patients, 95 ovarian cancer patients and 205 control subjects, for *XPD* exon 10 polymorphism using PCR-RFLP.

Results: We found AA variant genotype in 15,8% of ovarian cancer patients and 6,8% of healthy group. We observed that carriers of *XPD* exon 10 AA genotype have increased susceptibility of ovarian cancer (OR=2,57 95% CI 1,19-5,59; p=0,017), especially before the age of 53 years (OR= 3,87 95% CI 1,39-10,81; p= 0,010). Sixteen percent of cases of ovarian cancer cases younger than 53 could be attributed to the influence of this risk factor. We found AA variant genotype in 9,5% of breast cancer patients and 6,8% of healthy group. We did not find any association between *XPD* exon 10 polymorphism and breast cancer risk (OR= 1,44 95% CI 0,70-2,96; p= 0,319), even when we considered age of onset (47 years).

Conclusions: Our results suggest an important role for *XPD* exon 10 polymorphisms in the susceptibility to ovarian cancer. Further studies will help to confirm the influence of these genotypes in the determination of chemoprevention strategies, and its role in prognostic and response to chemotherapy.

595 POSTER

UGT1A1 polymorphisms correlate with adverse side effects and clinical response in metastatic colorectal cancer patients treated with Irinotecan

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Background: Irinotecan (CPT-11) is metabolized by esterase to form a SN-38, which is further conjugated by UGT1A1. A genetic polymorphism related to its enzymatic activity has been identified in the promoter region of the UGT1A1 gene. Individuals with an additional TA repeat in this region may be at increased risk of Irinotecan toxicity.

Material and Methods: Fifty eight patients with metastatic colorectal cancer (MCRC) were included in the study. Eleven patients were treated with CPT11; nine with CPT11 + Tomudex and thirty eight patients with CPT11 + 5FU. DNA extraction and UGT1A1 genotype were performed using previously described methods. We investigated the differences in the development of adverse side effects (grades III-IV diarrhea, neutropenia and asthenia), termination of therapy, reduction of dose intensity and clinical response, depending on the genotypes of UGT1A1 in MCRC patients treated with Irinotecan.

Results: Twenty patients (47%) were TA6/TA6; 25 patients (43%) were TA6/TA7, and 6 patients (10%) were TA7/TA7. All six homozygote 7/7 cases showed grade > III toxicity (4 cases diarrhea; 1 case asthenia, fever and bad performance status, and 1 case with neutropenia). Fifteen out of 25 (60%) heterozygous 6/7 cases showed grade III-IV toxicity (11 cases diarrhea and/or neutropenia; 3 cases with asthenia and bad performance status, and 1 case with septic shock due to pneumonia). Six out of 27 patients (22%) with a normal genotype 6/6 developed side effects (grade III-IV diarrhea and/or neutropenia). Termination of therapy or reduction of dose intensity had to be performed in 100% of patients homozygotes TA7/TA7, in 52% of patients with a TA6/TA7 genotype and in 22% of patients with a TA6/TA6 genotype. There was no mortality due to CT. Twenty one out of 27 patients,(78%)(95%-CI: 62%-93%), with severe toxicity had a homozygous or heterozygous UGT1A1 genotype. TA6/TA6 and TA7/TA6 patients had a better response rate and median time to progression than homozygous TA7/TA7 cases:36% and 27% vs 17%, and 8,3 months and 5,5 months vs 4 months, respectively.

Conclusions: The results suggest that the UGT1A1 genotype i) might be clinically useful for predicting severe toxicity in cancer patients treated with Irinotecan and ii) is related to the clinical response in the group of patients included in the study.

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Ha-ras oncogene induces the metastatic ability of transformed cells in vivo through RalGDS downstream signalling pathway.

