miRNA Biomarkers for Toxicology

SOT | CONTEMPORARY CONCEPTS in TOXICOLOGY

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ABSTRACTS
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ABSTRACT

**Poster No: 1**

**Exposure to the Genotoxic Chemical 1,3-butadiene Confers Tissue-and Strain Specific Alterations in microRNA Expression**

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MicroRNAs (miRNAs) are a class of critical posttranscriptional regulators of gene expression that can silence genes by blocking translation or inducing mRNA degradation. The role of miRNAs as mediators of toxicity has recently received increasing attention, but is not fully understood. In the present study, 1,3-butadiene (BD), a widely used industrial chemical and an environmental pollutant, was used as a model human and rodent carcinogen to study chemically-induced alterations of miRNA expression in mice. Although genotoxicity is an established mechanism of the carcinogenicity of BD, epigenetic alterations (DNA methylation and histone modifications) have also been observed in a tissue- and strain-specific manner in mice exposed to BD by inhalation. We hypothesized that tissue- and strain-specific changes in miRNA expression also occur after short-term exposure to BD, and that some miRNAs potentially regulate transcriptional response to BD. To test these hypotheses, we sequenced both mRNA and miRNA collected from the liver, lung, and kidney of male C57BL/6J and CAST/EiJ mice that were exposed to 0 or 625 ppm of BD by inhalation (6 hr/day, 5 days/week) for 2 weeks. While tissue-specific basal levels of miRNA expression were very similar between the two strains, the effect of BD on miRNA expression was more profound in C57BL/6J mice than in CAST/EiJ. This finding is in line with the previous observation that the C57BL/6J strain is more susceptible to BD-induced DNA damage and epigenetic effects. The greatest degree of change in miRNA expression was observed in the lung, followed by the kidney, and there were no differentially expressed miRNAs in the liver. Members of the miR-34/449 family, which are associated with DNA damage, were the most highly up-regulated miRNAs in the lung of BD-exposed C57BL/6J mice. Further, this family was identified as a regulatory hub based on the enrichment of mRNA targets of members of these families among the BD-induced down-regulated. Upregulation of miR-34/449 family members may explain the down-regulation of immune response genes observed in the lung of BD-exposed C57BL6 mice. Our results expand the current knowledge of miRNA responses across different tissues and strains, and present evidence for potential underlying mechanisms of BD-associated health effects.
Temporal and Dose-Dependent Increases in Exosomal RNA Precede Hepatocellular Toxicity in Human and Rodent Models of Drug-Induced Liver Injury

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Alterations in the abundance and composition of circulating liver-specific RNAs have been observed in human and rodent models of drug-induced liver injury (DILI). These RNAs remain stable in biofluids largely due to encapsulation within extracellular vesicles such as exosomes. Hepatocyte-derived exosomes (HDE) have been shown to modulate the phenotype of recipient cells, including Kupffer cells and cholangiocytes. Therefore, we hypothesize that HDE regulate an individual’s response to DILI by signaling to other cell types, which likely involves changes in HDE content prior to overt injury. To examine drug-induced alterations in HDE, the present work utilized the prototypical hepatotoxicant acetaminophen (APAP) to explore the time- and dose-dependent dynamics of HDE release. Exosomes were isolated using ExoQuick precipitation reagent. An in vivo study was conducted in male Sprague-Dawley (SD) rats administered APAP (0, 500 or 1400 mg/kg) by gavage (n=6). Plasma from the 500 mg/kg group revealed significant elevations in exosomal albumin mRNA (p<0.05) at 24 hr post-dose in the absence of increased plasma ALT. Exosomal and protein-bound microRNA-122 (miR-122) also increased, but did not reach statistical significance until hepatotoxicity was observed. To determine the fidelity of primary rat hepatocyte cultures to recapitulate these events, hepatocytes isolated from male SD rats (n=3) were exposed to a concentration range of APAP (0 - 30 mM) for 24 hr. Consistent with our in vivo findings, exosomes isolated from culture medium demonstrated significant albumin mRNA elevations at APAP concentrations as low as 1.1 mM (p<0.05), while ATP loss and LDH leakage were not significantly altered until 30 mM. The human relevance of these data was examined using primary human hepatocytes (n=7 donors) exposed to a sub-toxic APAP concentration (10 mM) for 24 hr. In the absence of overt necrosis, APAP exposure produced changes in exosomal albumin mRNA, and caused statistically significant elevations in exosomal miR-122 across all donors (p<0.001). We conclude that significant alterations in the liver-specific RNA content of HDE occur prior to hepatocellular injury, a finding that translates across in vivo and in vitro experimental platforms as well as across species.
Modeling Bacterial Mixtures in the Cervix during Pregnancy and the Association with microRNA Expression and Subsequent Gestational Age

