Nucleic Acids and Cancer I

PROGNOSTIC AND PREDICTIVE ROLE OF CIRCULATING TUMOR DNA

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Circulating tumour DNA has been detected in multiple forms from different cancers. We first identified circulating tumour DNA both in serum and plasma in different clinical stages of melanoma patients. Our studies have shown that there is an increasing level of circulating tumour-related DNA as related to higher stages of disease. Through various melanoma patients cohorts we have demonstrated that circulating tumour DNA can be detected as specific microsatellite markers (loss of heterozygosity), methylated promoter regions of DNA, mutations, and in differences of DNA integrity. In control healthy individuals these tumour-related aberrant DNA forms are not detected. Further studies have identified that these DNA aberrations can originate from any chromosome whereby, detection can be as double or single stranded DNA. In assessing the utility of detection of circulating DNA the sensitivity decreases with early stage disease dependent on the size of the lesion. In recent studies we have demonstrated that the integrity of DNA in serum correlates with disease stage and that circulating DNA can be used as prognostic markers to predict outcome of disease progression. Using multiple microsatellite markers approach we have shown utility in predicting disease outcome. Similarly, microsatellite markers have been shown as predictive markers of therapy. Circulating methylated genes as markers have also been shown to be predictive of therapeutic responses in patients. The studies on melanoma patients strongly suggest the utility of circulating DNA of different forms in staging, identifying disease progression and response to therapy. Using multiforms of circulating DNA we have demonstrated the clinical utility as independent prognostic factors in melanoma patient’s disease outcomes.

MOLECULAR ANALYSIS OF SERUM/PLASMA DNA FOR THE DETECTION OF LUNG CANCER

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Purpose: Aberrant promoter hypermethylation of several known or putative tumor suppressor genes occurs frequently during the pathogenesis of lung cancers and is a promising marker for cancer detection. We investigated the feasibility of detecting aberrant DNA methylation in the serum/plasma samples of lung cancer patients.

Experimental Design: To determine the analytical sensitivity, we examined the tumor and the matched serum/plasma DNA for aberrant methylation of fifteen gene promoters (APC, AIM, CyclinD2, CALCA, CDH1, DCC, p16, MGMT, RASSF1, MINT31, CyclinA1, ESR1, HIC1, PGP9.5, TIMP3) from 10 patients with primary lung tumors by using QMSP, a high-throughput DNA methylation assay. Additionally, we used a training set to identify the most important DNA methylation changes in the serum of a limited number of lung cancer patients. Serum/plasma DNA from age-matched non cancer patients was used as a control. In the training set, we detected the six most promising genes (APC, CDH1, MINT31, AIM, Rassf1A and AIM) for further analysis. Results: Promoter hypermethylation of at least one of the genes studied was detected in all 10 lung primary tumors; 9/10 (90%) APC, 4/10(40%) AIM, 8/10 (80%) CyclinD2, CALCA 9/10 (90%), 9/10 (90%) AIM, 9/10 (90%) Rassf1A, 5/10 (50%) MINT31, 7/10 (70%) AIM, 5/10 (50%) HIC1, 3/4 (75%) PGP9.5 and 4/10(40%) TIMP3. In every case, aberrant methylation in serum/plasma DNA was accompanied by methylation in the matched tumor samples. In the training set, promoter hypermethylation in serum/plasma DNA from lung cancer patients was found in: 9/25(36%) APC, 4/17(24%) AIM, 4/17(24%) CyclinD2, 12/17(71%) CALCA, 10/17(59%) CDH1, 3/17(18%) DCC, 0/25(0%) p16, 5/25(20%) MGMT, 2/25(8%) Rassf1A, 0/25(0%) MINT31, 2/20(10%) CyclinA1, 1/20(5%) ESR1, 10/20(50%), 3/20(15%) PGP9.5 and 10/20(50%) TIMP3. The methylation frequencies in controls were: 2/30(7%) APC, 2/63(3%) AIM1, 3/35(9%) CyclinD2, 23/35(66%) CALCA, 2/30(7%) CDH1, 0/136(0%) DCC, 0/30(0%) p16, 1/30(3%) MGMT, 1/30(3%) Rassf1A, 0/30(0%) MINT31, 0/10(0%) ESR, 0/10(0%) HIC1 and 0/30(0%) TIMP3.

