of genes such as *BNIP3, EGFR, AATF* and *NDRG1* did not change with telomere status, however genes such as *p53, p16, DAPK1, GADD45A* and *SHC1* showed a significant overexpression in the group of tumours in which telomere shortening was not 20% higher than in corresponding non tumour tissues.
Conclusion: Our data suggest a differential impact for senescence and cell death pathways in CRC and NSCLC, in relation to telomere function.

143 Expression and clinical significance of the Kv3.4 potassium channel subunit during the development and progression of head and neck squamous cell carcinomas

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Background: Increasing evidences indicate that ion channels are involved in tumour cell biology and the concept of ion channels as membrane therapeutic targets and diagnostic/prognostic biomarkers is attracting growing interest. Dysregulation of the voltage-gated potassium channel Kv3.4 has been linked to a human neuromuscular disease, periodic paralysis and Alzheimer's. In addition, increased Kv3.4 mRNA expression has been reported in oral and oesophageal squamous cell carcinomas. This prompted us to investigate the expression pattern and clinical significance of the Kv3.4 channel subunit in the development and progression of HNSCC.
Material and Methods: Kv3.4 mRNA levels were determined by real-time RT-PCR in both HNSCC tissue specimens and derived cell lines. Kv3.4 protein expression was evaluated by immunohistochemistry in paraffin-embedded tissue specimens from 84 patients with laryngeal/pharyngeal squamous cell carcinomas and 67 patients with laryngeal dysplasias. Molecular alterations were correlated with clinicopathological parameters and patient outcome.
Results: Increased Kv3.4 mRNA levels were found in 15 (54%) out of 28 tumours, compared to the corresponding normal epithelia and varied mRNA levels were detected in 12 HNSCC-derived cell lines. Increased Kv3.4 protein expression was observed in 34 (40%) of 84 carcinomas and also at early stages of HNSCC tumourigenesis. Thus, 35 (52%) of 67 laryngeal lesions displayed Kv3.4-positive staining in the dysplastic areas, whereas both stromal cells and normal adjacent epithelia exhibited negligible expression. No significant correlations were found between Kv3.4-positive expression in HNSCC and clinical data, however Kv3.4 tended to diminish in advanced-stage tumours. Interestingly, patients carrying Kv3.4-positive dysplasias experienced a significantly higher laryngeal cancer incidence than did those with negative lesions ($p = 0.0209$). In addition, functional studies using HNSCC cells revealed that Kv3.4 blockade by siRNA leads to the inhibition of cell proliferation via selective G2/M cell cycle arrest without affecting apoptosis.
Conclusions: These data demonstrate for the first time that Kv3.4 expression is frequently increased during HNSCC tumourigenesis and significantly correlated with a higher cancer risk. Our findings support a role for Kv3.4 in malignant transformation and provide original evidence for the potential clinical utility of Kv3.4 expression as a biomarker for cancer risk assessment.

144 UGT-expression in breast tissue from healthy women is associated with mammographic density

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Introduction: Mammographic density (MD) is one of the strongest risk factors for breast cancer and confers a four to six fold risk elevation of developing breast cancer, even after adjustment for other known breast cancer risk factors. The relative content of adipose, connective and epithelial tissue in the female breast determines the MD which in turn is assessed from film screen mammograms. Today, little is known about the biologic correlates of MD.
Material and Methods: Gene expression analysis using whole genome arrays was performed on breast biopsies from 79 women with no malignancy (healthy women) recruited through mammographic centres. To compare with findings in tumour samples, 64 newly diagnosed breast cancer patients were recruited. MD percentage was determined using a previously validated, computerized method on scanned mammograms. Significance analysis of microarrays (SAM) was performed to identify genes associated with MD.
Results: SAM identified 24 genes differentially expressed between high and low MD in the healthy women, including three uridine 5′-diphospho-glucuronosyltransferase (*UGT*) genes: *UGT2B7*, *UGT2B10* and *UGT2B11*. These genes had a reduced expression in samples from breasts with high MD compared with samples from breasts with low MD and reduced expression in breast cancers compared with healthy breasts. These *UGT* genes were the only genes among the 24 differentially expressed which had a similar expression in breasts with high MD and in breast cancers. The UGT enzymes inactivate several endogenous and exogenous compounds, including sex hormones. The reduced expression in breasts with high compared with low MD was most significant in the subpopulation with higher levels of female sex hormones (premenopausal women and postmenopausal women on hormone replacement).
Conclusions: Twenty-four genes associated with MD were identified. Three UGT2B genes had reduced expression in breasts with higher MD and breast cancers compared with healthy breasts. We hypothesise that reduced expression of *UGT* genes in women exposed to female sex hormones, increase MD and that this may be associated with an increased risk of breast cancer. Validation and further analysis of these genes is ongoing.