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Preterm birth affects 12% of births in the US and is associated with increased childhood mortality and morbidity. The cervix is an ideal tissue to study risk factors for preterm birth, such as bacterial vaginosis. MicroRNAs (miRNAs) are small non-coding RNAs that post-transcriptionally control gene expression and play an important role in the host response to microbes. No previous studies have examined bacterially-associated alterations in cervix miRNA expression during pregnancy as biomarkers for preterm birth. We analyzed whether the association of 5 common microbes in bacterial vaginosis (bacteroides, gardnerella vaginalis, mycoplasma, ureaplasma urealyticum, and mobiluncus) modeled as a complex biological mixture predicted both miRNA expression and subsequent gestational age. We obtained cervical swabs from 80 women between 18 and 20 weeks gestation in the same fashion as a Pap smear. Bacterial 16S rRNA and miRNA expression were quantified using the Nanostring nCounter Analysis System. To model the candidate bacteria as a mixture, we applied a weighted quantile sum (WQS) approach and then performed linear regression to examine the associations between expression of 74 miRNAs and gestational age adjusting for women's age, parity, education, and environmental tobacco smoke. We developed a novel framework for modeling bacterial populations in the cervix as a mixture. In adjusted models, the WQS bacterial mixture was associated with shorter gestational age (p=0.005). The bacterial mixture was also associated with altered expression of five miRNAs including miR-494, 371a, 888, 223, and 23a (p<0.05; q<0.3). The associations with miRNA expression were largely driven by the presence of bacteroides, gardnerella and mobiluncus. Previously, we and others have shown that miR-223 expression is associated with shorter gestations. These findings suggest that subclinical bacterial colonization in the cervix might be predictive of preterm birth and that miRNAs in the cervix could be mediators of this relationship. The role of bacterial mixtures and cervical miRNA expression in pregnancy outcomes deserves further study.
Poster No: 4

Serum miRNAs As Biomarkers of Nephrotoxicity in Female and Male Rats Fed a Diet Containing Melamine and Cyanuric Acid

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Circulating microRNAs (miRNAs) have been proposed as useful biomarkers of tissue injury and disease for multiple organs, including the kidney. We have shown previously that several serum miRNAs were affected by a 28-day combined dietary exposure to melamine (MEL) and cyanuric acid (CYA) in male NCTR F344 rats. The combination of these compounds is known to lead to the formation of crystals in the nephrons and consequent obstruction of renal tubules, which may result in acute kidney failure. In the current study, we expanded our investigation to female F344 rats. Similar to males, the miRNome of serum samples from females (n = 3-4/dose group) was screened using quantitative real-time RT-PCR (qRT-PCR) and several miRNAs were found to be differentially expressed (p-value < 0.05; fold-change ≥ 2.0) in the high dose group (240 ppm MEL & CYA) versus nvehicle control. Eight of the 32 down-regulated miRNAs in females were common to those identified as down-regulated in the males. In addition, and similar to the findings in males, miR-128-3p was down-regulated in female serum from the 180 ppm MEL & CYA dose group. The screening data were validated by confirmatory qRT-PCR (n = 10-12/dose group); three additional dose groups (30, 60, 120 ppm MEL & CYA) were included to characterize better the dose-response. As previously described (Gamboa da Costa et al., 2012), kidney lesions in these animals were increased in the 180 and 240 ppm MEL & CYA groups; however, blood urea nitrogen (BUN) and serum creatinine were increased only in the 240 ppm MEL & CYA group. Our current results support the use of serum miRNAs as biomarkers of nephrotoxicity and reinforce the observation that miR-128-3p may be a more sensitive biomarker of kidney toxicity than BUN or serum creatinine in rats. IAG FDA 224-12-0003/NIEHS AES12013.
Poster No: 5

