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