Conclusion: Our findings suggest that promoter hypermethylation in serum/plasma DNA can be detected in the majority of lung cancer patients. This approach needs to be evaluated in a larger test set to determine the role of this approach in early detection and surveillance of lung cancer.

MOLECULAR DIAGNOSTIC MARKERS FOR LUNG CANCER IN SPUMAN AND PLASMA SAMPLES.

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Purpose: Lung cancer is the leading cause of cancer deaths. This study was designed to select multiple DNA markers, which have high sensitivity and specificity to serve as biomarkers for early detection of lung cancer.

Methods: In the first study we examined the promoter methylation of three tumour suppressor genes by methylation-specific PCR (MSP), and the instability of eight microsatellite markers by loss of heterozygosity (LOH) and microsatellite instability (MSI) analyses in lung tumour tissues and matched sputum specimens from 79 lung cancer patients. In the second study we examined the promoter methylation of six tumour suppressor genes by MSP assay in lung tumour tissues and matched plasma specimens from 63 lung cancer patients.

Results: In the first study, of 79 cancer patients and 22 cancer-free individuals based on sensitivity, specificity, and concordance from each marker analyzed, we selected seven biomarkers, These were LOH of D9S286, D9S942, GATA49D12, and D13S170, MSI of D9S942, and methylation of p16INK4a and RASSF1A. In the second study, a regression model, which calculated by overall cases (63 cancer patients, 22 cancer-free individuals), achieved a sensitivity of 77%, a specificity of 90%, and a concordance of 79%. In addition, p16INK4a, RASSF1A, and RASSF1A genes had high promoter hypermethylation frequencies. The odds ratio of p16INK4a methylation and RASSF1A methylation in plasma was 5.56 (95% confidence interval, CI: 1.41–17.72, P=0.012) and 5.48 (95% CI: 1.40–37.00, P=0.014), respectively.

Conclusion: These biomarkers are potentially useful in the early detection of lung cancer.

QUANTITATIVE AND QUALITATIVE ANALYSIS OF PLASMA DNA IN COLORECTAL CANCER PATIENTS: A NOVEL DIAGNOSTIC TOOL

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High levels of cell-free circulating DNA and presence of cancer-related gene alterations at time of surgery have been demonstrated in plasma or serum of patients with different tumor types. Our aim was to verify whether levels and cancer-related gene alterations in cell-free DNA in plasma might indicate the presence of colorectal cancer.

We studied 70 patients with primary colorectal cancer and 20 healthy donors. Plasma samples were obtained from patients at the time of surgery. Plasma DNA was measured by using the Dipstick method. A subset of plasma and paired tumor samples were characterized for K-Ras mutations and p16INK4a promoter methylation.
At the time of surgery, cell-free plasma DNA levels in all patients were about 25 times higher, in comparison with healthy donors. In contrast, the CEA value of this cohort of patients was altered in only about 40% of cases. Moreover, our data show that cell-free DNA levels decreased progressively after surgery in tumor-free patients, while those developing recurrences or metastasis showed a concomitant increase in plasma DNA level.

These results were supported by qualitative analysis, such as the detection of K-Ras mutations and p16INK4a promoter hypermethylation.

Our preliminary data confirm that plasma tumor DNA levels: i) are significantly high in all patients with colorectal cancer, ii) show a progressive decrease during follow-up in tumor-free patients and iii) increase in patients with recurrence or metastasis.

Thus, quantitation of plasma cell-free DNA seems to represent a useful tool for the diagnosis and monitoring of colorectal cancer.

O16

CONCENTRATION OF EXTRACELLULAR DNA AND DEOXYRIBONUCLEASE ACTIVITY IN HUMAN BLOOD
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Deoxyribonuclease (DNAase) activity was determined by an immuno chemical method using 1 kbp PCR fragment of 28S rRNA gene labeled with biotinylated forward and fluorescein-labeled reverse primers. Undergraded PCR fragment was bound with avidin-coated 96-well EIA microtitre plates and detected with anti-fluorescein antibodies. Serial dilutions of DNase I were used to obtain calibration curve.

Concentration of DNA was measured using Hoechst 33258 dye after DNA isolation from blood plasma by guanidine thiocyanate/glass milk procedure.

