tumor stem cell marker CD133 is the marker of choice for identifying brain tumor stem cells in gliomas, CD133 has become a new target candidate for antibody-mediated therapy. However, the use of different anti-CD133 antibody clones possibly recognizing different CD133 splice variants with epitopes of different glycosylation status confuses the field. Furthermore, a dynamic CD133 tertiary structure has been proposed to render epitopes inaccessible in differentiating cells. All these factors are important when considering CD133 for future immunotherapy.

In the present study, we hypothesized that the use of different CD133 antibodies for identification of CD133 would yield discordant results. We investigated this using paraffin embedded sections of glioblastoma, kidney, pancreas and placenta tissue as well as glioblastoma and retinoblastoma cell lines. This material was stained with four different CD133 antibody clones and analyzed using light microscopy. In glioblastomas, ten consecutive tumors were analyzed using quantitative stereology on stainings of adjacent sections with each of the four CD133 clones.

Results revealed presence of $CD133^+$ niches in glioblastomas, often in close relation to blood vessels, using all four antibody clones. The distribution of identified niches did, however, rarely correspond among each antibody clone. Staining of glioblastoma single and niche cells was predominantly cytoplasmatic, which is opposed to the membranous staining observed in epithelial cells in kidney, pancreas and placenta tissues. Stereology revealed vast dissimilarities regarding fractions of $CD133^+$ niches and single cells among the CD133 antibody clones.

In conclusion, we report that discordant results are obtained when using different CD133 antibodies for identification of $CD133^+$ cells in paraffin sections, thereby possibly explaining current discordant CD133 observations in the literature. This may have important implications for CD133 as a new therapeutic target since it is not clear which tumor cell populations the different CD133 clones identify. Future CD133 immunotherapy should thus include comprehensive characterization of epitopes, splice variants and influence of differentiation on CD133 tertiary structure.

621 POSTER

The three-dimensional FISH with IHC can work on circulating tumor cells

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Background: Circulating tumor cells (CTCs) offer a non-invasive approach to characterize metastatic tumor cells. However, the low recovery rates of CTCs by methods performed in most clinical laboratory have limited their usefulness. Recent studies have suggested that discordance may exist between Her-2 status of a patient's CTC and that of primary breast tumor. To investigate Her-2 status in detail, we developed a new technique of the FISH analysis of CTCs. Most CTCs enrichment techniques are anti-EpCAM antibody based. However, some tumor cells express low or no EpCAM. So, we adopted the strategy independent of EpCAM antigenecity in order to reliably analyze genes even with very few CTCs.

Material and Methods: The method for Her-2 analysis that we established was based on three-dimensional multi-color imaging. Briefly, CTCs of tumor patients were concentrated by negative selection from peripheral blood by using antibodies against WBC, RBC, and platelets. The concentrated CTCs were labeled by fluorescent monoclonal antibodies against pan-cytokeratin and CD45. The specimens were then hybridized with FISH probes and were mounted in DAPI with antifade reagent. The preparations were screened for a series of Z-axis optical sections with a confocal microscope. To inspect the availability of our method, we analyzed the peripheral blood or effusion specimens of patients with gastrointestinal tumor. The study was approved by the Institutional Review Board of Japanese foundation for Cancer Research.

Results: CTCs (Cytokeratin+/CD45−/DAPI+) were easily discriminated from remaining hematopoietic cells (CD45+). And these immunocytochemical staining had no crossover on FISH signal by the benefit of confocal imaging. In addition, three-dimensional imaging reconstruction enabled counting of three-dimensional single signals, distinguishing between overlapping three-dimensional signals in three-dimensional single nuclei. Recovery rates of tumor cells spiked into normal blood averaged 79%. With the peritoneal/pleural effusion samples, the recovery rates of cytokeratin positive cells were significantly higher compared to conventional method using magnetic sorting by anti-EpCAM mAb (72% vs 9.2%, n = 6). Tumor cells in these effusion fluid expressed low or no EpCAM, so this discrepancy was considered to be cause by low EpCAM expression.