145 Role of collagen in the anti-metastatic activity of a ruthenium-based drug

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Background: NAMI-A, namely imidazolium tetrachorido (S-dimethylsulfoxide) imidazoleruthenate (III) is an anti-metastasic drug independent of conventional cell cytotoxicity. Interactions of NAMI-A with the components of extracellular matrix, including collagen, are thought to be crucial for its anti-metastatic action.
Materials and Methods: Structural changes in collagen and cultured cancer cells treated with NAMI-A were investigated by using a combination of X-Ray absorption spectroscopy (XAS), field-emission scanning electron microscopy (FE-SEM), Fourier transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM), X-Ray photoelectron spectroscopy (XPS) and gel electrophoresis.
Results: The *XAS* results showed that the incubation of collagen with NAMI-A at pH 7.4 resulted in a significant change in coordination environment, most of the Cl^- ligands being replaced with N- and O- donor ligands of the protein. The *SEM* observation demonstrated that the Ru treatment leads to the formation of Ru clusters of 25−35 nm between collagen fibrils, where the thin fibrils exhibit an increased axial D periodicity. The secondary structure of collagen was monitored by *FTIR*. When the native form of collagen was subjected to Ru treatment, the amide I band attributed to helical protein structures increased, and that attributed to random coil formation decreased. *Gel electrophoresis* confirmed the formation of crosslinks between collagen chains in Ru-treated collagen, as well as matrix metallo-proteinase (MMP) inhibition. The *TEM* results showed that the co-culture of lung cancer cells (A549) with Ru-treated collagen resulted in the formation of 20−35 nm particles along the plasma surface of cells' invasive protrusions. *XPS* revealed that these particles contained Ru.
Conclusion: Combination of the above results points to the formation of Ru clusters deposited among collagen fibrils, as well as of Ru-induced intra- and inter-molecular cross-links. The binding of Ru to collagen leads to an increase in the structural order, but does not destroy the triple helices of collagen. These changes cause inhibition of matrix metallo-proteinase (MMP) interactions with collagen, which is expected to contribute to the anti-metastatic activity of NAMI-A.

146 Identification of novel tumour-associated autoantibody signatures in gastric cancer

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Background: Gastric cancer, despite an overall global decrease in incidence, remains the third most common malignancy in Eastern Europe, and in more than 80% of the cases it is diagnosed at late stages when therapy is ineffective. Thus, the identification and validation of novel biomarkers for the early detection of gastric cancer would contribute significantly to the decrease of gastric cancer-related morbidity and mortality.
Material and Methods: We applied the T7 phage display-based SEREX technique to identify a representative set of antigens eliciting humoral responses in gastric cancer and gastritis patients. All identified antigens

were used for the production of phage displayed-antigen microarray that was applied to survey autoantibody profiles in patients with gastric cancer (n = 176), various gastrointestinal inflammatory diseases (n = 125) and healthy individuals (n = 148). The microarray data were analysed as qualitative – after normalisation the average serum antibody signal intensities in healthy donors (HD) were calculated for each antigen, and a threshold of 5 SD above the average signal intensities in HD was set to define antigens preferentially reacting with patients' sera.

Results: Serum autoantibody profiling of ~1322 element phage-displayed antigen microarray comprising all immunoselected antigens resulted in the identification of a panel of 232 antigens with potential diagnostic significance. The statistical data analysis resulted in the determination of a 60 antigen detector group that was able to discriminate between gastric cancer and healthy individuals with 81% sensitivity and 95% specificity (PPV 95%, NPV 81%), and between gastric cancer and gastrointestinal inflammatory disorders with 77% sensitivity and 90% specificity. Noteworthy, the sensitivity of the detection of stage I and II cancer was 77% and stages III and IV – 90%. Twenty-nine and 33 antigen detector groups were identified that were able to detect diffuse and intestinal type adenocarcinomas with 86% and 81% sensitivity, respectively, reaching 93% specificity in both cases, and the two groups were shown to encompass different sets of antigens.

Conclusions: Results of this study show that the serum autoantibody signatures have a potential to detect the presence of gastric cancer with significantly higher accuracy and earlier than any of the currently known serological markers and are promising candidates for the development of non-invasive serological tests.

147 Detection of circulating tumour cells in gastric cancer patients using telomerase-specific replication-competent adenoviral agent: a prospective feasibility study

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Background: Cancer of the digestive tract causes blood metastasis as well as lymphatic metastasis. Recent advanced techniques make it possible to detect circulating tumour cells (CTCs). CTCs are predicted to be involved in blood metastases. However, the relationships between CTCs and blood metastases are poorly understood.

In this study, we attempted to detect CTCs which may have potential for metastases in gastric cancer patients using telomerase-specific replication-seelctive adenovirus agent.

Material and Methods: Patients with clinical solitary gastric adenocarcinoma, underwent surgery at the Digestive Disease Center, Showa University Northern Yokohama Hospital between September, 2009 and January, 2010 were eligible for study. Patients aged over 81, those who received preoperative treatment, and those with the other organ neoplasm were excluded from this study.

Twenty-two patients (sixteen men and 6 women) fulfilled the inclusion criteria. The study was approved by the Institutional Review Board of the Showa University and each patient gave written, informed consent. The patients ranged in age from 39 to 74 years (average 55.6 years).