Impact of Data Normalization and Hemolysis in the Quantification of Serum MicroRNAs

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Circulating cell-free microRNAs (miRNAs) are promising biomarkers of disease and toxicity. Numerous publications suggest their superior specificity and/or sensitivity when compared with other traditionally used circulating biomarkers, for example, to assess nephrotoxicity (blood urea nitrogen and serum creatinine) or hepatotoxicity (alkaline phosphatase and alanine aminotransferase). The gold standard to quantifying miRNAs in biofluids is quantitative real-time RT-PCR, a technique widely available and easy to use; however, several extrinsic factors can have a negative impact on the outcome of the study. One such factor is the choice of the normalizer miRNA to correct the data, e.g., for differences in template input; another factor is the contribution of hemolysis to the make-up of the serum miRNome. In the current study, we screened the serum miRNome of NCTR F344 rats (n=11-12/sex) and identified miR-342-3p as a stable serum miRNA in both sexes; the expression level of this miRNA was moderate, making it a suitable candidate for a normalizer. We further confirmed its stability using a larger sample size (n=65-70/sex). We then assessed the impact of hemolysis in this and other serum miRNAs by quantifying specific miRNAs in non-hemolyzed rat serum samples that had been spiked with increasing volumes of hemolyzed blood. As expected, the serum levels of miR-451-5p, an erythrocyte-enriched miRNA, significantly correlated with that of hemoglobin, while miR-23-3p, a miRNA known to be unaffected by hemolysis, did not. The levels of miR-342-3p were also not affected by the hemolysis level. We conducted a similar correlation study using serum samples with different levels of hemolysis, but in which each sample originated from a different animal. No correlation was found between the hemoglobin levels and the levels of miRNAs, including miR-451-5p. These data suggest that, although hemolysis can impact the levels of certain miRNAs in serum, the biological variability between animals may outweigh this effect.
Poster No: 6

Serum microRNA Biomarkers of Acute Pancreatic Injury in the Dog

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Following identification and characterization of microRNA biomarkers of acute pancreatic injury in rats and mice, studies were designed to characterize the response of the pancreas-enriched microRNAs miR-216a, miR-216b, miR-217, and miR-375 and microRNAs enriched in brain or liver in a canine model of caerulein-induced acute pancreatic injury. Studies were run with one set of four male beagles 6 to 8 months of age to pilot the use of subcutaneous caerulein, for assay development, and to test the time course and dose response of microRNAs of interest compared to gold standard assays. A caerulein dose range finding study with 1 dog per dose was run first, followed by equivalent dosing of all dogs at 3 progressively lower doses, with 4-5 weeks between dosing. Serial sampling was taken pre-treatment and at 1, 3, 6, 12, 24, 48, 72 hours, and 1 week post-treatment. The four pancreas-enriched microRNAs showed a dose-related increase in serum levels that was detectable at 1 hr, peaked at 3-6 hours, and returned to near baseline levels by 24 to 48 hours post-treatment for total caerulein doses of 5, 25, 50, 100, or 150 µg/kg. Serum amylase demonstrated similar dose response and temporal response to the microRNAs, but with a reduced dynamic range of response and a slower return to baseline (48 to 72 hours). Serum pancreatic specific lipase did not consistently show a dose response, was more influenced by individual variation, and took longer to return to baseline at higher exposures. Follow on experiments using naïve groups of 4 dogs should help to confirm responses and link biomarker level changes to histologically identifiable changes in acinar and islet cell morphology.
ABSTRACT

Early, Sensitive, and Mechanistic Detection of Drug-Induced Kidney Injury in Humans Using Urinary KIM-1, miR-21, -200c, and -423