The average concentration of circulating DNA (cirDNA) in plasma of healthy men did not exceed 16 ± 7 ng/ml of total blood, and is similar to that in plasma of healthy women (15 ± 13 ng/ml). The average DNase activity in plasma of healthy men was 0.405 ± 0.524 units/ml (n =10) and in healthy women was 0.360 ± 0.249 units/ml (n =10). This difference was not significant.

Increased concentration of cirDNA in blood plasma of patients with colon (n = 8) and stomach (n = 8) cancer (102-297 and 178-527 ng/ml, respectively) was accompanied by low DNase activity. In both groups activity of DNase was lower than the detection limit of the assay (2*10-5 units/ml plasma).

Thus, decrease in DNase activity in plasma of patients with malignant gastrointestinal disease can cause increase in cirDNA concentration.

O17

CIRCULATING SERUM AND SALIVARY TRANSCRIPTOME BIOMARKERS FOR ORAL CANCER DETECTION
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Specific circulating mRNA biomarkers in serum or plasma are widely investigated for cancer detection in patients with colorectal, breast, lung and thyroid cancers and malignant melanoma. Aim: The aim of this study was to test the hypothesis that discriminatory mRNA profiles exist in serum and saliva of oral squamous cell cancer (OSCC) patients.

Methods: Serum and saliva were collected from patients with primary T1/T2 OSCC (n=32). The same number of normal subjects were matched with cancer patients and used as controls. Precautionary methodologies and techniques were developed to halt RNA degradation and to maximally recover human mRNA from saliva and serum. The quality and quantity of RNA were assessed by different RNA assays. HG-U133+2.0 microarrays were used to profile transcriptome patterns between cancer patients and controls. Quantitative PCR (qPCR) was used to validate the selected transcripts that showed significant difference in saliva and serum. Multiple statistical strategies (dChip, RMA and MAS 5.0) were used to build prediction models to identify the combinations of serum and saliva RNA biomarkers that can best discriminate cancer patients from controls.

Results and Conclusions: Our optimized methods recover up to 10,000 species of human mRNA both in serum and saliva. The RNA harvested was of sufficient quality for PCR, qPCR and microarray applications. Both serum and saliva exhibit unique gene transcriptome profiles. The risk model yielded a predictive power (ROC) of 95% by using only the salivary transcriptome and 88% by using only the serum transcriptome for OSCC detection. These results indicate that transcriptome biomarker profiling from bodily fluids can be exploited to robust, high-throughput and reproducible tools for cancer detection.

O18

DNA-BASED SCREENING ASSAYS FOR MUTATION AND LOSS OF HETEROZYGOSITY (LOH) ASSESSMENT IN PLASMA DNA OF BREAST CANCER PATIENTS: POTENTIAL USE FOR EARLY SCREENING AND MONITORING RESPONSE TO THERAPY
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We evaluated the potential utility of occult circulating tumor DNA as a molecular marker of disease in subjects previously diagnosed with breast cancer. In the initial pilot study, an in-depth screening panel was designed to assess both LOH and mutation status in primary tumours and plasma of lung cancer patients at various disease stages with the goal of identifying a panel of markers for future use in screening and monitoring. DNA was extracted from primary tumours, matched plasma and peripheral lymphocytes from 60 breast cancer patients. Eight genes (PIK3CA, AKT1, AKT2, PTEN, TP53, KRAS, EGFR, and ERBB2) were scanned for mutations, and eight microsatellite markers covering chromosomal regions 1p, 8q, 10p, 14q, 16q and 17p/q were used to assess LOH. Variations were identified with a denaturing high-performance liquid chromatography (DHPLC) platform that uses post-separation fluorescence technology, enabling the detection of variants that represent < 0.1 – 1.0% of the total analyzed DNA. Using this approach, at least two somatic events were detected in 48/60 (82%) and one in 100% of the tumours. At least one event was detected in 46/60 (77%) plasma DNAs, which included early stage samples. All markers detected in plasma were also observed in the matched primary tumor. The results emphasis the heterogenous pattern of genomic alterations and that mutation and LOH scanning provides an attractive approach to breast cancer screening. The thoroughness of the approach may have important implications for screening and staging, and disease monitoring during and following therapy.