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Circulating Nucleic Acid in Plasma and Serum

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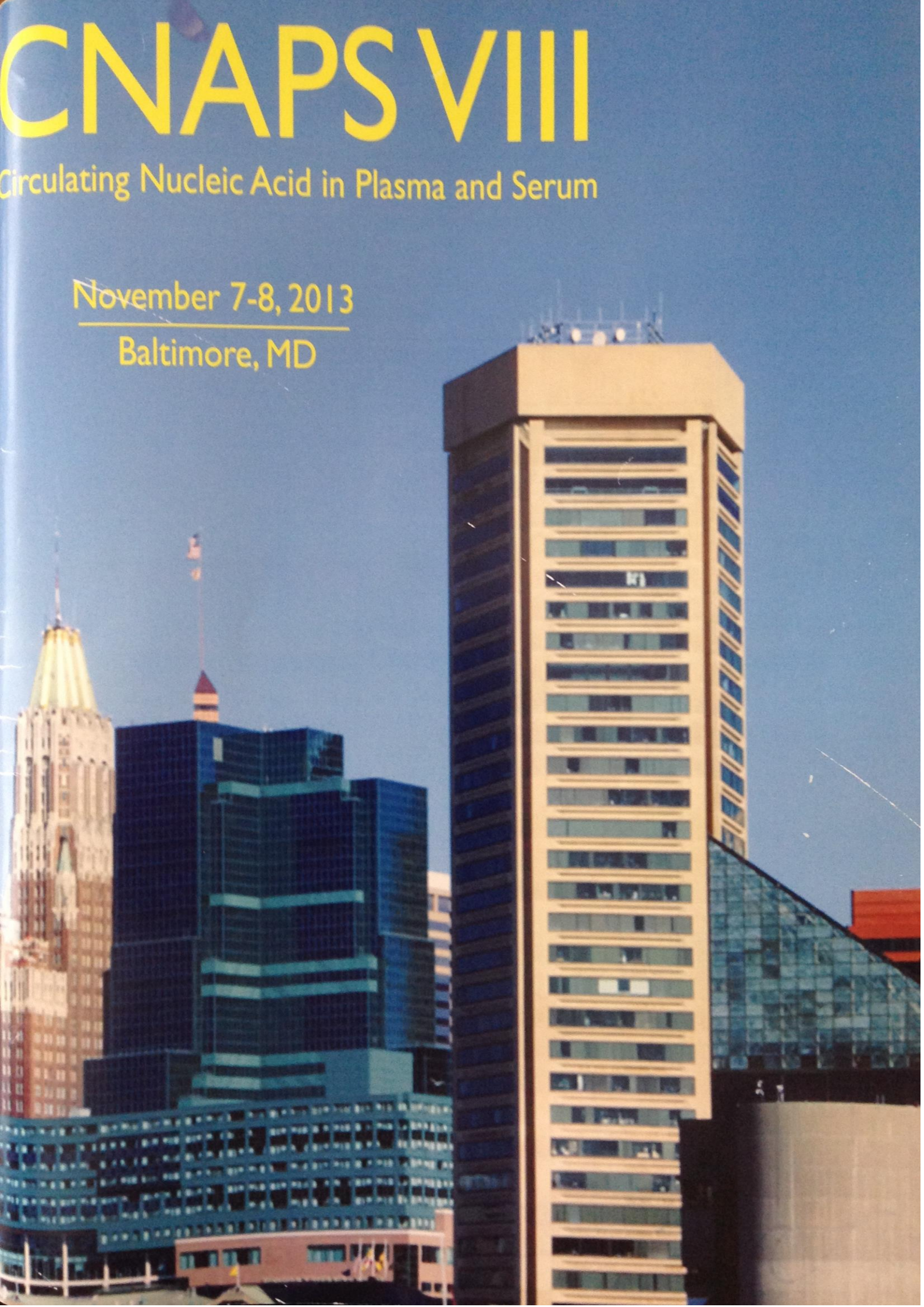

A Salivary Transcriptomic Platform to Predict Oral Feeding Maturity in the Premature Newborn: The NOuRISH Panel

Jill L. Maron MD, MPH, Jooyeon S. Hwang, Brendan Lee, Subash Pathak MS, Gil Alterovitz PhD

Background: Currently, there is no objective assay that can accurately assess mature oral feeding skills in the premature newborn. This results in short and long-term feeding associated morbidities, prolonged hospitalizations, and millions of health care dollars spent annually.

Objective: To test the accuracy of a novel, objective salivary transcriptomic diagnostic platform (NOuRISH: Neonatal Oral-feeding Readiness In Salivary High-throughput diagnostics) for the noninvasive determination of oral feeding maturity in the neonatal population.

Methods: Data derived from comparative whole transcriptomic microarrays performed on salivary samples collected from preterm infants pre- and post-oral feeding success (n=12) were used to generate the diagnostic platform. Predictive gene targets were indentified in a non-biased fashion using a Bayesian network with a n-fold cross-validation model. An area under ROC of 0.90 for the Bayesian network with 200 best features was obtained. To test the network's accuracy at predicting feeding status, a diagnostic platform composed of 24 genes, inclusive of 2 reference genes, was generated. Four hundred salivary samples derived equally between 200 unsuccessful and 200 successful oral feeders were tested on this high-throughput multiplex RT-qPCR platform. All samples were run in duplicate with appropriate controls. Samples were prospectively collected and retrospectively correlated to feeding status at time of collection. Statistical analyses were performed on normalized gene expression data to determine the accuracy of each gene at predicting feeding status. Genes were analyzed in both a binary fashion (+/- expression), as well as by their mean relative expression between successful and unsuccessful oral feeders.