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Up to 1/3 of hospitalized acute kidney injury (AKI) patients can be attributed to drug toxicity. Additionally, nephrotoxicity is also an issue during drug development. Clinical diagnosis of AKI still utilizes serum creatinine (SCr) acknowledged as insensitive and nonspecific. In an attempt to identify biomarkers (BMs) for improved AKI diagnosis, we investigated a multidimensional approach that included Kidney Injury Molecule-1 (KIM-1) and candidate miRNAs (miR-21, -200c and -423) in a longitudinal cohort of mesothelioma patients (n=108) receiving cisplatin (Cp) therapy. All BMs were increased after treatment, also in patients without clinical AKI diagnosis. The three miRNAs were significantly increased (p<0.01) as early as 4h after Cp administration although >90% of the patients were not diagnosed with AKI based on SCr levels until 12h. Using in situ hybridization we detected an increased expression of miR-21 in injured tubules in biopsy sections from AKI patients, suggesting the kidney to be the likely source of this miRNA in urine. Complementary, in vitro experiments mimicked in vivo findings with significantly increased (1.7-3.1 fold; p<0.001) levels of all three miRNAs in the medium of primary human proximal epithelial cells after a 24h treatment with Cp. Furthermore, target analysis supports the potential of miRNA profiles in urine to reflect pathological processes in the kidney. In summary, we provide evidence for the value of using more than one BM and thus combining the sensitivity of KIM-1 along with early diagnostic and mechanistic potentials of candidate miRNAs for non-invasive detection of drug-induced AKI in humans.
Poster No: 8

Development and Qualification of Assays to Detect Circulating miR-122 in Rat and Human Serum to Support Preclinical and Clinical Studies

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MicroRNAs (miRNAs) are small, double-stranded, non-coding RNA molecules approximately 18-25 nucleotides in length in their mature form. Due to their relative stability in circulation, tissue enrichment, and dynamic range of detection, miRNAs are being explored extensively for new biomarkers of disease, drug efficacy, and safety. miRNA-122 (miR-122) is highly expressed in hepatocytes and enriched approximately 10,000-fold in the liver relative to other organs. Increased levels of miR-122 in serum or plasma have been associated with drug-induced liver injury, viral hepatitis, and autoimmune hepatitis. As such, we have begun to evaluate circulating levels of miR-122 as an exploratory biomarker of liver injury in preclinical and clinical studies. Assays to detect miR-122 in human and rat serum were developed using qRT-PCR based detection on the Fluidigm™ Biomark HD platform with commercially available reagents and kits with minor modifications. Assay linearity, dynamic range, precision, and accuracy were assessed accordingly as part of a fit-for-purpose qualification. Circulating levels of miR-122 were measured in serum from healthy human volunteers (N=92) and naive or vehicle treated Sprague Dawley rats (N=71). As there is no consensus on best practices for normalization of circulating miRNAs, serum miR-122 levels were reported relative to either volume of sample, the expression of exogenous spike-in control (miR-Cel-39), or to the average expression of a panel of potential endogenous control miRNAs in circulation. Lastly, we evaluated miR-122 levels in rat serum samples from preclinical studies where liver injury was observed microscopically and in human serum from patients with abnormal/elevated liver enzymes. The biological variability of miR-122, the effects of different normalization approaches, and correlations to liver transaminase levels and/or liver injury will be presented. In conclusion, we have developed robust assays to quantify the relative expression of miR-122 in rat and human serum and are continuing to evaluate this exploratory biomarker in both the preclinical and clinical settings.
ABSTRACT

Invited Speaker Abstracts are listed with poster numbers in the 100 series and will not have poster presentations.

Poster No: 9

**In vivo Inhalation Exposures to Jet A and JP-8 Alter Brain miRNA Expression Profiles**

J. Frey\(^1,2\), K. Henderson\(^1,2\), K. Mumy\(^3\), C. Gut\(^3,4\), J. Reboulet\(^3,4\), M. Grimm\(^3,4\), C. Mauzy\(^1\)

