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

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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.

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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

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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