hypothesis that amifostine increases polyamine synthesis in these cells, reducing the amounts of L-arginine that can be metabolised by nitric oxide (NO) synthase to NO. It is well known that spermidine does not affect migration, while we have previously shown that decreased levels of NO inhibit HUVEC migration. Therefore, the decrease in migration seems to be due to a decrease in NO production by these cells. Finally, amifostine reduced tyrosine nitration of the cytoskeletal proteins actin and tubulin, in a time dependent manner. This last action could be due to the reduced amounts of NO or to other, not yet identified mechanisms.

Conclusions: Collectively, our results suggest that amifostine acts on endothelial cells through pathways that affect the redox status of the cells, either by producing H_2O_2 or by modulating NO production.

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Microvessel density of bone metastasis is dependent on the cancer type and therapy

J. Timár[1], T. Lrincz[2], J. Tóth[1], M. Szendri[2]. [1] *National Institute of Oncology, Tumor progression, Budapest, Hungary;* [2] *Semmelweis University, Orthopaedics, Budapest, Hungary*

Background: Bone may provide an extremely fertile microenvironment for angiogenesis. Experimental investigations indicate angiogenesis as a major regulator of bone metastasis development. However, no studies have investigated angiogenesis in bone metastases of human cancers.

Methods: We have evaluated microvessel density in bone metastases of various cancer types and compared to their primary tumors in paraffin samples of 39 patients. Microvessel density (MVD) was determined by using the hot spot method and the endothelial marker, CD34. Patients were chemotherapy-naïve except a subgroup of breast cancer cases.

Results: Two patterns of modulation of the angiogenic phenotype in the bone emerged in this study which seem to be cancer type specific: decreased angiogenic potential characterizing 45% of renal cell cancers and breast cancers of high vascularity in their primary, and increased angiogenic potential characterizing 40% of lung adenocarcinomas and breast cancers of low vascularity in their primary lesion. Analysis of the breast cancer cases indicated no differences in VEGF expression, hormone receptor status or histology between the two groups of primary tumors. However, when we have analysed these cases for possible cause for the different angiogenic responses we found that those cases where MVD decreased in bone metastases were all but one have been treated by chemo- or hormone therapy.

Conclusions: Our data demonstrate that 1. the vascularization of cancer metastases is different from that of the primary tumors, 2. patterns are different in the case of various cancer types. Among factors modulating MVD in bone metastases the unique microenvironment as well as the therapeutic interventions both have to be considered.

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1p chromosomal deletion and candidate genes mapping in liver fluke related cholangiocarcinoma.

P. Jearanaikoon[1,2,3], T. Limpaiboon[1,2,3], B. Sripa[3,4], V. Bhudhisawasdi[3,5], K. Uchida[6]. [1] *Khon Kaen University, Clinical Chemistry, Khon Kaen, Thailand;* [2] *Khon Kaen University, Center For Research & Development In Med Diagnostic Laboratory, Khon Kaen, Thailand;* [3] *Khon Kaen University, Liver Fluke & Cholangiocarcinoma Research Center, Khon Kaen, Thailand;* [4] *Khon Kaen University, Pathology, Khon Kaen, Thailand;* [5] *Khon Kaen University, Surgery, Khon Kaen, Thailand;* [6] *University of Tsukuba, Biochemistry & Molecular Oncology, Tsukuba, Japan*

Background: We have characterized genetic alteration in the development of liver fluke related cholangiocarcinoma which is commonly found in northeastern region of Thailand. Genomic wide aberration have been previously examined in 30 cases of cholangiocarcinoma patients (Uchida K,et al. unpublished data). They found the most frequent chromosomal loss at 1p36-qter with the frequency of 35%. To identify the possible candidate gene on this region, deletion mapping were investigated in cancerous tissues using quantitative PCR.

Material and methods: Five Sts markers covering 1p36-pter were firstly screened in 23 cancerous tissues using lightcycler- DNA Master SYBR GreenI (Roche). All samples were run in triplicates with an acceptable CV of less than 10%.

Results: Large deletion was spanned between these markers with 48-60% of all 23 cases. Nine out of 23 cases were selected as representatives for further fine mapping study. Gene copy number was quantitated using 6 gene markers designed in accordance with candidate gene present on this region. At least 4 gene markers demonstrated high deletion frequency. The most frequent deletion (7/9cases) was found at p73 locus.

Conclusions; Our data provided deletion information on 1p36-pter in liver fluke related cholangiocarcinoma. The possible candidate gene was represent as potential marker for further investigation. The expression of p73 will be investigated for the involvement in malignant progression of cholangiocarcinoma.

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Gene expression profiling in papillary thyroid carcinoma: Are there different pathways of carcinogenesis?

