The molecular analysis of the disease is difficult and expensive due to complexity of FA pathway, diversity of genetic structure and the absence of a genotype–phenotype correlation. DEB screening test is regarded as the gold standard. The breakage in chromosomes is determined in peripheric blood cultures, spontaneous and DEB stimulated. In FA the break ratios are 0.02–0.85 and 1.06–23.9 per cell in spontaneous and DEB stimulated cultures respectively. The cases with 0.10–1.00 break per cell in DEB stimulated cultures may cause contradiction. Having been firstly suggested by Akiko Shimamura et al. in 2002, the monoubiquination of FANCD2 has been reported as an alternative to DEB test. In this study patients with FA prediagnosis were analyzed for the monoubiqutiniation of FANCD2 protein, a new screening method for FA. The possibility of replacing this method with DEB screening test as a proposal in FA diagnosis algorithm has been questioned according to the results of this study.

H45 GENOME WIDE EXPRESSION ANALYSIS IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA (T-ALL)

[1]O Hatırnaz, [1]M Sayitoğlu, [1]Y Erbilgin, [2]FJT Staal, [3]JJM van Dongen, [1]U Özbek *[1]Department of Genetics, Institute of Experimental Medicine Research, Istanbul University, Istanbul, Turkey; [2]Department of Immunology, Erasmus Medical Center, Rotterdam, The Netherlands; [3]Department of Immunohematology and Bloodtransfusion, Leiden University Medical Center, Leiden, The Netherlands*

T-lymphocytes are developed via precursor cells in bone marrow and migrate to thymus by different signals and mature there. Childhood T-ALL occurs in 10–15% of all childhood leukemia and treated as high-risk group patients. Many different pathways and signaling molecules found to play a role in T-ALL development, but there is still not enough data to clarify the mechanism. According to these findings we aimed to study the expression profiles of 33 Turkish childhood T-ALL patients. As control, thymocyte subsets were used (SP4, SP8, ISP, DPCD3 + /CD3-, DPCD3-) to represent different steps of T-cell development. Following the RNA isolation, cRNA synthesis was performed, biotin labeled cRNAs were fragmented and hybridized overnight to GeneChip HU-133 Plus2.0 (Affymetrix, USA) microarray chip and scanned on GeneChipScanner3000 (Affymetrix). Raw microarray data is normalized by the RMA method. Following normalization, the analysis was performed using BRB Array tools 3.7.1. The data that shows less than 20% expression values, with at least a two-fold change in either direction from the genes median value is excluded. Two-way clustering algorithm, complete linkage, Euclidian distance correlation was used to cluster genes and samples. Patients and control subsets were distributed differentially in the dendogram. Furthermore a T-cell specific tumor-suppressor gene, was down-regulated in patients when compared to control subsets. This down regulation was also confirmed by real-time PCR in T-ALL cell lines. Real time PCR analysis are ongoing for a wide range of patient samples and the expression of the candidate gene is being detected in normal thymus tissue and primary patient materials.

H46 DETECTION OF MICRORNA (MIRNA) GENE EXPRESSION DIFFERENCES BY USING STEM-LOOP RT-POLYMERASE CHAIN REACTION (PCR) METHOD

[1]N Mavi, [1]M Sayitoglu, [1]O Hatirnaz, [1]U Ozbek *[1]Institute for Experimental Medical Research*

MicroRNAs (miRNAs) are small non-coding RNAs of 18–25 nucleotides in length that regulate expression of target genes through sequence specific hybridization to the 3′untranslated region (UTR) of messenger RNAs and either block translation or direct degradation of their target messenger RNAs. In our previous studies we showed that WNT signaling is deregulated in childhood leukemias and the key molecule Beta-catenin is highly upregulated. To clarify the role of miRNA in this up regulation, we aimed to detect CTNNB1 specific miRNAs gene expressions by Stem-Loop RT-PCR method. First, we performed bioinformatic analyses and found out that mature hsa-miR-214 is CTNNB1 specific miRNA. Total RNA was isolated by trizol method from the diagnostic material of B-cell and T-cell ALL patients bone marrow samples. Four cell lines were also used as controls (Reh, SupB27, DND41, Molt4). Following the RNA isolation miRNA specific cDNA synthesis was performed by Stem-Loop PCR method using a consensus RT primer and miRNA specific forward primer. After the synthesis of miRNA we performed real time PCR to detect the gene expression levels. Relative hsa-miR-214 levels were found to be significantly decreased in ALL patients and cell lines. In ALL patients expression levels were lower than cell lines. These results confirmed our hypothesis that hsa-miR-214 is related to CTNNB1 which we found significantly upregulated in our previous findings. This method can be easily used to examine the relative expression levels of miRNAs. Further experiments will be done to confirm these findings.