Conclusions: High recovery rate of CTCs and three-dimensional imaging made it possible to analyze Her-2 gene status easily and accurately. Using this methods, we are currently working on the investigation of Her-2 state in CTCs derived from patients with gastrointestinal tumor and its relation of treatment prognosis.

622 POSTER

Detection of genetic alterations in patients with hepatocellular carcinoma (HCC) in Coimbatore population

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Hepatoma or Hepatocellular Carcinoma (HCC) is the fourth leading cause of cancer deaths in the world. Hepatoma is one of the most common and highly malignant tumour worldwide, with a high incidence in developing country. The aim of the present study was to identify the Chromosomal aberrations in hepatoma patients to assess whether peripheral blood had non-random cytogenetic aberrations as observed in tumor samples. The study was conducted on the peripheral blood of 65 hepatoma patients (aged 30 to 85 years male) undergoing hepatic resection of liver tumour with curative intent. In the present study all the experimentals and controls were analysed chromosomal alterations using conventional G-banging. We sought to identify those changes that may be associated with development and progression of HCC. In the present investigation, HCC patients had significantly increased aberrant metaphases compared to controls. Hepatoma samples revealed frequent aberrations in chromosomes 1, 8, 17, 13, 16 and 20. Our finding of a high incidence of 1q gain and frequent deletion in the short arm of chromosome 8 strongly suggested this aberration was associated with the development of this disease. Chromatid breaks were seen on chromosomes 1, 2, and 4 while chromatid gaps were on chromosomes 1, 2 and 3. The identified altered chromosomal regions may harbour tumour suppressor genes or Oncogenes that are involved in the multistep process of carcinogenesis or disease pathology. Aberrations of diverse sites indicate that the patients probably have a constitutional chromosomal instability which participates in cancer predisposition and there is involvement of some common genes in tumor initiation and development. The results of this study might help in providing important clues and to add better knowledge in the location of relevant genes on specific altered regions of chromosomes. Comprehensive elucidation of the specific genes and molecular pathways involved in progression from pre neoplastic lesion to frank neoplastic in the protracted process of hepatocarcinogenesis will facilitate development of new strategies for prevention and therapy. Identification of molecular pathways that drive the proliferation of neoplastic hepatocytes may enable development of drugs that can specifically target and kill those cells.

623 POSTER

FDG PET/CT as an imaging biomarker for patients with metastatic renal cell carcinoma

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Background: In this era of molecular targeting therapy when various systematic treatments can be selected, prognostic biomarkers are required for the purpose of risk-directed therapy selection. Numerous reports of various malignancies have revealed that 18-Fluoro-2-deoxy-D-glucose (^{18}F-FDG) accumulation, as evaluated by positron emission tomography, can be used to predict the prognosis of patients. The purpose of this study was to evaluate the impact of the maximum standardized uptake value (SUVmax) from 18-fluoro-2-deoxy-D-glucose positron emission tomography/computed tomography (^{18}F-FDG PET/CT) on survival for patients with advanced renal cell carcinoma (RCC).

Methods: A total of 26 patients with advanced or metastatic RCC were enrolled in this study. The FDG uptake of all RCC lesions diagnosed by conventional CT was evaluated by ^{18}F-FDG PET/CT. The impact of SUVmax on patient survival was analyzed prospectively.

Results: FDG uptake was detected in 230 of 243 lesions (94.7%) excluding lung or liver metastases with diameters of less than 1 cm. The SUVmax of 26 patients ranged between 1.4 and 16.6 (mean 8.8 ± 4.0). The patients with RCC tumors showing high SUVmax demonstrated poor prognosis ($P = 0.005$ hazard ratio 1.326, 95% CI 1.089–1.614). The survival between patients with SUVmax equal to the mean of SUVmax, 8,8 or more and patients with SUVmax less than 8.8 were statistically different ($P = 0.0012$). Multivariate analysis with classical risk factors revealed that SUVmax was an independent prognostic factor ($P = 0.032$). SUVmax demonstrated a tendency to predict the survival compared with the Memorial Sloan-Kettering Cancer Center classification ($P = 0.070$ vs 0.12). This is the first report to evaluate the impact of SUVmax on advanced RCC patient survival. However, the number of patients and the follow-up period were still not extensive enough to settle this important question conclusively.

