The aim of this study was to examine the effect of the ACE inhibitor ramipril (RAM) on tumour growth and metastases as well as the underlying mechanisms.

Material and methods: A murine Lewis Lung tumour and metastasis-model was used. C57BL/6J mice were inoculated subcutaneously with $2x10^5$ tumour cells on day 1. Treatment was initiated the following day as RAM 30mg/kg/day in the drinking water, cyclophosphamide (CTX) 100mg/kg intraperitoneally (i.p.) day 2, doxorubicin (DOX) 2.4mg/kg i.p. day 2-5 or combinations of RAM+CTX or RAM+DOX. Tumour size was recorded three times per week and mice were sacrificed on day 28. The lungs were processed for stereological determination of metastasis volume. Tumours were collected and examined for expression of MMPs at mRNA and protein levels by RT-PCR and Western blot. Some of the lungs were examined for MMP expression at the protein level as well.

Results: Based on time to reach a tumour volume of $800mm^3$ Kaplan-Meier plots were constructed for each treatment group. Compared to saline-treated controls, RAM treatment significantly increased time to tumour volume $800mm^3$ (23 days versus 21 days, $p<0.0001$, log rank test) and significantly decreased the volume of lung metastases ($P=0.002$, Mann-Whitney test). RAM+CTX and RAM+DOX significantly increased time to tumour volume $800mm^3$ (25 days versus 21 days, $p<0.0001$ and 25 days versus 23 days, $p=0.0013$, log rank test). Lung metastasis volumes were also significantly reduced by the combined treatment regimens ($p=0.003$ and $p=0.015$, Mann-Whitney test) compared to treatment with CTX or DOX alone.

MMP-2 and MMP-9 were measured at the mRNA level in tumour extracts and at the protein level in tumour and lung extracts. RAM had no effect on the mRNA level of either MMP. In tumours the MMP-2 and MMP-9 protein expression were similar in all treatment groups. In lungs RAM-treatment tended to increase MMP-2 protein expression and decrease MMP-9 protein expression.

Conclusion: Treatment with RAM significantly inhibited tumour growth and lung metastasis formation. The effect of two different cytostatic agents on these parameters was increased when RAM was added, suggesting an additive or even synergistic effect.

631 POSTER

Nitric oxide up- regulates cyclooxygenase-2 expression through the cAMP- response element in its promoter in a head and neck cancer cell line

S.W. Park[1], M.W. Sung[2]. [1] *Seoul National University College of Medicine, Cancer Research Institute, Seoul, Korea;* [2] *Seoul National University College of Medicine, Department of Otorhinolaryngology - Head and Neck Surgery, Clinical Research Ins, Seoul, Korea*

Background: We previously observed the over-expression of cyclooxygenase-2 (COX-2) and the increased production of prostaglandin E by nitric oxide (NO) in several cancer cell lines. In this study, we investigated the mechanisms of interaction between the NO and COX-2 pathways in head and neck cancer cells.

Material and Methods: For our experiments, we used plasmids containing partial COX-2 promoter region and the fusion *trans*-activator plasmids (pFA-ATF-2, pFA2-CREB, and pFA2-cJun from stratagene) and performed western blotting and promoter-luciferase assay.

Results: cAMP-response element (CRE) was identified as a critical factor of COX-2 expression in SNU-1041. It was found that at least three transcription factors (TFs) - CREB, ATF-2 and c-jun, could bind to CRE of the COX-2 promoter and that their activities were increased by SNAP, a NO donor. Also we found that the activation of soluble guanylate cyclase, p38 and JNK by NO might play an important role in COX-2 over-expression through the up-regulation of these three TFs. The effect of dibutyryl-cGMP on COX-2 expression was similar to that of SNAP and was blocked by a p38 inhibitor, not by a JNK inhibitor. In addition, we found that dibutyryl-cGMP might activate CREB and ATF-2, whose activities were increased by p38, but not c-jun. Moreover, NO induced JNK signaling followed by the activation of c-jun and ATF-2 in cGMP-independent manner.

Conclusions: These results imply that NO generated endogenously at low concentrations may affect many gene expressions, including COX-2, which can promote the growth and survival of tumor cells.

632 POSTER

Genomic DNA amplification of Decoy receptor 3 (DcR3) correlates with cancer progression of well-differentiated colorectal adenocarcinoma.