H47 INVESTIGATION OF POSSIBLE RELATIONSHIP BETWEEN SURVIVIN GENE PROMOTER POLYMORPHISMS AND LUNG CANCER

[1]A Eren, [1]E Coskunpinar, [1]C Kelten, [2]E Aynaci, [2]P Yildiz, [3]A Turna, [1]I Yaylim Eraltan *[1]Molecular Medicine Department, Istanbul University, DETAE, Istanbul, Turkey; [2]Yedikule Chest Diseases and Thoracic Surgery Training Hospital, Istanbul, Turkey; [3]Thoracic Surgery Department, Istanbul University, Cerrahpasa Medical School, Istanbul, Turkey*

The survivin gene is located on human chromosome 17q25 which is composed of 142 amino acids. Survivin is one of the first reported inhibitors of apoptosis proteins (IAPs), which is an important family of proteins that regulate apoptosis. A common polymorphism at the survivin gene promoter (-31 G/C) has been shown to influence survivin expression and the risk for cancer. The genetic variant -31G/C in the survivin promoter region has been identified to be associated with over expression of survivin at both protein and mRNA levels in cancer cells. The over expression of survivin was found to be associated with disease development, recurrence, and prognosis in various malignancies, including cancers. Survivin gene polymorphism may affect the survivin production and activity, thus providing a sensitivity for the development of lung cancer. In this study, 44 diagnosed lung cancer patients and 47 healthy controls were examined. They had no chronic illness or malignancy. For the genotyping studies, the polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) methods were used. Promoter (-31G\C) genotype distribution was in patient group (n = 44), GG: 72% (32), CG: 18% (8), CC: 9% (4), and in control group (n = 47), GG: 74% (37), CG: 20% (10), CC: 0% (0), (p = 0.105). In light of these results, in our study group CC genotype of the survivin gene is a risk factor for occurrence of the lung cancer in the Turkish population. This study will continue with a larger series of patients and controls in the next period.

H48 EVALUATION OF BRG1 MRNA EXPRESSON WITH CLINICOPATHOLOGICAL DATA IN ORAL SQUAMOUS CELL CARCINOMAS

[1]E Gunduz, [1,2]M Gunduz, [3]L Beder, [1]K Demircan, [4]K Shimizu *[1]Departments of Medical Genetics, Faculty of Medicine, Fatih University, Ankara; [2]Departments of Otolaryngology, Faculty of Medicine, Fatih University, Ankara, Turkey; [3]Department of Otolaryngology, Wakayama Medical University, Japan; [4]Department of Molecular Genetics, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Japan*

Activation of oncogenes and inactivation of tumor suppressor genes (TSGs) is a critical step during carcinogenesis. Inactivation of TSGs occurs through deletion of one allele and mutation in the other allele or decreased mRNA expression. Loss of heterozygosity (LOH) analysis is a sensitive method to detect deletions of specific chromosome regions, which are considered to harbor putative TSGs. By this method we previously demonstrated the frequent deletions of several chromosomal loci and identified candidate TSGs such as ING1, ING3, ING4, Caspase-6 and BRG1 in head and neck cancer. On the other hand, recent researches showed that alterations of chromosomal loci and genes both at genetic (microdeletion, chromosomal rearrangement etc) and epigenetic (methylation, decreased mRNA expression etc) could be used as a predictive marker for the prognosis of the patients, for the behaviour of the tumor and its response to treatments such as chemotherapy and radiotherapy. We recently detected high allelic loss of 19p13 region and identified BRG1 gene as a candidate TSG in 39 oral cancer samples. In that study, we also analyzed mRNA expression status in these cancer samples. In the current study, we analyzed the clinicopathological data of the patients and compared with mRNA expression status of BRG1. Our results demonstrated that increased expression of BRG1 could be linked to worse prognosis contrary to our expectations. Patients with increased BRG1 expression revealed worse survival and more recurrence. This result could be related with selective deletion of splicing variants of BRG1 in cancer and its possible nucleocytoplasmic translocation and altered mRNA expression level. In conclusion, the current study suggests that level of BRG1 mRNA expression could also be used as a predictive marker in oral cancer.

H49 CURCUMIN AND BAY 117085 ATTENUATES ADAMTS9 GENE EXPRESSION IN HUMAN CHONDROCYTES

[1]K Demircan, [2]S Hirohata, [1]E Gündüz *[1]Department of Medical Genetics, Faculty of Medicine, Fatih University, Ankara, Turkey; [2]Molecular Biology and Biochemistry Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama, Japan.*

We previously demonstrated that ADAMTS9 is an IL-1beta- and TNF alpha-inducible gene that appears to be more responsive to these proinflammatory cytokines than are other aggrecanase genes. Furthermore, these cytokines had a synergistic effect on ADAMTS9 (Arthritis Rheum, 52, 2005), and ADAMTS9 activation regulated via NFATc1 in OUMS-27 chondrosarcoma cells and in human chondrocytes (Mol Cell Biochem, 323, 2009). Researchers suggest that the inhibition of ADAMTSs in musculoskeletal diseases will form part of arthritis therapies. ADAMTS9 was first discovered in 2000 and has many physiological functions in the human body. ADAMTS9 is a matrix metalloproteinase and plays a critical role in the remodeling of extracellular matrix by cleaving aggrecan and versican. ADAMTS9 also plays a role in certain cancers as a putative tumor suppressor gene, but its signal transduction mechanism has not been clarified yet. Many reports demonstrated some relationship between arthritis related genes and nuclear factor-κB (NF-κB) signalling pathway. Studies with curcumin (one of the NF-κB inhibitors) and green tea extract recently focused on ADAMTS genes. NF-κB plays a role in gene regulation in inflammation. As interleukin 1 (IL-1 beta) is known to exert numerous effects on cartilage metabolism through the NF-κB pathway, we hypothesized that NF-κB inhibitors is involved in ADAMTS9 gene regulation. Human chondrocytes were cultured in the presence of IL-1beta with specific inhibitors of NF-κB signaling pathways. ADAMTS9 mRNA levels were quantitatively measured by real time PCR. NF-κB phosphorylation was checked by western blotting. Real time PCR results showed that NF-κB pathway inhibitors (Curcumin and BAY 117085) attenuated cytokine-induced ADAMTS9 mRNA expression very effectively. Curcumin attenuated NF-κB phosphorylation in a dose dependent manner, not BAY 117085. These results indicated that NF-κB may play an important role in IL-1beta – induced expression of ADAMTS9 in human chondrocytes. Collectively

