Discussion: Specific and non-random chromosome rearrangements in Burkitt's lymphoma cell lines have been reported previously. Our results demonstrated some well characterized chromosome abnormalities and also some variations in both the numerical and structural chromosomal abnormalities from those reported in other studies. Some of these chromosome abnormalities also have reported from Burkitt's lymphoma patents. Therefore characterizing these abnormalities might be of great importance in understanding the progression of the disease.

598 POSTER

Identification of two loci of frequent allelic deletions on chromosome 6 involved in cervical cancer progression

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Several regions of frequent allelic deletions on chromosome 6 were identified by loss of heterozygosity (LOH) analysis in 145 cervical carcinomas (CC) and cervical intraepithelial neoplasias (CIN) using 30 microsatellite markers. More than 50% of CC cases had allelic deletions at 6p21.3 within the region of major histocompatibility complex (MHC) and at 6q16-21. Some of these frequently deleted microsatellites are located in introns of recently described but not fully characterized genes and we analyzed the structure of two of them. Predicted exon-intron structure of these genes were characterized according to expressed sequences deposited in NCBI database and published data. The first gene is located at 6p21.3 in the region of MHC class III and is frequently deleted in CIN and early stages of CC, so can be designed as EDCC gene (early deleted in cervical carcinomas). The predicted size of this gene is about 10kb with at least 3 exons and its function is still unknown. The second gene, located at 6q16-21 was deleted mostly in invasive cervical cancer and was designed as LDCC gene (lately deleted in cervical carcinomas). The predicted gene spans across 700kb of genomic sequence with at least 15 exons. It contains two domains of enzyme belongs to lipid metabolism and associated with posttranslational protein modification. The data of expression of these genes in normal and cervical cancer RNA samples with and without allelic deletions will be presented.

599 POSTER

Promoter hypermethylation of hMLH1 gene in liver fluke related cholangiocarcinoma

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Background: Cholangiocarcinoma is a malignant tumor arising from bile duct epithelium. It is a leading cancer in Northeast Thailand where the liver fluke *Opisthorchis viverrini* is highly endemic. Many epidemiological and experimental studies suggest that liver fluke infection causes chronic inflammatory and enhances the susceptibility of bile duct epithelium to carcinogenic chemicals leading to genetic and epigenetic damages in the cells. Genetic aberration of DNA mismatch repair gene *hMLH1* has been described in liver fluke related cholangiocarcinoma. However, hypermethylation of the *hMLH1* gene promoter has never been reported in this cancer. This study aimed to elucidate an epigenetic mechanism underlies *hMLH1* gene inactivation in liver fluke related cholangiocarcinoma.

Material and methods: DNA methylation patterns in the *hMLH1* promoter were determined in 55 intrahepatic cholangiocarcinoma and matching normal liver tissues using methylation-specific PCR (MSP).

Results: Hypermethylation of the *hMLH1* promoter occurred in 25 of 55 cholangiocarcinoma patients (45.5%). Of 31 cases whose genetic alterations (LOH or MSI) of *hMLH1* gene (D3S1611) were previously determined, 7 cases showed positive for both methylation and D3S1611 alteration whereas 11 cases showed methylation positive without D3S1611 alteration (Table 1).

Discussion: This study suggests that genetic and epigenetic mechanism plays an important role in *hMLH1* gene inactivation in liver fluke related cholangiocarcinoma and *hMLH1* gene inactivation might be a pivotal cause of cholangiocarcinogenesis.

Table 1. Correlation between D3S1611 alteration and *hMLH1* promoter hypermethylation

D3S1611 alteration	Methylation positive	Methylation negative	Total
Positive	7 (22.6%)	1 (3.2%)	8 (25.8%)
Negative	11 (35.5%)	12 (38.7%)	23 (74.2%)
Total	18 (58.1%)	13 (41.9%)	31

600 POSTER

The expression of the transcript isoforms oh human Arg gene is differently regulated in different cell types

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Background: The products of the Arg and Abl genes belong to the Abelson family of non receptor tyrosine protein kinases and both have high similarity. Arg has alternatively spliced amino terminal chains and the protein isoforms are defined IA and IB, the IB forms have a myristoilation site (Proc. Natl. Acad. Sci. 1990, 87, 5802). In the C-terminal domain Arg has two actin binding sequences. Arg is ubiquitously expressed with a higher expression in nervous tissue and Arg protein has a cytoplasmic localization. Arg expression increments during granulocytic and macrophage-like differentiation of HL-60 cells and in the maturation of B lymphoid cells. Altered Arg expression has been described in colon, pancreas and bladder carcinoma. Arg gene rearrangement has been reported in acute leukemias (Blood 1999, 94, 4370). To gain insight into the biological function of Arg we determined the relative abundance of the different forms of Arg transcripts.

Materials and methods: With Real-time PCR we analyzed different cell lines and primary cell cultures. The Arg mRNA expression in cells was measured as $2^{-\Delta CT}$, a quantitative value representing the amount of Arg transcripts.