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Background: Earlier a collection of v-src transformed cell lines were isolated as a result of independent infection of primary hamster fibroblasts with different stocks of Rous sarcoma virus. All lines had a typically transformed phenotype and were highly tumorigenic for the inoculated animals [Deichman et al. 1989]. However, remarkable differences were found in the metastatic activity of transformed cells: after s.c. injection the majority of cells within two months induced about 150 metastatic nodules in the lung and/or other organs of inoculated hamsters (high metastatic lines, HM). On the other hand, after s.c. inoculation of HET-SR cells (single low metastatic, LM) metastatic nodules were practically not observed. Amount of the v-src protein and its kinase activity were approximately equal in both HM and LM cell types. [Tatosyan et al., 1996]. Introduction of activated Ha-ras^{V12} oncogene into LM cells leads to significant stimulation of metastatic potential of recipient cells.

Material and Methods: v- src-transformed hamster fibroblasts with low metastatic potential. Transfection >f by different mutant variants of Ha-ras oncogene. Analysis of spontaneous (SMA s.c. injection of the cells) metastatic characteristics of the transfectants *in vivo*. Comparative immunoblot analysis of different signal transduction proteins supposed to be involved in metastatic processes.

Results: In order to identify intracellular signaling chains and proteins involved in the metastatic process of transformed cells we have used three different effector-loop mutant forms of Ha-ras each of which activates a single downstream effector pathways. ERK pathway selective protein $RasV^{12}35S$ had no effect on metastatic activity of transfected cells (no more than 3-4 nodules *per* animal). Identical results were obtained with transfected PI3K kinase pathway protein $RasV^{12}40C$. In contract the $RasV^{12}37G$ an activator of RalGDS was extremely effective *in vivo* inducing about 120 metastatic nodules. The production and tyrosine phosphorylation of several proteins, involved in major signal transduction pathways were compared in all transfected cell lines. No differences have been seen in Src, PI3K, PKB, MEK-1 and Rac-1 activity. At the same time the level of Rho and Rac production, as well, as the phosphorylation of ERK1/2 and were changed in cells, expressing different phenotype.

Conclusions: The described collection of new cell lines with modulated metastatic properties is unique model system for identification of specific genetic and molecular factors responsible for invasive and metastatic behavior of tumor cells. RalGDS downstream effector pathway has major contribution to metastatic properties of transformed cells. We found several intracellular signaling proteins modulating their activity according with metastatic phenotype.

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A cytogenic study of Burkitt's lymphoma cell lines; Daudi, Namalwa and Raji

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Introduction: Since 1960 and description of the Philadelphia chromosome in CLL, the association of specific structural and numerical chromosome abnormalities with certain types of malignancies has been appreciated. Relatively little progress has been achieved in the study of human lymphoma cell lines, especially the cytogenic changes and the degree of heterogeneity observed in different karyotypic variants of the subpopution of these cell lines. Therefore, the study of karyotypic evolution of lymphoma cells in direct preparations and on serial *in vitro* passages may be important in demonstrating karyotype stability under the new growth environment and to evaluate any cytogenetics evolution taking place during the *in vitro* establishment of the cell lines.

Materials and Methods: The cell lines were cultured in RPMI 1640 supplemented with 10% FBS and were subcultured every 3-4 days by dilution (1:4) of the culture medium. The cells were harvested by centrifugation after 2-3 days for Daudi and Raji and after 4 days for Namalwa cell lines. The cell pellets were resuspended in hypotonic solution and then fixed in the freshly made fixative. After preparing slides from the cells and trypsin treatment, the slides were stained in freshly prepared leishman's stain. The observed chromosomal abnormalities were then reported using ISCN 1995. In some cases FISH were applied to confirm results of G-bandings. In these cases mostly PCR amplified band specific fluorescent-labeled probes and for some abnormalities chromosome arm painting (CAP) probes were used.

Results: Chromosome analyses were successfully achieved on preparations from all cell lines. Overall analysis of the cell lines demonstrated that, in Daudi and Raji the chromosome number of majarity of the cells were 46, this number for Namalwa was 45. The most common chromosome abnormality detected in all cells analyzed was translocation between chromosome 8 and 14 with classical breakpoints (q24;q32), but many other abnormalities were also detected. The most important of these were additional material on the short arm of chromosome 11 (Daudi), additional material on the q35 of chromosome 4 (Raji) and an HSR attached to the long arm of one of the chromosome 1q (Namalwa) among others.

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.

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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.

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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

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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.

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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