original dizygotic existence of homologous chromosomes still make cancer genome sequencing to be a difficult subject. It has been demanded to establish a convenient method to manipulate long stretch of individual chromosomes for analyzing their sequence information respectively.
Materials and Methods: A novel methodology has been developed to amplify single chromosomes for genotyping. A key feature of this methodology is a solid-phase multiple displacement amplification, that is an enzymatic reaction of Phi29 DNA polymerase, within a solidified agarose gel. It consists of following seven steps. (I) Lysis of limited number of cultured cells within a heated agarose gel solution to release chromosome molecules. (II) Careful aliquoting of small volume gel solutions containing limited number of chromosome molecules. (III) Solidification of the gel on ice. (IV) Solid-phase multiple displacement amplification of the gel-immobilized individual chromosome molecules. (V) Recovery of the amplified materials by heating. (VI) Screening of target chromosomes by real-time QPCR. (VII) Multi-loci SNP typing using newly developed on-plastic chip allele-specific primer extension method (Michikawa et al., Anal Sci 2006; 22: 1537–1545).
Results: Utilization of agarose gel as a reaction matrix enabled reliable amplification-ready limited dilution of DNA to the level that homologous chromosomes hardly locate together. Aggregation of chromosomes while diluting process was reduced by incubating the gel solution at alkaline pH and at high temperature. Separation of chromosomes thus achieved provided reliable determination of multi-loci genotypes on each amplified homologous chromosome. Using this methodology, we could have successfully determined haplotypes of multiple SNPs in human ATM region that spans 240 kilobase pairs.
Conclusions: The methodology developed in this study is effective for genotyping long stretch of individual homologous chromosomes. Since amplified materials are easily recovered in a solution as PCR-ready form, this methodology can be used for various purposes. Further application as of demanding chromosome-wide sequencing is considerable.

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High expression of FGF19 in hepatocellular carcinoma (HCC) is associated with poor prognosis

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Background: Hepatocellular carcinoma (HCC) is one of the most aggressive solid tumors associated with poor prognosis. Fibroblast growth factor (FGF) signaling mediates cell-to-cell communication in development and organ homeostasis in adults. Of the four FGF receptor (FGFR) tyrosine kinases, only FGFR4 is expressed in mature hepatocytes. There have been numerous reports correlating up regulation or amplification of FGFR4 and a variety of human cancers. FGF19, a member of FGF family, has unique specificity for FGFR4, but its role in human cancer is not known.
Materials and Methods: We investigated mRNA of human FGF19 and FGFR4 expression in 40 HCC specimens using quantitative reverse transcription polymerase chain reaction analysis. Further, we investigated FGF19 and FGFR4 expression by immohistochemistly in 40 patients with HCC. We analyzed the correlation between patients clinicopathological characteristics and FGF19 mRNA expressions by non-parametric analysis and Kaplan-Meier method.
Results: Compared with corresponding noncancerous liver tissues, FGF19 was remarkably expressed in HCCs ($P < 0.05$). Immunohistochemical staining also showed increased FGF19 protein in HCCs. Meanwhile FGFR4 was not significantly overexpressed in HCCs. FGF19 expression was not associated with any of the general clinicopathological parameters, including age, tumor size, histological grade, and histological type. With regard to prognosis, both of the disease free survival and overall survival time for patients in the high FGF19 mRNA ratio group ($n = 20$) was significantly poorer when compared with low FGF19 mRNA ratio group ($n = 20$, $P = 0.021$).
Conclusion: These results suggest that FGF19 mRNA expression has a prognostic significance for the survival of postoperative patients with HCC. FGF19 may be critically involved in the development of HCCs.

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IFN-gamma induces transient MHC I expression in neuroblastoma cells – influence of suppressor of cytokine signaling (SOCS) 1

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Background: Major histocompatibility complex (MHC) class I expression is an obligate condition for cells concerning their recognition by the immune system. In differentiated neuronal cells, protein overexpression of the suppressor of cytokine signalling (SOCS) family downregulates MHC I expression constantly. However, little is known on the role of SOCS proteins in MHC class I regulation in neuroendocrine differentiated tumour cells. The aim of this study was to determine the effect of different cytokines on the expression of MHC class I and II molecules as well as SOCS1 and SOCS3 in the human neuroblastoma cell line SH-SY5Y.
Materials and Methods: FACS analysis and RT-PCR were used to detect MHC class I/II and SOCS1/3 expression, respectively. MHC expression was measured after 6, 12, 24 and 48 h of treatment with the cytokines IFNγ, IL-1β or TNFα. Cellular levels of SOCS1 and SOCS3 mRNA in SH-SY5Y cells were determined after 0.5, 1, 2, 4, 8, 16 and 24 h treated with IFNγ only. To assess a synergistic effect of cytokines on either MHC or SOCS expression, SH-SY5Y cells were incubated with combinations of the cytokines for 24 h and analyzed by FACS or RT-PCR.
Results: Neither MHC class I nor MHC class II expression was detectable in untreated SH-SY5Y cells and they expressed detectable levels of SOCS1 and SOCS3 mRNA. Incubation with IFNγ resulted in an induction of MHC class I molecules with a maximum after 12 h of stimulation and a constant decrease after this time point. SOCS1 expression increased significantly after 4 h when it reached saturation. The SOCS3 mRNA level was not modified by IFNγ treatment. Expression of MHC class II remained unaffected. Combinations of cytokines including IFNγ, showed an effect comparable to a treatment with IFNγ alone indicating no role of IL-1β or TNFα on either MHC or SOCS expression.
Conclusions: These data show that MHC class I in neuroblastoma cells is controlled by SOCS1 and, in contrast to differentiated neurons, can be induced by IFNγ treatment. However, IFNγ also induces SOCS1 expression, which might be responsible for the downregulation of MHC class I expression as part of a classical negative feedback loop.