M. Kawahara, Y. Koyama, C. Kanbayashi, V. Valera, T. Iiai, H. Okamoto, T. Suda, K. Hatakeyama. *Niigata University Graduate School of Medical and, Division of Digestive and General Surgery, Niigata, Japan*

Background: Decoy receptor 3 (DcR3), a member of tumor necrosis factor receptor (TNFR) superfamily, shows inhibitory effect on Fas- mediated apoptosis. We have reported the relationship between DcR3 mRNA overexpression and the progression of well-differentiated colorectal adenocarcinoma (17th Meeting of the European Association for Cancer Research, abstract #403). In the present study, we examined the relationship between DcR3 gene amplification and Fas mRNA expression, and also examined the correlation of DcR3 gene amplification with apoptotic cell death to clarify its effect(s) in human well- differentiated colorectal adenocarcinoma.

Materials and methods: Tissue specimens were obtained from 27 patients with well-differentiated colorectal adenocarcinoma who underwent operations at Niigata University Hospital between 1998 and 2002. Genomic DNA of cancer tissue was extracted from paraffin embedded sections by microdissection under light microscope. Total cellular RNA, extracted from tissue samples of both cancer and nomal mucosa, were reverse-transcribed to synthesize cDNA. Quantitative real- time PCR was carried out to determine genomic DNA amplification of DcR3 and mRNA expression of Fas by standardizing with b-globin gene. In 19 patients, cancer cell death was examined visibly by in situ enzymatic labeling of DNA strand breaks using Apoptag in situ oligo ligation (ISOL) kit. Statistical analysis was performed by Mann-Whitney U-test, Kruskal- Wallis test, and Spearman's correlation coefficient by the rank test, and the statistical significance was defined as $P<0.05$.

Results: Genomic DNA amplification of DcR3 was found in 23 cases (85.2%), and was significantly increased in patients with tumor invasion deeper than subserosa or non-peritonealized perirectal tissues ($P=0.0014$), and in the vascular invasion-positive patients ($P=0.014$). There was no significant correlation between DcR3 gene amplification and other clinicopathological features including Fas mRNA expression. By ISOL in situ cell death detection assay, DcR3 gene amplification was significantly increased in apoptotic cells-negative patients compared to positive patients ($P=0.0412$).

Conclusions: These results suggest that DcR3 gene amplification in well-differentiated colorectal adenocarcinoma may be one of the factors for evasion of apoptosis, and may be involved in cancer progression effecting variables such as depth of tumor invasion and vascular invasion.

633 POSTER

Specific activation of Akt3 in ovarian cancer

B.E. Cristiano, N. Marmy Conus, K.M. Hannan, I. Campbell, R.B. Pearson. *Peter MacCallum Cancer Centre, Trescowthick Research Laboratories, East Melbourne, Australia*

Background: The serine/threonine protein kinase Akt exists as three isoforms; Akt1, Akt2 and Akt3. Akt, which is activated in response to mitogenic stimuli, may contribute to tumourigenesis at multiple levels with the kinase shown to play prominent roles in several processes considered hallmarks of cancer including the regulation of proliferation, cell survival, invasiveness and angiogenesis. Recent studies suggest deregulation of specific Akt isoforms may be involved in individual tumour types including ovarian, breast and pancreatic cancers. Levels of Akt1 and Akt2 activity have been shown to be amplified in 6 and 36% of primary ovarian tumours, respectively. However, this work has been limited by lack of specific reagents for the Akt isoforms. This study has investigated the role of Akt3, in parallel with Akt1 and Akt2, in ovarian cancer.

Materials and Methods: The expression of each Akt isoform was assessed by western blotting using isoform specific antibodies. Akt activity was determined by direct assay using the specific peptide substrate RPRAATF, by isoform-specific immunoprecipitation assays and by western blotting with phosphospecific antibodies. Expression of Akt2, Akt3 and activated Akt (representing all 3 isoforms) was assessed in primary ovarian tumour samples by immunohistochemistry.