NF-κB pathway inhibitors are suggested as therapeutical agents for the treatment of cartilage diseases.

H50 ASSOCIATION OF HPC2/ELAC2 AND SRD5A2 POLYMORPHISMS WITH PROSTATE CANCER

[1,2]M İzmirli, [3]B Arıkan, [1]Ü Kılıç, [2]D Alptekin *[1]Department of Medical Biology, Bezmiâlem University, İstanbul, Turkey; [2]Department of Medical Biology, Çukurova University, Adana; [3]Department of Molecular Biology, Çukurova University, Adana, Turkey*

Prostate cancer can be determined as the alteration of the balance between cell proliferation and cell death in the prostate bladder which causes malign increase of the organ volume. The epidemiologic factors causing prostate cancer can be hormonal, diet, environmental and genetical. There are a number of genes causing prostate cancer. Some of them are ELAC2, 5α-reductase type II (SRD5A2), HPC1, PCAP, CAPB, HPCX, AR and PSA genes. The ELAC2 gene has been mapped to chromosome 17p11.2 and SRD5A2 gene located at chromosome 2p22-23. The aim of this study is to investigate relationship between Ser217Leu and Ala541Thr polymorphisms of the ELAC2 gene and Ala49Thr and Val89Leu polymorphisms of the SRD5A2 gene and prostate cancers. For this purpose, regions where polymorphisms are located in were amplified with PCR method. Polymorphisms were determined by using available restriction enzymes with Restriction Fragment Length Polymorphism (RFLP) method. A control group was also included in the study. In this study, a statistically significant relationship has been found between ELAC2 gene Ser217Leu ($p = 0.004 < 0.05$) and SRD5A2 gene Ala49Thr ($p = 0.002 < 0.05$) polymorphisms and prostate cancers. However, the relationships between Ala541Thr ($p = 0.668 > 0.05$) polymorphisms of ELAC2 gene and Val89Leu ($p = 0.587 > 0.05$) polymorphisms of SRD5A2 gene and prostate cancer has not been found statistically significant.

Keywords: Prostate cancer, ELAC2 gene, SRD5A2 gene.

H51 INVESTIGATION OF MIR-21, MIR-141 AND MIR-221 IN BLOOD CIRCULATION OF PATIENTS WITH PROSTATE CANCER

[1]M Kovancılar, [2]F Yaman, [2]E Darendeliler, [2]Y Dizdar, [1]N Dalay, [1]U Deligezer *[1]Department of Basic Oncology, Istanbul University Oncology Institute, Istanbul, Turkey; [2]Department of Radiation Oncology, Istanbul University Oncology Institute, Istanbul, Turkey*

Introduction: To predict the progression of prostate cancer (PCa) novel predictive markers are needed. One of the emerging fields in cancer research is microRNAs (miRNAs). miRNAs are small non-protein coding molecules that regulate basic cellular processes and are frequently dysregulated in cancer. A possible use of the circulating miRNAs in blood of cancer patients as potential diagnostic/prognostic biomarkers is one of the active research fields. Our aim was to investigate whether three cancer-associated miRNAs, miR-21, -141 and -221, in blood circulation would enable to discriminate metastatic PCa from localized disease.

Patients and Methods: Peripheral blood was drawn the patients (n = 51) at the end of the therapy, the results were comparatively analyzed in subgroups of patients with local/local advanced (n = 23) or metastatic PCa (n = 25). We extracted total RNA from plasma, and used special cDNA synthesis kit and assays to quantitate miRNAs.

Results: Levels of miR-21 and -221 in the patients were higher than in healthy controls ($p = 0.001$) while for the miR-141 no difference was observed ($p = 0.2$). The pattern of three individual miRNAs among the patients were also different ($p = 0.001$). The miR-21 displayed highest levels with a median of 1.51; miR-221 intermediate levels (median 0.71) while the miR-141 displayed lowest levels (median 0.051). All three miRNAs were present in significantly higher levels in the circulation of the patients with metastasis than in those with local/local advanced PCa.

Conclusion: In conclusion, the analysis of miR-21, -141 and -221 in circulation of PCa patients at the post-treatment phase allows to distinguish metastatic PCa from localized/local advanced disease.