Results: All tested cells contained the different forms of Arg mRNA, but their relative abundance varied. Based on the abundance of the different forms of Arg mRNA the cells can be grouped in different categories. In hematopoietic cell lines AllP0, Raji, LP-1 (B cells), Jurkatt, Molt-4 (T cells), HL-60, GFD8, K562, U937 (myeloid cells), and in donor lymphocytes, granulocytes and monocytes the IB isoforms are about 20 fold higher than IA forms. In epithelial cell lines and in primary cultures of renal cortex and renal carcinomas (clear cells) that derives from kidney cortex the IA forms are higher than IB forms. Also in fibroblastic cell line HEL-299 the Arg IA mRNA is higher than IB mRNA. In A172 glioblastoma cell line and, of note, in HL-60 cells differentiated to macrophage-like cells with TPA, the IA forms are 2-3 fold higher than the IB forms.

Conclusions: These observations show that the expression of the type-specific Arg mRNA is differently regulated in tissues with a pattern that can be typical. This expression pattern can be used to characterize the cells deriving from different tissues. The role of the IA and IB forms during cell differentiation needs to be investigated further.

601 POSTER

p53 codon 72 polymorphism in basal cell carcinoma of skin

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Background: A common polymorphism at codon 72 of exon 4 of p53 tumor suppressor gene has been reported to be associated with increased heritable susceptibility to several cancers.

Subjects and Methods: In this study we investigated the frequency of p53 codon 72 polymorphism in 91 patients with Basal Cell Carcinoma (BCC) of skin compared to 205 healthy normal individuals. DNA extracted from peripheral blood lymphocytes was examined by an allele-specific polymerase chain reaction.

Results: 34(37.4%) BCC patients and 75(36.6%) normal individuals had *Arg/Arg* genotype while 10(11%) BCC patients and 40(19.5%) normal individuals had *Pro/Pro* genotype. The frequency of heterozygotes in BCC and healthy individuals were 51.6% and 43.9%, respectively. In total, there was no significant difference in the p53 genotypes in patients and controls. However, there was an apparent increase in *Arg/Arg* genotype among those BCC patients who had a history of occupational sun-exposure compared to non-exposed patients (46.3% vs. 23.1%, P = 0.11). The increase in *Arg* allele among sun-exposed patients was marginally significant (69.4% vs. 53.8%, P = 0.07). Comparison of the genotype frequencies between sun-exposed patients and normal controls confirmed the accumulation of *Arg/Arg* genotype in these patients (46.3% vs. 36.6%, P = 0.09). In addition, the frequency of *Arg* allele was significantly higher in sun-exposed patients compared to controls (69.4% vs. 58.5%, P=0.05) **Conclusion:** Our results

suggest that *Arg* allele at codon 72 of p53 gene might affect the risk of ultraviolet-induced Basal Cell Carcinoma.

602 POSTER

Investigating the role of Smad4 in TGF-beta signaling using high density microarrays

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Transforming growth factor-beta is a multifunctional growth factor whose best-known function is to inhibit cell growth and suppress tumor formation. Loss of TGF-beta growth inhibition is one of the most common cellular events in the pathogenesis of human breast, pancreatic and colon cancers. TGF-beta signals through a heteromeric signaling complex consisting of Smad2, 3 and 4. Disruption of the Smad signaling complex often leads to tumor formation. We have used both 60-mer oligonucleotide and cDNA microarrays to investigate the role of Smad4 in the TGF-beta controlled transcription program in tumor cells. These high density DNA microarrays, generated using Agilent's SurePrint inkjet technology, were used to profile global transcriptional regulation in breast, colon and pancreatic Smad4-null tumor cell lines in response to TGF-beta. Data from both microarray types showed a high degree of correlation in demonstrating that TGF-beta induces transcriptional activation and repression of genes involved in signal transduction, cell adhesion and transcriptional regulation across the range of cell lines tested. Data from a number of studies is presented comparing expression profiles from Smad4-null tumor cell lines to those from either Smad4-transfected cell lines or normal cell lines. These data indicate that the composition of the Smad signaling complex controls the specificity of TGF-beta signaling.

603 POSTER

Polymorphic (CAG)n and (GGC)n in androgen receptor and breast and ovarian cancer risk in BRCA1/2 carriers and non-carriers

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Introduction. The androgen receptor (AR) is involved in the regulation of hormone-responsive genes and variation within the gene is hypothesized to play a role in breast and ovarian cancer susceptibility. We therefore examined whether *AR* repeat alleles modify cancer risk in *BRCA1* and *BRCA2* mutation carriers and familial breast and ovarian cases in comparison with age-matched control group.

Patients and Methods. Results were generated from 109 cases with mutation in BRCA1 and BRCA2, 60 first-degree familial cases without mutation within BRCA1/2 and 113 controls. Genomic DNA was PCR amplified using fluorescently labeled primers. The fragments were run on a 5% denaturing polyacrylamide gel, and amplicon length was determined relative to size standard by automated fluorescence detection. As in previous studies, $(CAG)_n$ repeat lengths of <22 were classified as short, and those of >=22 were classified as long. For $(GGC)_n$ repeats, those < 17 were classified as short, and those >= 17 were classified as long.