Results: A screen of isoform expression in 8 ovarian cancer cell lines and a non-tumourigenic control cell line, revealed Akt1 expression to vary across the cell lines, whereas Akt2 was detected in only one cell line (OVCAR3). Expression of Akt3 also varied across the cell lines with marked overexpression in 2 of 9 cell lines tested (OVCA429 and DOV13). Total Akt and Akt3 specific activity was shown to correlate with overexpression of

Akt3 in these cell lines. This increased activity also correlated with faster proliferation rates and increased survival in the absence of serum. Treatment of the cells with the PI3K inhibitor LY294002 resulted in increased cell death in those cells with high Akt activity. Akt isoform expression was assessed in 53 primary ovarian tumour samples revealing high Akt3 expression in 33% mucinous, 59% serous, and 66.6% endometrioid tumours. High Akt2 expression was observed in 11% mucinous, 18% serous but not in endometrioid tumours. Positive phospho-S473 staining, representing active Akt, correlated with high Akt3 expression.

Conclusion: These results suggest that Akt3 may play an important role in ovarian tumourigenesis.

634 POSTER

The cytochrome p450 (CYP) family 1 at early stages of carcinogenesis.

A. Vaiman. *Institute of Carcinogenesis, N.Blokhin Cancer Rese, Laboratory of Tumor Cell Genetics, Moscow, Russian Federation*

Background: CYP1 plays an important role in activation of environmental carcinogens including polycyclic aromatic hydrocarbons, PAH and some drugs. It was suggested that PAH is present in tobacco smoke and participates in tumor development in smokers. There are many studies of CYPs in cancers, however the data on regulation of CYP isoforms at early stages of carcinogenesis are limited. Using two models of cell immortalization and transformation, we studied mRNA expression and activity of CYP1 enzymes as well as the expression of *AHR* and *ARNT* genes that encode the regulators of CYP1 expression.

Material and methods: cultivation of cell culture, RNA isolation, RT-PCR, benzo/a/pyrene-hydroxilase activity assay, cell transfection, cell survival assay.

Result: In the embryo rat fibroblasts (RF) constitutive level of *CYP 1A1* mRNA was not detectable, whereas *CYP1B1* mRNA was expressed. After cells immortalization with Rauscher virus (F-27/RLV), mRNA level of *CYP 1A1* became high, and *CYP 1B1* level increased in comparison with RF cells. The F-27/RLV cells oxidized benzo/a/pyrene more effectively and were more sensitive to toxic effects of benzo/a/pyrene and 7.12-dimethylbenz/a/anthracene than RF cells. In spontaneously immortalized embryonic rat fibroblasts (Rat1) we found high expression of *CYP1B1* mRNA compared to RF cells. Treatment with TCDD increased *CYP 1B1* mRNA level in both rat cell lines. Unlike RF, Rauscher immortalized cells with relatively high level of CYP1 expression were sensitive to transforming effect of benzo/a/pyrene. In transformed clones levels of *CYP1A1* and *CYP1B1* mRNA were lower compared with F-27/RLV cells. Benzo/a/pyrene - hydroxylase activity decreased in transformed cells. In Rat/ras transformed cells obtained after transfection of *N-ras*^{asp12} gene, the constitutive expression of *CYP1B1* mRNA disappeared in comparison with Rat1 cells. The mRNAs of proteins which take part in the regulation of enzymatic induction of CYP1 (AHR and ARNT) were the same in all cell models studied.

Conclusion: Constitutive and inducible levels of *CYP1B1* mRNA increase after immortalization. Transformation of immortalized cells provokes disappearance of *CYP1B1* expression. Since *AHR* and *ARNT* expression are similar in all cells studied, we suggest that other factors besides AHR and ARNT take part in CYP1 regulation.

Paediatric oncology

635 POSTER

Second tumors after treatment for Hodgkin's lymphoma (HL) in children

S. Safonova, A. Malinin, Yu. Punanov. *Cancer Research Institute, Paediatric, St.Petersburg, Russian Federation*

Second malignant tumors (SMT) are one of the most severe complications in children treated for HL. The aim of the study was to investigate the incidence of second tumors in children with HL.

Materials and methods: We observed 450 pts. in CR after treatment of HL from 1972 to 1997. The period after treatment was 60-360 months (med.=134,5). The age of pts was from 2 to 18 years (med.=12,5 yrs; M/F=2,6/1). Histological subtypes nodular sclerosing and mixed cellularity were predominance. The III and IV stages of HL were reveal in 70% patients. Practically all children were undergo combined treatment HL, which were include chemotherapy (MOPP, COPP, ABVD, OPPA, PCVP) and radiotherapy (20-50 Gy).