H52 IN OUR MYELODYSPLASTIC SYNDROME CASES, THE POSSIBLE ROLE OF AUTOUMMINE CONDITIONS IN DISEASE PROGRESSION

[1]S Guran, [2]S Kozan, [2]D Torun, [3]C Beyan, [2]Y Tunca, [3]A Ifran, [3]K Kaptan, [3]AU Ural, [3]O Nevruz, [3]F Avcu, [3]T Cetin, [4]AE Kurekci, [1]YK Terzi, [2,5]D Gül, [2]M Bahce *[1]Department of Medical Biology, Gülhane Military Medical Academy, Etlik-Ankara; [2]Department of Medical Genetics, Gülhane Military Medical Academy, Etlik-Ankara; [3]Department of Haemataology, Gülhane Military Medical Academy, Etlik-Ankara; [4]Pediatric Haematology, Gülhane Military Medical Academy, Etlik-Ankara; [5]High Science Council Member, Gülhane Military Medical Academy, Etlik-Ankara, Turkey*

The myelodysplastic syndromes (MDS) are a heterogeneous group of disorders characterized by ineffective hematopoiesis, impaired maturation of hematopoietic cells, progressive cytopenias, and dysplastic changes in the bone marrow. This syndrome can generally be seen in elderly patients. Although the possible etiologic factor is not known in most cases, a drug used in a treatment protocol may cause this syndrome (seconder MDS-sMDS) in few cases. Some cases may transform to acute myeloid leukemia (AML). In these cases, the prognosis is worse (AML/MDS). The genetic tests are important for the diagnosis and prognosis. In this manuscript, the possible role of the diseases in the progression of MDS and AML transformed from MDS (MDS/AML) were analyzed from the pedigrees of all families. All the required genetic tests had been applied for the diagnoses of 71 MDS, MDS/AML patients. Among 71 MDS and MDS/AML patients 45.07% RCUD, 1.40% RARS, 35.21% RCMD, 15.48 RAEB, 2.81% MDS associated with isolated del (5q) had been diagnosed. Twenty-one patients (29.58%) revealed different cytogenetic abnormalities. MDS/AML had been diagnosed in nine patients, six of which had chromosomal abnormalities classified in ''poor'' prognostic risk group and in one which had chromosomal abnormality classified in intermediate risk group. Only in two cases, classified in good prognostic group with – Y clonal chromosomal abnormality and normal karyotype, AML progression had been observed. Three cases classified in poor prognostic risk group had been survived without any progression. In eight patient/families (11.26%), autoimmune diseases had been diagnosed including rheumatoid arthritis (RA), scleroderma, psoriasis, Sjogren syndrome. The MDS patients

in our series who had autoimmune diseases may not be correlated with any subgroup of MDS and good/poor prognosis. Only one patient with Sjogren syndrome and 47, XX, +22 findings, had MDS/AML diagnoses in disease progression. According to our findings, autoimmune diseases were significantly high in our case/family histories. Medications used in the treatment of autoimmune diseases may be the cause of s MDS but the medications used in these cases may not be the possible cause of all these MDS cases. Moreover, genetic predisposition to the autoimmune diseases may be another risk factor in the progression of MDS.

H53 ANALYSIS OF GENETIC VARIATIONS CAUSED BY ENVIRONMENTAL FACTOR AFLATOXIN IN HEPATOCELLULAR CARCINOMA

[1]CB Akyerli, [1]D Agirbasli, [3]T Acun, [4]O Sahin, [1,2]MC Yakicier *[1]Department of Medical Biology, School of Medicine, Acibadem University, Istanbul, Turkey; [2]GENETIKA Genetic Laboratories, Istanbul, Turkey; [3]Department of Molecular Biology and Genetics, Bilkent University, Ankara, Turkey; [4]German Cancer Research Center (DKFZ), Heidelberg, Germany;*

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third leading cause of cancer death worldwide. Despite recent advances in the diagnosis and treatment of HCC, it has a poor prognosis and therefore, it is very important to understand its molecular pathogenesis. HCC often contains a somatic mutation at codon 249 in TP53 (R249S). There is evidence that R249S occurs as the result of mutagenesis by aflatoxin in a context of HBV chronic infection. The aim of our study is to investigate the other genetic variations caused by environmental factor aflatoxin. We have sequenced the most common 115 cancer genes by using FEBIT's highly automated technology in MAHLAVU and PLC/PRF cell lines both known to carry TP53 R249S mutation. Many missense and frameshift mutations were found to be common in both cell lines. Three genes (*FANCD2* – Fanconi anemia, complementation group D2; *NF1* – Neurofibromatosis 1; *SDHC* – succinate dehydrogenase complex, subunit C, integral membrane protein, 15 kDa) carrying G->T mutations were selected for further analysis. Our experiments were extended to include 18 HCC patient samples. Eight of these patients had TP53 R249S mutation and ten of them carried no TP53 mutation, but they were obtained from a region that has high aflatoxin exposure. *FANCD2*, *NF1* and *SDHC* genes were screened in all HCC patients by sequencing analysis. As a result, no mutations were detected in these targeted genes. Our investigations continue to cover further analysis of the remaining genes.