Results. Within the group of BRCA mutation carriers there was a significant difference in CAG cumulative repeat size between women with and without ovarian carcinoma (82.4% and 62.7% of CAG>=43, respectively, OR 2.78, $p<0.05$). GGC size was related to breast cancer presence: cumulative GGC>=45 was found in 33.3% of breast cancer cases and 57.6% of patients without breast cancer (OR 0.37, $p<0.05$). When the group of mutation carriers was compared to healthy subjects and familial breast cancer cases, there was no observed difference in CAG cumulative length, while a significant decrease in frequency of GGC cumulative >=33 was revealed: 45% in the group of mutation carriers vs. 67.4% in healthy subjects and 71.7% in familial breast cancer patients (OR 0.4 and 0.32, respectively; $p<0.005$). This study, one of the first to examine both $(CAG)_n$ and $(GGC)_n$, suggests a role of CAG long repeat for the development of ovarian cancer in BRCA mutation-carriers, while long GGC repeats seem to protect against breast cancer in these patients. In addition, our data show that long GGC repeat (>=17 repeats) is less common between breast and ovarian cancer cases when compared to general.

Conclusion. These results imply that CAG and especially GGC repeat length can potentially serve as a useful marker to identify a subset of individuals at higher risk of developing breast and ovarian cancer in BRCA1/2 mutation carries and familial cases.

604 POSTER

HER2 polymorphism and the risk of breast cancer

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Introduction: Breast cancer is a major public health problem around the world, and its carcinogenesis is not yet well understood. The Human Epidermal growth factor Receptor-2 (HER2) seems to play an important role in the development of this neoplasia, and genetic alterations in this gene, such as point mutations and polymorphisms have been detected in breast cancer patients. The aim of our study was to analyze the frequency of a single nucleotide polymorphism in the *HER2* gene in a southern European population.

Materials and Methods: The study included 161 patients who were diagnosed with breast cancer in the Portuguese Institute of Oncology Porto. DNA was extracted from peripheral blood of these patients. As control, the same experience was performed in blood samples from 142 healthy donors. DNA extracted from peripheral blood was submitted to Polymerase Chain Reaction (PCR) followed by Restriction Fragment Length Polymorphism (RFLP), in order to identify the possible *HER2* genotypes; Ile/Ile, Ile/Val and Val/Val. The restriction fragments were analyzed in a 3% agarose gel, stained with ethidium bromide.

Results: We found that the frequency of the Ile/Val genotype was higher in cases (39.1%) than in controls (24.0%), and the same was observed with the Val/Val genotype (4.4% and 2.8%, respectively). A twofold increase in risk of breast cancer was found among women who are carriers of a Val allele genotype Ile/Val and Val/Val genotypes (OR = 2.1; 95% CI: 1.3-3.4; p = 0.002).

Discussion: Our results indicate an association between the presence of the Val allele in the *HER2* polymorphism and the risk of breast cancer. Further studies are needed to evaluate the role of this polymorphism in the behavior of breast cancer.

605 POSTER

Reversible deposition of allele-specific primers by excess of complementary oligonucleotides drastically improves the reliability of allele-specific PCR

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Background: Allele-specific PCR (ASPCR) is considered to be a very straightforward approach for detection of single nucleotide polymorphisms (SNP), however its application remains somewhat limited due to insufficient reliability. Here we suggest a simple modification of ASPCR, that broadens the range of conditions in which ASPCR retains both high specificity and high sensitivity.

Material and methods: The idea of the method is based on the reversible deposition of allele-specific primers by addition of the corresponding complementary oligonucleotides. Since the bulk of the primers is diverted towards the excess of the competitor, DNA template has access to the primer only temporarily, when the latter is released from the depository duplex. Once annealing to the target sequence has occurred, the fate of the primer heavily depends on whether its 3' nucleotide matches or mismatches. In the case of match, even temporary hybridization to the DNA template is followed by immediate primer extension, due to residual activity of Taq polymerase in the annealing temperatures. Thus the matched primer becomes longer, and loses the ability to dissociate from the template. On the contrary, the extension of the 3' mismatched primer is compromised, thus increasing its chances to dissociate from the DNA template before the elongation occurs. Noticeably, the association/dissociation between allele-specific primer and its corresponding complementary oligonucleotide is absolutely reversible, because neither of the partners undergoes any modification. Therefore, despite the increased ASPCR specificity due to primer deposition, the absolute amount of allele-specific primer remains sufficient to support effective DNA template amplification even in the later stages of reaction.

Results: The suitability of this modification was proven using several examples of complicated ASPCR genotyping, such as TNF-alpha (G/A), DPD (G/A), XRCC1 (C/T), and CHEK-2 (C/T) allele discrimination. Conven-