Results: Second tumors were found in 20 pts (4,4%). Malignant tumors were in 12 pts (2,7%), and benignant tumors 8 pts (1,8%). Among SMT were: stomack cancer -3, breast cancer -2, thyroid cancer -1, liposarcoma -1, glyosarcoma -1, malignant schwannoma -1, rhabdomyosarcoma -1, acute myeloblastic leukemia -1. SMT were reveal in period from 30 to 340 months to beginning the treatment HL (med. =165 months). Mortality rate for patients with SMT compose 41,7%. Second benignant tumors (SBT) were diagnosed in 8 pts during 46-300 months (med.=153). Thyroid adenoma were in 5 pts, breast fibroadenoma 2, papillomatosis of larynx 1. One patient suffered from 2 SBT thyroid adenoma and neurinoma.

Conclusion: SMT more often reveal in patients with HL, which treated in age older 10 years (8 pts from 12) and in women (8 pts from 12). All the second solid tumors (SMT and SBT) are localized in zone of radiotherapy with dose 40 Gy. Thyroid and breast tumors are the most frequent in structure of second tumors.

636 POSTER

Neuroblastoma in adolescents and adults: analysis of a mono-institutional series of 33 consecutive patients.

M. Podda[1], D. Polastri[1], M. Massimino[1], P. Collini[2], L. Gandola[3], L. Piva[4], M. Terenziani[1], M. Casanova[1], F. Fossati Bellani[1], R. Luksch[1]. *[1] Istituto Nzionale dei Tumori, Pediatric Oncology, Milano, Italy; [2] Istituto Nzionale dei Tumori, Pathology, Milano, Italy; [3] Istituto Nzionale dei Tumori, Radiotherapy, Milano, Italy; [4] Istituto Nzionale dei Tumori, Surgery, Milano, Italy*

Background: Neuroblastoma (NBL) is the most common extra-cranial solid tumor in children. More than 95% of patients at diagnosis are younger than 10 years. Adolescents and adults have a grave prognosis, but may have a more indolent course.

Material and methods: From 1980 to 2002, 33 patients (stage I= 3, stage II= 6, stage III=8, stage IV=16) with newly diagnosed NBL older than 12 have been admitted at Istituto Nazionale dei Tumori of Milan. Median age was 17 yrs (range 12-69); M/F ratio was 1.2. Symptoms were present and disregarded for many months in the majority of cases: the mean time frame between the onset of symptoms and diagnosis was 15 months. Site of the primary tumor was retroperitoneum in 19 cases, mediastinum in 5, pelvis in 1, cervical in 1, while 6 had an esthesioneuroblastoma; 1 case the primary tumor was unknown. LDH was elevated in 15/33 pts. *Treatment applied*: surgery alone for stage I; post-operative radiotherapy for stage II; stage III and IV received chemotherapy regimens including anthracycline + cyclophosphamide + vincristine ± ifosfamide ± etoposide± cisplatinum. In addition, 10/16 stage IV pts were submitted to sequential hemi-body irradiation as consolidation treatment. Radiotherapy and/or surgery on primary and metastases were decided on individual basis.

Results: 20/33 relapsed: 0 stage I, 1 stage II, 4 stage III, 13 stage IV, and 18 relapsed died. The median follow-up is 42 months (range 12-264). EFS and OS probability at 5 years are shown in the table:

	Stage I	Stage II	Stage III	Stage IV
EFS	1	0.67	0.40	0
OS	1	0.83	0.56	0.12

In this series a consistent number of late relapse/progression were observed: time to progression/relapse ranged from 3 to 58 months and the time from relapse to death from 2 to 75 months. In univariate analysis, together with the stage, the only statistically significant prognostic factor is LDH level at diagnosis: an elevated LDH negatively predicted the outcome (5 yrs OS: normal 54%, pathological 0; p 0.0089).

Conclusions: Localized NBL (stage I and II) in adolescents and adults have the same good prognosis of children. For pts with locally advanced and metastatic disease, late events were frequently observed, thus suggesting a lower biological aggressiveness of the disease in this subset. Nevertheless, the prognosis for these patients is dismal.

637 POSTER

Evidence for a redox mechanism of action of prednisolone in childhood acute lymphoblastic leukaemia through the identification of the novel gene CGI-31.

A. Burke, P. Kearns, N. Goulden. *University of Bristol, Pathology and Microbiology, Bristol, United Kingdom*

Glucocorticoids are the most important drugs used in the treatment of acute lymphoblastic leukaemia and poor response to these drugs during