H54 LINCRNA EXPRESSION IN HEPATOCELLULAR CARCINOMA

[1]D Agirbasli, [1]CB Akyerli, [3]T Acun, [4]A Keller, [1,2]MC Yakicier *[1]Department of Medical Biology, School of Medicine, Acibadem University, Istanbul, Turkey; [2]GENETIKA Genetic Laboratories, Istanbul, Turkey; [3]Department of Molecular Biology and Genetics, Bilkent University, Ankara, Turkey; [4]Febit biomed gmbh, Heidelberg, Germany*

Large intervening non-coding RNAs (lincRNAs) are recently discovered regulatory molecules. They are evolutionarily conserved in mammals, suggesting their critical role in important cellular processes. The aim of this study is to define the potential role of lincRNAs in hepotocellular carcinoma (HCC) development by gene expression profiling in tissue samples and cell lines. LincRNA chips were used for expression analysis in three normal liver tissues, three HCC samples from the same patient and in 14 cell lines. LincRNAs exhibited differential expression between normal and tumor states. The expression difference was significant in one lincRNA which specificly separated the cell lines into two groups. To the best of our knowledge, this is the first study to show differential expression of lincRNAs in HCC, suggesting their potential role in HCC development. Further studies are needed to elucidate the mechanism of action of lincRNAs in HCC development and also to determine their potential use as biomarkers.

C01 IDENTIFICATION OF POINT MUTATIONS AND LARGE REARRANGEMENTS IN THE BRCA1 GENE IN 667 TURKISH UNSELECTED OVARIAN CANCER PATIENTS

[1]D Aktas, [2]M Gultekin, [1]S Kabacam, [1]M Alikasifoglu, [3]AT Turan, [3]G Tulunay, [3]MF Kose, [4]F Ortac, [2]K. Yuce, [1]E Tuncbilek, A Ayhan[2] *[1]Hacettepe University Medical School, Department of Genetics, Ankara, Turkey; [2]Hacettepe University Medical School, Department of Gynecology and Obstetrics, Ankara, Turkey; [3]Etlik Zübeyde Hanim Women's Health Training and Research Hospital,Department of Gynecologic Oncology, Ankara, Turkey; [4]Guven Hospital, Department of Gynecology and Obstetrics, Ankara, Turkey*

Objective: The aim of this study was to evaluate the prevalence and spectrum of a known founder mutation, 5382 insC, and large genomic rearrangements (LGRs) in BRCA1 in ovarian cancer patients in Turkey. The additional aim was to determine the genetic testing strategy in Turkish breast/ovarian cancer family.

Methods: Six hundred and sixty-seven ovarian cancer patients from five large geographical regions in Turkey, 61 of which had family history of breast/ovarian cancer, were tested for the mutation 5382 insC by mutagenically separated polymerase chain reaction and direct sequencing of the entire coding sequence and the splicing sites. Additionally, multiplex ligation dependent probe amplification (MLPA) was performed for large mutational scanning of BRCA1 gene in unselected ovarian cancer.

Results: In this study, BRCA1 point mutations were observed in 1% of all patients and 9.8% of familial cases: 5382insC, unique novel missense variant-G1748S and unclassified splice site variant IVS20 + 5A>T. 5382insC was observed in two patients. However, G1748S, previously unreported, was found in four patients and thus led to the conclusion that this mutation may be unique to Turkey. A splice site variant, IVS20 + 5A>T, was detected in three patients, with two of them including G1748S and IVS20 + 5A>T, together. Using MLPA, six different distinct LGRs in BRCA1 were observed: the deletion of E1A-1B-2, E11, E17-19, E18, E18-19 and duplication of E5-9. The prevalence of LGRs in this study was 40.9% among patients with family history. The deletion of E1A-1B-2 was the common mutation and patients with this deletion were referred to us from four different geographical regions in Turkey. Therefore, it was hypothesized that this deletion covering E1-2 is common in Turkey.

Conclusion: LGRs in BRCA1 were strongly associated with positive family history amongst the Turkish population. On the basis of these findings it can be recommended that a low-cost screening for LGRs in BRCA1 may be the first-line mutation detection method in families with strong breast/ovarian cancer history in Turkey.

C02 THE EFFECTS OF THE IL-1RA, IL-4 AND TNF-BETA GENE POLYMORPHISMS ON ETHIOPATHOGENESIS AND PROGNOSIS OF TRANSITIONAL CELL BLADDER CARCINOMA

[1]S Bozdogan, [1]A Dursun, [2]G Bozdogan, [2]I Donmez, [2]NA Mungan *[1]Department of Medical Genetics, Zonguldak Karaelmas University, Medical Faculty, ZONGULDAK; [2]Department of Urology, Zonguldak Karaelmas University, Medical Faculty, ZONGULDAK*