Results: Salivary volume collected from each newborn was ~10 µL. The NOuRISH platform successfully amplified 97.5% of samples and 95% of target genes. Eight genes were found to be statistically significant in this experiment: four genes in the binary analysis and four genes based on relative gene expression differences between feeding groups. Genes identified were involved in the developing peripheral and central nervous systems, hypothalamic regulation of feeding behavior, head morphogenesis, and visual development.

Conclusion: Transcriptional analysis of neonatal saliva identifies objective, novel, and informative biomarkers that accurately predict mature neonatal oral feeding skills. Multiple developmental systems may be monitored in real-time from a single, noninvasive sample source. These data lay the foundation for integrating neonatal salivary diagnostics into the NICU to improve clinical care by reducing feeding related morbidities and their associated medical costs.

Exosomal lncRNA Levels may Help Discriminate Prostate Cancer from Bening Disease

Mustafa ISIN

Background: Prostate cancer (PCa) and bening prostatic hyperplasia (BPH) are the diseases most frequently observed in elder men and limited information is present in the literature concerning their molecular basis. PCa is diagnosed via biopsy and patients undergo biopsy according to their serum prostate specific antigen (PSA) levels. However, serum PSA levels are affected by prostate volume, age inflammation, trauma and some drugs and false negative results may be observed. This points out to a fact that there is no such highly specific test for PCa. In our study, we aimed to investigate exosomal levels of lncRNAs GAS5 and LincRNA-p21 which are involved in the prevention of uncontrolled cell proliferation. For this purpose, urine samples were collected from patients after digital rectal examination.

Materials and Methods: 20 patients diagnosed with PCa and 19 with clinic BPH were enrolled. Exosomal RNA was isolated from participants' urine samples and cDNA synthesized. GAS5 and LincRNA-p21 levels were investigated by real-time PCR method and the results were statistically evaluated with SPSS ver.15.

Results: We observed higher lncRNA levels in PCa patients then the BPH patients (median values for LincRNA-p21; 0.305 and 0.164, and for GAS5 were; 1.986 and 1.433). Furthermore, we observed that both lncRNAs were highly specific for discriminating between PCa and BPH (lincRNA-p21 AUC=0.778, GAS5 AUC=0.800, CI:%95).

Conclusion: Our results indicate that exosomal LincRNA-p21 and GAS5 levels found in urine samples are higher in PCa patients and their levels may be used in the discrimination between PCa and BPH.

The Investigation of Quality Check Program in Plasma Samples

Katsutoshi Shoda

Backgroud: MicroRNAs (miRNAs) play important roles in variety of biological processes, and have been detected in many body fluids. It was, however, proved that the amounts of miRNAs in the plasma of hemolyzed samples are affected by many miRNAs originated from blood cells, and the macroscopic color tone or measurement of absorbance in plasma would be related with hemolysis. So we tried to examine the more objective method about the quality check of plasma samples.

Method: The first, we set up the permitted level about the color tone and examined the absorbance at 405nm wavelength in the 480 clinical samples. And next, we examined the amounts of candidate miRNAs (miR16, 19b and 223), which show the increased or equal levels in the miRNA microarray of fresh and hemolyzed plasma, by PCR method.

Result: The ROC analysis was obtained by the absorbance at 405nm wavelength and the macroscopic color tone. The AUC was 0.926 and cut off value of absorbance was 1.25. The amounts of candidate plasma miRNAs (miR16, miR19b) in hemolyzed sample were significantly high (p=0.001). And also in the clinical samples, which absorbance was near the cut off value, the amounts of these miRNAs (miR16, miR19b) showed the higher levels.

Conclusion: We think it will be very important to establish the easy and objective method for the quality check of plasma sample affected by hemolysis.

Diagnostic values of plasma miR-18a in gastric cancer patients, and evaluation of miRNA releasing mechanism in cell culture model

Masahiro Tsujiura, Shuhei Komatsu, Daisuke Ichikawa, Shoji Hirajima, Ryo Morimura, Hirotaka Konishi, Hiroki Takeshita, Tomohiro Arita, Tsutomu Kawaguchi, Hiroaki Nagata, Atsushi Shiozaki, Hitoshi Fujiwara, Kazuma Okamoto, Eigo Otsuji

Objectives: To improve the clinical outcomes of cancer patients, early detection and accurate diagnosis of diseases are necessary. Therefore, the importance of developing a useful biomarker should be emphasized. Recently, accumulating reports have been proven the potent usefulness of circulating microRNAs (miRNAs) as diagnostic and prognostic biomarkers in a variety of human cancers. However, the biological roles and significance of circulating miRNAs have not yet been fully elucidated, especially regarding the origin, kinetics and metabolism. In this study, we hypothesized that plasma miR-18a, a component of miR-17-92 cluster, could contribute to a novel plasma biomarker in patients with gastric cancer (GC), and evaluated the possible release mechanism of miRNA into the surrounding environment in cell culture model.

Methods: We focused on miR-18a based on the finding that it was reported as highly expressed in GC tissues among miR-17-92 cluster. (1) The clinical significance of plasma miR-18a levels in GC was evaluated by comparing the findings between GC patients (n=104) and healthy controls (n=65) by quantitative RT-PCR method. (2) To verify the potential of monitoring tumor dynamics, 22 paired plasma samples were obtained before and after curative gastrectomy. Subsequently, the miR-18a expression levels of those samples were investigated by plasma miR-18a assay. (3) By using miR-18a expressing GC cell line, the relationship between the miR-18a levels in cultured medium and plated cell number was examined, mimicking the interaction between the primary tumor cells and the adjacent bloodstream.

Result: (1) The plasma concentrations of miR-18a were significantly higher in GC patients than in controls ($P < 0.0001$). The receiver-operating characteristic (ROC) plots aimed at assessing the feasibility of this assay provided a strong separation