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Irradiation-induced side-effects in the lung: establishment of a murine model for analysis of physiological and histological alterations

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Background: Pneumonitis and fibrosis are dose-limiting side effects of radiation therapy. Unfortunately, the underlying mechanisms are still unclear. To study the putative connection between radiation-induced tissue damage and the development of pneumonitis, we recently established a murine model for radiation-induced pneumonitis.
Materials and Methods: 4−6 week-old female C57BL6/J mice were adapted to a total-body plethysmograph and subsequently enrolled into the study at a body weight of approximately 20 g. Following anaesthesia, mice were placed in holders and their right hemithorax were irradiated with a single dose of 0/12.5/22.5 Gy using a linear accelerator ($n \geqslant 5$ mice/dose group). Thereafter, pathognomonic alterations of pneumonitis were subsequently analysed at defined time points (d1-d84).
Results: Mice developed characteristic histopathological alterations indicative for pneumonitis as judged by alveolar wall thickness, interstitial edema, interstitial and peribronchial inflammation already at day 21. These alterations were paralleled by increased breathing frequency and pulmonary resistance. Moreover, increased leakage of albumin into bronchioalveolar lavage fluid was observed. In addition, the invasion of inflammatory cells was studied histologically as well as by measuring the myeloperoxidase content in the lung.
Conclusions: This model can now be used to study the role of specified signalling molecules involved in cell death induction, damage recognition and/or immunoregulation by means of genetically defined mice strains. The detailed knowledge of the underlying mechanisms is a prerequisite for the design of radioprotective treatment.

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The aurora kinase inhibitor MK-0457 (VX-680) demonstrates anticancer activity alone or in combination with docetaxel (Dtx)

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Background: MK-0457 (VX-680) reversibly inhibits aurora kinases A, B and C (Ki's of 0.7, 18 and 4.6 nM, respectively), FLT3 (Ki 30 nM), wild type

and T315I mutant BCR-ABL (Ki's of 30 and 40 nM, respectively) and Jak2 (Ki 123 nM). Clinical investigation of MK-0457 in patients with solid and haematologic malignancies is ongoing. Aurora kinase activity is essential for microtubule spindle assembly and cytokinesis. The effects of MK-0457 and the microtubule stabilizer Dtx alone or in combination were evaluated in cancer cell lines.
Methods: A panel of eighteen NSCLC cell lines (wild-type, mutant, or p53 null) and a pair of genetically engineered p53 wild-type or p53 null alveolar epithelial cell lines were exposed to MK-0457 and/or Dtx. Cell Titer Glo (Promega) was used to measure cell viability. Cell cycle profiles were evaluated by FACS analysis. Colony formation assays were also performed (in soft agar and on plastic). The Bliss Independence method was used to assess the combinatorial effects of MK-0457 and Dtx.
Results: NSCLC cell lines showed variable single agent sensitivity to MK-0457 (IC50 range: 50 nM to >5 mM) and Dtx (0.4 to 10 nM). By FACS analysis, MK-0457 induced G2/M arrest and polyploidy, characteristic of aurora kinase inhibition. The combinatorial effects of MK-0457 and Dtx ranged from antagonism to synergy and were sequence, concentration, and cell context dependent. In viability assays, simultaneous exposure to MK-0457 and Dtx did not reveal synergy, however sequential exposure yielded synergy at low concentrations of both agents. Simultaneous exposure to MK-0457 and Dtx in long-term colony formation survival assays enhanced cell death compared to either single agent.
Conclusions: MK-0457 in combination with Dtx may result in synergistic anticancer activity, particularly in long-term CFU survival assays. Evaluation of MK-0457 and Dtx combination regimens in xenograft models is warranted.