Introduction: Transitional cell bladder tumours usually show local proliferation and recurrence and have a potential to become invasive and metastatic. The aim of our study was to set forth the contingent relation between IL-1Ra, TNF-β and IL-4 gene polymorphisms and clinical condition at the time of diagnosis, progression of illness and the respond to intravesical immunotherapy in patients with bladder carcinoma.
Patients and methods: DNA samples were obtained from 100 patients with bladder carcinoma and age, gender and smoking habits matched 102 healthy control subjects. Their IL-1Ra, IL-4 and TNF-β gene polymorphisms were determined by using PCR-RFLP method. Patient and control groups were compared for IL-1Ra, IL-4 and TNF-β gene polymorphisms. Bladder carcinoma patients group was compared according to stage, histopathological grade, tumour size and number, and smoking condition for the same polymorphisms.
Results: While allele distribution frequency of IL-1Ra and IL-4 gene polymorphisms was significantly different between patients with bladder tumour and control groups, allele distribution of TNF-β gene was not statistically significant. There was no significant difference in allele distribution of the three genes in both groups regarding stage, tumour size, number of tumours and smoking condition. Although allele distribution of IL-4 gene showed significant difference considering histopatological grades, allele distribution of IL-1RN and TNF-β were not different. Between the two groups that are sensitive or resistant to intravesical BCG treatment, it was proved that regarding polymorphic allele distributions of the three genes, there was no statistically significant difference.
Conclusion: In the future, clinical improvements on diagnosis, treatment and prognosis of bladder carcinoma is expected owing to development of more sensitive and specific tests for genetic polymorphisms of cytokines that are effective on inflammation.
Key words: Bladder carcinoma, gene polymorphism, inflammation, IL-1Ra, IL-4, TNF-β, immunotherapy.

C03 LOSING OF ABL GENE PRODUCTS IN A PATIENT WITH THERAPY RELEATED RECURRENT ACUTE LYMPHOBLASTIC LEUKEMIA

[1]MS Yildirim *[1]Department of Genetics, Selcuk University Meram Medical Faculty, Konya, Turkey*

After therapy recurrent leukemia usually shows complex karyotypic abnormalities. The therapy related leukemias morphologically and cytogenetically differ from de novo acute leukemias. Monosomy 7 and structural abnormalities of chromosomes 5 and 7 are common in secondary leukemias. A 46 year old female was diagnosed as having ALL and had been received chemotherapy. When first diagnosed, Fluorescence in situ hybrization (FISH) studies identified no detectable level of bcr/abl rearrangement. Two years after the first application, illness was recurrent. Loss of abl gene revealed in the FISH studies. Late of Philadelphia chromosome has been observed in patient with various malignancies such as acute myeloblastic leukemia, acute lymphoblastic leukemia and solid tumor but alone abl gene loss was not shown. This patient was applied three cycles aggressive treatment. After one mount of recurrent diagnosis, patient died. This situation may be considered as a result of treatment. The patient is the first case in the literature.

Consequently, lead to genetic aberration on patients of aggressive treatment and its clinical implications always are considered.

C04 JAK2 V617F MUTATION IN HEMATOLOGICAL DISORDERS IN TURKISH POPULATION

S Guntekin, A Bakır, MA Ergun, EF Percin *Gazi University Faculty of Medicine Department of Medical Genetics, Besevler Ankara Turkey*

A point mutation in the JAK2 gene, resulting in a substitution of valine for phenylalanine (JAK2 V617F) has been associated with myeloproliferative disorders such as; polycythemia vera, essential thrombocythemia, idiopathic myelofibrosis, myelodysplastic syndromes, chronic myelomonocytic leukemia, systemic mastocytosis, chronic neutrophilic leukemia and eosinophilic disorders. In this study, we investigated the relation of JAK2 V617F mutation in hematological disorders, in Turkish population by real time PCR. The mutant allele was quantitated using a standard calibration curve, revealing $< 50\%$ and $\geq 50\%$ mutational load groups. Regarding erythrocyte, hematocrite, platelet and leukocyte levels, a significant relation was found among wild type (wt), $< 50\%$ and $\geq 50\%$ groups. Also, there was a statistically significance between wt and $\geq 50\%$ groups regarding hemoglobin levels. Finally, an increase in the mutational load has been shown to induce erythrocyte, hematocrite, and leukocyte levels, except platelet levels. As a conclusion, routine screening of JAK2 mutation appears to be indicated in patients with myeloproliferative disorders.

C05 MOLECULAR DISCRIMINATIVE DIAGNOSIS OF BENIGN THYROID NODULES AND PAPILLARY THYROID CARCINOMAS

[1]YZ Igci, [1]A Arslan, [2]E Akarsu, [3]S Erkilic, [1]M Igci, [1]S Oztuzcu, [4]B Cengiz, [1]B Gogebakan, [1]EA Cakmak, [5]AT Demiryurek *[1]Department of Medical Biology, University of Gaziantep, Faculty of Medicine, Gaziantep, Turkey; [2]Department of Endocrinology and Metabolism, University of Gaziantep, Faculty of Medicine, Gaziantep, Turkey; [3]Department of Pathology, University of Gaziantep, Faculty of Medicine, Gaziantep, Turkey; [4]Department of Physiology, University of Gaziantep, Faculty of Medicine, Gaziantep, Turkey; [5]Department of Pharmacology, University of Gaziantep, Faculty of Medicine, Gaziantep, Turkey*