Conclusions: The survival of patients with advanced RCC can be predicted by evaluating their SUVmax using ^{18}F-FDG-PET/CT. ^{18}F-FDG-PET/CT has potency as an "imaging biomarker" to provide helpful information for the clinical decision-making.

624 POSTER

Establishment of a large panel of "early" colon carcinoma xenografts as a preclinical tool for identification of predictive biomarkers

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Cancer is the result of an accumulation of several genetic and environmental factors promoting tumour growth. Therefore, only a quite small fraction of the patients derive benefit from novel targeted drugs. This is, to a large extent, due to the molecular variability among the tumours of the same classification. Each tumour is individual, and every patient can, at least to some extent, react differently to a particular treatment.
Human tumour xenografts directly derived from patient cancer specimen can provide a preclinical research alternative considering both heterogeneity and individuality of malignomas. Xenografts allow to test novel antitumour agents in a fast and standardised manner and provide sufficient tumour tissue, even post treatment, for the search of corresponding predictive biomarkers.
It was the aim of our project to establish a relevant number of human colon carcinoma xenograft models to perform a preclinical biomarker study. 240 primary colon carcinoma tissue samples were collected during two years by a network of four clinics using a standardised procedure. Tumour pieces were transplanted onto immunodeficient mice immediately after surgery. A panel of 148 stably passagable colon cancer xenografts could be established as permanent tumour models. These patient-derived colon cancer models feature a high coincidence with the original tumour regarding histology and genome-wide gene expression profiling.
In ongoing experiments these models are subjected to an extensive characterization, including gene expression analysis, sequencing for mutations, and determination of response to classical as well as novel targeted compounds. Interim analysis of available results determined the following response rates: Oxaliplatin 7%, Cetuximab 25%, and Bevacizumab 3%. An integrated data analysis will be performed and should lead to the identification of candidate markers of response or resistance for final characterization and validation in clinical studies.

625 POSTER

Optimization of microRNAs detection in urine samples of patients with bladder and prostate cancers

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Background: Detection of cancer in its early stages is a key factor in improving patients' survival rate and lowering the cost of treatments. Unfortunately, the current methods for bladder/prostate cancer diagnosis are suffering from low sensitivity and specificity. Therefore, the discovery of novel tumor markers with high specificity and sensitivity is of great interest in cancer research.
MicroRNAs (miRNA, mir) are small endogenously-produced, non-coding RNAs with an important role in regulating gene expression. Recent studies show that miRNAs expression profiles represent significant tumor-specific changes that are unique for most cancers. The latest achievement in detecting miRNAs in peripheral blood was an important step to utilize miRNAs as a novel class of tumor markers. Detection of cancer specific miRNAs in urine would be another step to achieve. The latter is especially important for early detection and screening of the patients with bladder and prostate cancers.
Purpose: Detection and optimization of mir-21, mir-141, mir-127 and mir-205 oncogenic miRNAs in urine of patients with bladder and prostate cancers.
Methods: 4 ml of urine samples from patients as well as age-matched bladdere/prostate cancer free volunteers (control group) were aliquoted in eppendorf tubes and stored in −80ºC. RNA extraction was carried out using 2 distinct methods; Trizol and RNX solution. RNA concentration and optical absorption in 260 and 280 nanometer were measured by Nanodrop instrument. Presence of mir-21, mir-141 and mir-205 were quantified in fresh and frozen samples by real-time RT-PCR.
Results: miRNA extraction from different samples by Trizol and RNX were compared and optimized. After doing some modifications in extraction method and adding a protease K treatment step, extracted RNAs were used in real time RT-PCR. Presence of mir-21, mir-141 and mir-205 was detected in the urine of control and patient groups. The level of mir-21 in extracted RNAs using a modified Trizol method was significantly higher than RNX method. Interestingly, the levels of mirRNAs expression were much higher in the frozen urines compared to the fresh ones. Mir-21, mir-141 and mir-205 showed a differential pattern of expression in normal persons compared to the cancer patients.
Conclusions: We have succeeded to set-up a protocol to easily detect and quantify miRNAs in urine samples. Based on our preliminary data, microRNAs seem to be good biomarkers for early detection of cancers.