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Preliminary microscopic evaluation of 64CuATSM as a PET radiotracer for tumour hypoxia

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Background: Tumour hypoxia results in a more aggressive phenotype together with resistance to treatment. 64CuATSM is being investigated as a positron emitting tomography (PET) radiotracer for hypoxia. Validation would provide clinicians with a non-invasive technique for tumour hypoxia assessment. This information could be used as a tool for determining most appropriate cancer therapy, prognostic information and subvolume delineation for radiotherapy dose escalation to radioresistant hypoxic regions. We looked for a correlation between 64CuATSM uptake and immunohistochemical marker of hypoxia pimonidazole in a tumour model.
Methods: Five BD-9 rats had syngeneic P22 carcinosarcoma allografts implanted subcutaneously in the left flank. After 14 days when the tumours had reached a size of 1.5 cm (approx) in diameter the rats were selected for study. Pimonidazole (60 mg/kg) i.p. was given at time = 0 hours. Anaesthetisia was administered i.p. and venous and arterial access was obtained. At time = 3 h the rats received a bolus i.v. injection of 64CuATSM (mean dose 37.17 MBq). At time = 4.25 h the animals were sacrificed and tumours resected. The tumours underwent rapid formalin fixation, wax embedding and 5 μm sections were taken. These were placed in a cassette and exposed to a phosphor screen for detection of 64CuATSM distribution. A StormTM phosphor imager obtained images at 10 days using ImageQuantTM software. The same slides were then stained for pimonidazole with HypoxyprobeTM-1 (Chemicon International). The distribution of 64CuATSM from autoradiographic detection was compared with pimonidazole distribution using linear unmixing tool TRI2 (Gray Cancer Institute in-house software) after microscopic image capture by an in-house spectral imager. Paint Shop Pro 7TM was used to orient and co-register the two distributions and ImageJ software (National Institutes of Health) was used to compare autoradiographic and pimonidazole intensity levels on a pixel by pixel basis.
Results: There was no statistically significant correlation (range −0.108 to 0.0382) between pimonidazole and 64CuATSM distribution.
Conclusion: 64CuATSM uptake in P22 carcinosarcoma in this animal model is not representative of hypoxia in the time scale indicated. It has been correlated with immunohistochemical markers of hypoxia on a microscopic level in some, but not all, rodent tumour models however in this model it is likely that other factors that determine tumour distribution of 64CuATSM dominate.

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Loss of IFN gamma sensitivity is accompanied by constitutive expression of SOCS3 and attenuation of SOCS genes induction in melanoma

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Background: The resistance to interferons (IFNs) limits their anticancer therapeutic efficacy but no attempts were made to correlate expression levels with IFN sensitivity.
Purpose: We aimed to investigate the relationship between IFN sensitivity and expression of STAT and SOCS genes.
Material and Methods: We used two subclones of human malignant melanoma WM1158 line that differ in IFNγ sensitivity. Repeated cloning of the parental cells resulted in the isolation of resistant WM1158R and sensitive WM1158S sublines.
Results: We studied the evolution of an IFN-resistant state in vitro using melanoma sublines. We found that the cells became less sensitive to antiproliferative effect of IFNγ after prolonged cultivation. While IFNγ retarded the growth of WM1158S by 80−90%, the growth of WM1158R was significantly less inhibited. The growth properties and cell morphology of both subclones in the absence of IFNγ were similar. The antiproliferative effect of IFNγ was further studied in several additional melanoma lines. These cells can be categorized into high (WM1158S, WM39, WM1552C), medium (WM1158R) and low (WM9, 1205Lu) sensitive ones.
We investigated transcription of STAT1−6 and SOCS1−3 genes as well as phosphorylation of STAT1 protein. WM1158R differed from WM1158S by a constitutive expression of SOCS3, weak SOCS1−3 induction after IFNγ, and short duration of cytokine activatory signal. Similar correlations were observed in additional melanoma lines differing in IFN sensitivities. At the protein level, IFNγ induced strong and prolonged STAT1 activation at S727 in WM1158R while this phosphorylation was less pronounced in WM1158S. On the other hand, phosphorylation of Y701 was stimulated regardless of the sensitivity phenotype.
Conclusions: Prolonged maintenance of melanoma cells in cell culture may lead to reduction of their sensitivity to IFNγ. At the molecular level, this process is associated with increased constitutive expression of SOCS3 whose levels are no longer or marginally influenced by IFN signals. Our data suggest that changes in the SOCS3 expression are tightly bound with the progression of melanoma cells from IFN sensitive to IFN resistant phenotype and may account for a growth advantage of melanoma in vivo at its advanced stages.
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Improved in vitro and in vivo anti-tumor efficacy of glucosylceramide-enriched liposomal doxorubicin

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Introduction: The continued evolution of liposomal therapeutics has resulted in new agents with remarkable antitumor efficacy and relatively mild toxicity profiles. Anti-cancer drugs generally have intracellular targets, implicating transport over the plasma membrane. For amphiphilic agents, such as the anthracycline doxorubicin, this occurs by passive diffusion. We investigated whether exogenous short-chain sphingolipid analogues improve doxorubicin influx in vitro as such and when co-administered in a liposomal formulation. Furthermore, the efficacy and toxicity of sphingolipid-modified liposomal doxorubicin on tumor growth in vivo were studied.
Material and Methods: Combinations of drugs and lipid analogues were co-administered to various (tumor) cell lines, and subsequent drug accumulation in cells was quantified. For in vivo studies, BALB/c nude mice were subcutaneously inoculated with A431 squamous carcinoma cells. The anti-tumor efficacy of sphingolipid-modified liposomal doxorubicin was compared to standard liposomal doxorubicin in a dose-escalation study. Tumor growth and regression, as well as changes in bodyweight were measured for a period of 2 weeks.