Papillary thyroid carcinoma (PTC), which emerges as a result of somatic or germinal mutations, is the most common thyroid

cancer type with an approximate frequency of eighty percent. Fine needle aspiration biopsy (FNA) is the most effective method in discriminating benign from malignant lesions. Up to 30% of FNA results are indeterminate or suspicious. These patients with suspicious or indeterminate results usually undergo surgery. According to the post-operational histopathological analysis, up to 30% of them carry a diagnosis of cancer which ultimately makes many of these operations unnecessary. For this reason, one of the greatest challenges in thyroid cancer research is to develop an adjunct to FNA to clarify the indeterminate lesions as benign or malignant. As for now, none of the potential immunohistochemical and genetic biomarkers or epigenetic mechanisms was proven to be totally succeeded in discrimination of these lesions. In this study potential biomarkers of PTC in gene expression level were investigated using DD-PCR method in 3 groups of totally 42 persons. Tissue distributions of the samples were 15 benign thyroid nodules, 14 follicular variant of PTC (FVPTC), and 13 classic variant of PTC (CVPTC). As a result of the study, using 21 genes' expression levels it was proven to be discriminated benign nodules and PTC lesions including its 2 subtypes (FVPTC or CVPTC). It is suggested that using the data obtained, not only new biomarkers can be developed in thyroid cancer diagnosis but also a chance of better understanding of thyroid cancer biology will be possible.

C06 CYTOGENETICAL ANOMALIES IN PATIENTS WITH AML-M4

[1]G Bagatır, [1]S Palanduz, [1]S Ozturk, [1]K Cefle, [2]A Palanduz, [1]HS Goksoy *[1]Department of Internal Medicine, Istanbul University, Istanbul Faculty of Medicine; [2]Department of Family Medicine, Istanbul University, Istanbul Faculty of Medicine*

Aim: Various cytogenetical anomalies have been described in acute myelomonocytic leukemia. In this study we analyzed the karyotypes of our patients with AML-M4.
Material and methods: Eleven patients with AML-M4 were included (seven female and four male). Bone marrow samples were obtained before the beginning of chemotherapy.
Results: Chromosomal anomalies were detected in all of the patients. Complex karyotype was observed in 45.5%. The chromosomal anomalies seen alone or together with were inv(16) (54.5%), t(3;5) (18.2%), 3q- (18.2%), 5q- (18.2%), 6q- (18.2%), 17p- (9%), 11q+ (9%), t(9;22) (9%).
Discussion: Inv(16) has been accepted as a marker of good prognosis. Complex translocations and t(3;5) point out poor prognosis. Routine karyotype analysis is recommended before deciding between chemotherapy and allogenic bone marrow transplantation.

C07 A STUDY OF CLASTOGENITY: AN EVALUATION IN PATIENTS WITH FANCONI ANEMIA AND THEIR FIRST DEGREE RELATIVES

[1]G Bağatır Karabudak, [1]Ş Öztürk, [1]Ş Palanduz, [2]A Palanduz, [1]A Uçur, [1]A Bayrak, [1]K Çefle *[1]Department of Internal Medicine, Istanbul University, Istanbul Faculty of Medicine; [2]Department of Family Medicine, Istanbul University, Istanbul Faculty of Medicine*

Aim: Sensitivity to clastogenic effect of DNA cross- link agents is a diagnostic marker in Fanconi anemia. In patients not exhibiting the classical stigmates of Fanconi anemia, DNA crosslink sensitivity tests with chemical agents such as DEB (diepoxibutan) or MMC (Mitomycin C) is recommended. In this study we aimed to determine clastogenity sensitivity with MMC in patients with Fanconi anemia and their first degree relatives.
Methods: This study was conducted in the Laboratory of Medical Genetics at the Department of Internal Medicine. Patients with a differential diagnosis of Fanconi anemia or aplastic anemia were included. We obtained venous blood samples and performed 2 cell cultures from each sample. We added no agents to the first and MMC (final concentration to be 0.01μg/ml) to the second culture 48 hours before harvest. We obtained metaphase plaques with standard cytogenetic methods, prepared conventional giemsa slides and analyzed at least 100 metaphase under light microscope.
Results: Twenty-three patients were included in the study. The diagnosis of Fanconi anemia was approved when breakage number per metaphase was ≥1 in spontaneous cultures. We observed positive results in 6 of the 23 patients (26%). The ratio was 1.14 in spontaneous culture and 3.22 in induced culture. Triradial and tetraradial exchange figures were detected in the metaphase plaques in 4 of the 6 patients. The first degree relatives of these 6 patients were also investigated. Positive results were demonstrated in 3 of them: the breakage number per metaphase was 0.5 in spontaneous culture and 2.45 in induced culture.
Conclusion: The breakage number per metaphase is ≥1 in both spontaneous and induced cultures in aplastic anemia. The demonstration of the similar high ratio in the first degree relatives of patients with aplastic anemia points out the inherited mechanism of this DNA repair defect.