B. Jarzab[1], M. Wiench[1], J. Wloch[2], K. Fujarewicz[3], K. Simek[3], M. Oczko[1], A. Czarniecka[2], S. Szpak[1], E. Gubala[1], A. Swierniak[3]. [1] *Center of Oncology - MSC Memorial Institute, Dpt. of Nuclear Medicine and Endocrine Oncology, Gliwice, Poland;* [2] *Center of Oncology - MSC Memorial Institute, Clinic of Oncological Surgery, Gliwice, Poland;* [3] *Silesian Technical University, Gliwice, Poland*

The aim of the study was to evaluate the expression profiles in papillary thyroid carcinomas (PTC) by means of high density DNA microarrays. The molecular mechanisms leading to different types of thyroid tumors are not completely understood. RET protooncogene activation, as a consequence of chromosomal rearrangement, is regarded at present as the most important initiating event in the development of papillary carcinomas. However, in those papillary thyroid tumors which are RET-negative the molecular mechanisms of carcinogenesis are unknown. Nine samples of PTC together with the corresponding normal tissues were frozen immediately after excision. Total RNA was isolated using RNeasy Total RNA Midi and Mini Kits. The RET gene rearrangements were found in four cases by RT-PCR. All samples were hybridized to Human Genome U133A arrays as recommended by Affymetrix. Three different approaches have been used to analyze gene expression data. In the first four methods of gene selection and Support Vector Machine technique with a linear kernel for classification were applied. In the second Singular Value Decomposition and hierarchical clustering algorithm and in the third Affymetrix Data Mining Tool software were used. Very similar results were obtained by all three methods giving a clear separation of gene expression profiles in tumors and normal samples. There were 99 genes overexpressed and 93 genes underexpressed in tumor samples, among them genes previously indicated by Huang et al (2001), particularly SCYA21, TFF3, CITED1, FABP4, LAMB3, SCEL, DPP4. Also, other differentially expressed genes, unreported so far, were found: EVA1, LRB1B, CDH3, gastrointestinal tumor-associated antigen GA733-1, prostate differentiation factor, low density lipoprotein receptor-related protein, TMPRSS, CDH16, PCSK2 and solute carrier NaPiIIb. Expression patterns observed in RET-positive and RET-negative tumors exhibited some differences which were related to expression of both thyroid-specific genes (i.e. PDS), cancer-related (i.e. CAXII, PCNA, PICOT) and still unknown genes (i.e. FLJ10044, FLJ10359). This differences may represent distinct pathways of carcinogenesis Our results obtained so far corroborate the rather stable molecular profile of PTC as postulated by Huang and form a starting point for the further studies of molecular markers in RET-negative thyroid cancers.

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The role of XPD exon 10 polymorphism in susceptibility to ovarian and breast cancer

S. Costa[1], D. Pereira[2], D. Pinto[1], A. Vasconcelos[1], H. Rodrigues[2], R. Medeiros[1]. [1] *Instituto Portugues Oncologia -Porto, Lab. Patologia Molecular, Porto, Portugal;* [2] *Instituto Portugues Oncologia -Porto, Dep. Oncologia Medica, Porto, Portugal*

Background: Breast and ovarian cancer are two neoplasia with important incidence and mortality in women all over the world. The mechanisms involved in the carcinogenesis of these cancers are not well understood. Nucleotide excision repair (NER) is a crucial pathway in the maintenance of genome stability. Variants of several DNA repair genes, including gene *XPD*, have been described. This protein has a dual function, both in nucleotide excision repair and in basal transcription. The *XPD* exon 10 polymorphism is characterized by a G to A change, being responsible for aspartic acid to asparagine amino acid substitution in the coding region of the *XPD* gene.The purpose of this study was to evaluate the role of *XPD* exon 10 polymorphism as genetic indicator of susceptibility to breast and ovarian cancer.

Materials and Methods: We have used a case-control study. We analysed DNA samples from 499 unrelated individuals, 199 breast cancer

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.

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

E. Marcuello[1], A. Menoyo[2], E. Del Rio[2], M. Gomez-Pardo[2], M. Balcells[3], M. Baiget[2]. [1] *Hospital de Sant Pau, Medical Oncology, Barcelona, Spain;* [2] *Hospital de Sant Pau, Genetics, Barcelona, Spain;* [3] *Almirall-Prodesfarma, Oncology, Barcelona, Spain*

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.

E. Tchevkina, A. Martinjuk, A. Komelkov, A. Tatosyan. *Cancer Research Center, Onconenes Regulation Laboratory, Moscow, Russian Federation*

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

M. Salehi[1], R. Salehi[1], M.H. Goyns[2]. [1] *Isfahan Medical University, Medical School, Isfahan, Iran;* [2] *School of Sciences, Sunderland University, Sunderland, UK*

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.