Peripheral blood samples (7.5 ml) were obtained from the patients before surgery, and were infected with telomerase-specific replication-competent adenovirus expressing green fluorescent protein (GFP) (OBP-401; Telome Scan) by incubation in the medium for 24 hours. Circulating tumour cells whose fluorescence can be detected were counted under fluorescence microscopy. And, it was confirmed that GFP fluorescence positive cells (GFP positive cells) were cancer cells by Immunohistochemistry staining.

The disease was pathologically staged with TNM staging system. The stage group value comparisons were performed with the Kruskal-Wallis test.

Results: Total 22 samples were examined. All values were presented as median; the value of stage IIIA group was reported as mean.

The pathological stages were IA in 12 patients, IB in 4 patients, IIIA in 2 patients, and IV in 4 patients. We detected GFP positive cells in all 22 samples. The numbers of GFP positive cells in the samples from patients at stage IA, IB, IIIA, and IV were 8.5 (range, 1–518), 3 (range, 1−10), 12.5 (range, 1−24), and 19 (range, 5−42). Although the value tended to increase with stage progression, there was no significant difference (P = 0.37).

The values in 3 patients with pathological multiple lesions at stage IA were 7, 13, and 518. On the other hand, the values in 4 patients with distant metastases were 5, 6, 32, and 42.

Conclusions: The GFP positive cells were detected in all blood samples from 22 gastric cancer patients, independently of cancer stage. There is possibility of early exact diagnosis of gastric cancer from only blood samples. In contrast, the numbers of GFP positive cells did not clearly show cancer stage. Therefore, the next study to investigate changes in numbers of GFP positive cells through a treatment is designed. Furthermore, we will analyze individual CTCs function after GFP positive cell separation.

148 In vivo preclinical evaluation of the topoisomerase I inhibitor camptothecin in human triple negative breast cancer xenografts

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Background: Triple negative breast cancers (TNBC) have a poor outcome and harbor early relapses despite a high chemosensitivity. New treatments are therefore warranted to improve the prognosis of TNBC.

Material and Methods: Using well characterized human primary TNBC xenografts (1), we have investigated the efficacy of the toposisomerase I inhibitor camptothecin (CPT11). CPT11 was intraperitonealy administered at a dosage of 50 mg/kg at day 1 every 3 weeks for 2 to 3 cycles. Tumour volume was measured twice a week and Relative Tumour Volumes (RTVs) from start of treatment were then calculated.

Results: The four human TNBC HBCx-4B, HBCx-11, HBCx-15, and HBCx-12B xenografts have been treated by CPT11, with an optimal tumour growth inhibition (TGI) of 100%, 91%, 100%, and 90%, respectively. In two models, HBCx-4B and HBCx-15, 100% and 87% of complete remission (CR) have been observed. As shown in the Table, CPT11 was as or more efficient than standard chemotherapies [doxorubicin + cyclophosphamide (AC), docetaxel (D), capecitabin (Cap.), or cisplatin (CDDP)], particularly in the HBCx-4B resistant xenograft.

	HBCx-4B		HBCx-11		HBCx-15		HBCx-12B	
	TGI	CR	TGI	CR	TGI	CR	TGI	CR
CPT11	100	100	91	0	100	87	90	0
AC	40	0	75	0	100	100	35	0
D	20	0	40	0	42	0	32	0
Cap.	45	0	57	0	71	0	55	0
CDDP	34	0	/	/	96	93	51	0

Spontaneous lung metastases occurrence is ongoing histopathological assessment in the spontaneously metastatic BC174 xenograft.

Conclusions: Altogether, these results suggest that topoisomerase I inhibitors could be efficiently used in TNBC. Further clinical trials are therefore warranted to confirm in cancer patients the efficacy of these cytotoxic agents.

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149 Prediction of response to cancer therapy from functional magnetic resonance image parameters – an artificial neural network approach

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Background: In modern cancer medicine, anatomical magnetic resonance imaging (MRI) is routinely used in diagnostics, treatment planning and assessment of therapeutic efficacy. During the past decade, functional imaging techniques like diffusion-weighted (DW) MRI and dynamic contrast-enhanced (DCE) MRI have increasingly been included into imaging protocols, allowing intratumoural information about underlying vascular, molecular and physiological mechanisms, not available in structural images, to be extracted. Separately, pre-treatment and early changes in functional parameters obtained from DWMRI and DCEMRI have shown potential in prediction of ultimate therapy response. We hypothesized that the combined use of several functional parameters may increase the predictive power.

Material and Methods: We challenged this hypothesis by using an artificial neural network (ANN) approach, exploiting nonlinear relationships between individual variables, which is particularly suitable in treatment response prediction involving complex cancer data. A clinical scenario was elicited by human prostate cancer xenografts treated with combinations of androgen-deprivation therapy and radiotherapy. DWMRI and DCEMRI from pre-radiation and on days 1 and 9 following radiation, in addition to tumour volumes and the established biomarker prostate specific antigen (PSA), were used as inputs to a back propagation neural network (BPNN), both separately and combined.

Results: The use of DWMRI parameters together with tumour volumes and PSA as inputs to the BPNN model revealed a correlation coefficient