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.