C08 DETECTION OF TYROSINE KINASE MUTATIONS OF THE EGFR FROM THE SERUM OF TURKISH NON-SMALL CELL LUNG CANCER PATIENTS

[1]Akca H, [1]Demiray A, [1]Tokgun O, [2]Yokota J *[1]Pamukkale University School of Medicine Department of Medical Biology, Denizli/Turkey; [2]National Cancer Center, Tokyo/Japan*

Introduction: In non-small cell lung cancer (NSCLC), somatic mutations of epidermal growth factor receptor (EGFR) are associated with dramatic responses to the EGFR tyrosine kinase inhibitors, such as gefitinib or erlotinib. EGFR mutations are generally detected in tumor tissue, should be adequate for pathologic review to evaluate cellular heterogeneity. If EGFR mutations can be observed in serum DNA, this could serve as a noninvasive source of information on the genotype of the original tumor cells that could influence treatment and the ability to predict patient response to tyrosine kinase inhibitors.
Materials and methods: For this purpose; serum genomic DNA will obtain from 33 Turkish patients with NSCLC and EGFR exon 18, 19, 20 and 21 were amplified by nested PCR and specific mutations were detected by restriction fragment length polymorphism and results were confirmed by direct sequencing.
Results: We detected G719X mutation in exon 18, frame deletion in exon 19, T790M mutation in exon 20, L858R mutation in exon 21. EGFR mutations were detected in 9 patients (27%).

Conclusion: Our results indicate that EGFR mutations can detect from serum of the NSCLC patients, and this is noninvasive and fast method for detection of EGFR mutations.

C09 EVALUATION OF PTEN AND MCL-1 EXPRESSIONS IN NSCLC EXPRESSING WILD-TYPE OR MUTATED EGFR

[1]Z Cetin, [2]G Ozbilim, [3]A Erdogan, [1]G Luleci, [1]SB Karauzum *[1]Akdeniz University, Faculty of Medicine, Department of Medical Biology and Genetics, Antalya, Turkey; [2]Akdeniz University, Faculty of Medicine, Department of Pathology, Antalya, Turkey; [3]Akdeniz University, Faculty of Medicine, Department of Thoracic Surgery, Antalya, Turkey*

Signaling pathways activated by epidermal growth factor receptors (EGFRs) are important in lung carcinogenesis. New treatment strategies with EGFR-targeting drugs provided improvements in management of lung cancer. However, molecular mechanisms underlying resistance to these drugs need to be evaluated. Surgically resected samples were obtained from 50 patients with non-small-cell lung cancer. PTEN, Mcl-1 and EGFR protein expression levels were evaluated by Western-blot. Direct sequencing was performed to investigate EGFR tyrosine kinase domain mutations. We detected c.2235-2249 (pGlu746-Ala750del) mutation in exon 19 in two patients with adenocarcinoma histology. Elevated expression levels of both Mcl-1 isoforms (Mcl-1S and Mcl-1XL) and EGFR proteins were found in 15 (30%) and 23 (46%) of the cases, respectively. Reduced PTEN protein expression levels were observed in 17 (34%) of the cases. PTEN expression level was reduced in 26% of cases that showed increased EGFR expression. Also, increased expression of Mcl-1 protein was observed in 26% of cases with EGFR overexpression. One of the cases harboring pGlu746-Ala750del mutation had increased levels of Mcl-1 and decreased PTEN expression levels. Our results indicate that, in addition to lack of PTEN expression, elevated levels of the Mcl-1 protein might be one of the important intrinsic mechanisms protecting non-small–cell lung cancer cells from apoptosis induced by several compounds. Therefore, EGFR mutations in conjunction with evaluation of Mcl-1 and PTEN expression levels in large cohorts might provide important clues for improvements of new treatment strategies in non-small–cell-lung cancer management.

C10 DIFFERENTIAL MRNA LEVELS OF METASTASIS RELATED GENES *NM23-H1*, *KAI1* AND *MKK4* IN OMENTAL METASTASIS OF EPITHELIAL OVARIAN CANCER

[1]T Bilgen, [2]G Erdogan, [3]T Simsek, [4]H Gulkesen, [2]E Pestereli, [2]S Karaveli, [1]G Luleci, [1]I Keser *[1]Department of Medical Biology and Genetics, School of Medicine, Akdeniz University, Antalya-07059, Turkey; [2]Department of Pathology, School of Medicine, Akdeniz University, Antalya-07059, Turkey; [3]Department of Obstetrics and Gynecology, School of Medicine, Akdeniz University, Antalya-07059, Turkey; [4]Department of Biostatistics, School of Medicine, Akdeniz University, Antalya-07059, Turkey*

Epithelial ovarian cancer (EOC) is the leading cause of death among gynecological malignancies. We aimed to define the changes at mRNA levels of *NM23-H1, KAI1*, and *MKK4* genes which are known as metastasis suppressor genes in paired primary and omental metastatic tumor samples of EOC in Turkish patients. Forty-one (31 fresh and 10 paraffin-embedded tissues) patients who underwent primary surgery because of epithelial ovarian carcinoma were included in this study. Gene expression patterns of three metastasis suppressor genes were analyzed using Quantitative - Real Time - Polymerase Chain Reaction (Q-RT-PCR) in normal ($n = 38$), primary malign tissues ($n = 41$), and its metastatic lesion ($n = 35$) on omentum. We found that mRNA level of *NM23-H1* was significantly higher in metastatic samples compared to primary tumors ($p = 0.009$). The mRNA level of *MKK4* was significantly lower in primary tumor samples compared to normal tissues ($p = 0.024$). Our findings showing up-regulation of *NM23-H1* and *KAI1* genes, which have been described as metastasis suppressor, may contribute to clarify previous uncertain situations regarding their roles in EOC metastasis. Based on our results, we can also conclude that *MKK4* is an important target for EOC because of decreased mRNA levels in both primary and metastatic tumor samples.