626 POSTER

Molecular characterization of circulating tumor cells using a highly sensitive method of enrichment based on the CellSearch CTC profile kit

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Background: The concept of "liquid biopsy" refers to analysis of rare cells in the blood such as Circulating Tumor Cells (CTC) and represents a powerful tool for molecular characterization of tumors for which biopsies are not available. An urgent need exists for improved technologies for isolation and molecular characterization of CTCs for low EpCAM-expressing cancers. Herein, we demonstrate a new approach for isolation and characterization of CTCs.
Materials and Methods: We developed a highly sensitive method for CTC characterization based on integration of the following platforms: CellSearch CTC Profile Kit for CTC isolation, immunofluorescent analysis using LSC for CTC detection and enumeration, and an automated platform for FISH analysis. The efficiency of CTC isolation by CellSearch Profile Kit was compared to that of FDA-cleared CellSearch CTC enumeration kit from Veridex, LLC. CTCs isolated from the blood of patients with non-small cell lung cancer (NSCLC), breast and prostate cancers were subjected to FISH analysis for detection of c-Met and IGF1R amplification, PTEN deletion and TMPRSS2-ERG fusion.
Results: In a side-by side comparison using blood from prostate cancer patients (EpCAM-positive), Profile Kit/LCS method recovered up to 470% more CTCs compared to standard CellSearch CTC enumeration kit. Zero CTCs were recovered by standard CTC Kit, whereas $\geqslant$39 CTCs were recovered by the Profile Kit in 4 prostate cancer cases, and $\geqslant$29 CTCs were recovered in 3 hepatocellular carcinoma (EpCAM-low). CTC detection in patients with head and neck, renal cell, basal cell, prostate, NSCLC, sebaceous gland and ovarian cancers demonstrated that the frequency of successful CTC detection was consistently higher with Profile Kit/LSC method: 79 of 90 (88%) were CTC-negative using standard CTC kit, while only 37% of patients were CTC-negative by Profile Kit/LSC method. We further validated an integrated method of CTC analysis by FISH. Using new method, NSCLC, prostate and breast cancer CTCs were interrogated by FISH and found to carry genetic abnormalities in c-Met, IGF1R, PTEN and TMPRSS2-ERG.
Conclusion: We developed a new method that offers higher CTC recovery and provides a broader capability for downstream molecular characterization of cancers. We report for the first time on the ability to conduct FISH characterization of NSCLC and breast cancer CTCs for abnormalities in c-Met and IGF1R at the single cell level.

627 POSTER

PI3K- and ERK-pathway biomarker comparison by IHC, IF/AQUA™ and RPPA upon AKT inhibition

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Background: Identification of robust mechanism-based biomarkers is increasingly important in preclinical and clinical studies. Classical immunohistochemistry (IHC) is an operator-biased technique that does not provide precise protein quantification. In the current study we have evaluated the reliability of immunofluorescence (IF)/AQUA™ and reverse phase protein arrays (RPPA) methodologies in measuring markers of PI3K pathway inhibition. Our ultimate goal is to incorporate these biomarkers in the phase I study with GDC-0068.
Methods: We have used xenografts from trastuzumab resistant breast BT474-Tr and PC3 prostate cells. Ten PI3K- and ERK-pathway biomarkers were analyzed upon treatment with a pan-AKT inhibitor, GDC-0068.