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Jet fuel exposure is a known occupational exposure hazard among military personnel, with exposures initiating neurological symptoms. We hypothesize that microRNA expression changes occur in specific brain regions from repeated exposure to jet fuels, altering gene expression to signaling pathways. Fisher 344 rats were exposed to 4 doses (0, 500, 1000, 2000 mg/m\(^3\)) of jet fuel administered as an aerosol/vapor combination of either Jet Fuel 8 (JP-8) or Jet A in whole body inhalation chambers. Molecular changes were anchored to phenotype alterations using post-exposure neurobehavioral testing (Morris water maze and acoustic startle reflex). Total RNA was isolated post-exposure from the prefrontal cortex, hippocampus, and cerebellum regions dissected from harvested brain tissue. The isolated RNA samples were analyzed using Affymetrix GeneChip 3.0 miRNA arrays, with the resultant data examined using GeneChip-compatible Expression Console Software v1.2 software (Affymetrix). Differential miRNA expression in the brain was seen between control/JP-8 in prefrontal cortex (miR-429, miR-200a, miR-200b), hippocampus (miR-301a, miR-29c, miR-21), and cerebellum (miR-301a, miR-153, miR-29c). Interestingly, while some overlap in expression alterations was seen, the miRNA signature to Jet A exposure contained unique specific miRNA species: prefrontal cortex (miR-667, miR-328b-3p), hippocampus (miR-352, miR-9, miR-301a, miR-199a-5p, miR-30e), and cerebellum (miR-301a, miR-199a-5p, miR-352). To further explore the biological significance of these molecular changes, pathway analysis was conducted using the Ingenuity IPA software (Qiagen). The result of this study will likely provide novel insight into the molecular mechanism of jet fuel neurotoxicity, as well as the knowledge needed for warfighter protection from the adverse effects of jet fuel exposure.
ABSTRACT

Invited Speaker Abstracts are listed with poster numbers in the 100 series and will not have poster presentations.

Poster No: 10

MicroRNA Profiling Identifies Potential Biomarkers of Hepatobiliary Injury following Exposure to Several Toxicants in the Rat

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MicroRNAs (miRNAs) are promising biomarkers of drug-induced liver injury; however, to date, candidate identification has mostly been limited to profiling in acetaminophen-induced toxicity. In this study, plasma miRNA alterations were quantified in male Sprague Dawley rats orally administered either a single dose of α-naphthylisothiocyanate (ANIT; 50 mg/kg) or daily doses of FP004BA (100 mg/kg/day) or respective vehicles, and in male Wistar rats orally administered methapyrilene (MPy; 30 and 80 mg/kg) or vehicle. Results were phenotypically anchored to the temporal progression of hepatobiliary injury and compensatory biliary hyperplasia which these compounds are known to elicit. Rats sacrificed 6, 24, 48, 72, 120, or 168 hours (h) post dose with ANIT (n=6/treatment/time point) displayed significant hepatobiliary injury from 24h to 72h post-dosing; hyperplasia was observed from 48h post-dose through study termination. Rats administered FP004BA, a Bayer Pharma AG proprietary compound, for 1, 3, or 14 consecutive days (d; n=6/treatment/time point), developed progressive hepatobiliary injury following 1 and 3d, biliary hyperplasia following 3 and 14d, and fibrosis following 14d. MPy- and vehicle-treated rats (n=6/treatment/time point) were sacrificed following 3, 7, or 14 daily doses of compound; a recovery group was sacrificed 10 days following 14 consecutive administrations of MPy or vehicle. Histopathological findings included periportal necrosis, inflammation and biliary hyperplasia; all findings increased dose and time dependently. Plasma miRNA profiling revealed that approximately 60-120 miRNAs were altered in blood, depending on the chosen selection thresholds, in each study. 25 miRNAs were commonly altered by the three toxicants examined in this investigation, independent of time and directionality. In addition to miR-122-5p and -192-5p, miR-802-5p, -200a-3p, -30a-5p, and -30e-3p were consistently elevated during hepatobiliary injury caused by all toxicants, suggesting that these species may be potential biomarker candidates for hepatobiliary injury.
Poster No: 11

Regulation of Secreted miRNAs and P450 Isoforms in Acetaminophen Metabolism in HepaRG™ Cells and Acetaminophen Overdose in Children

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Acetaminophen (APAP) is an important cause of acute liver injury. In children, APAP overdose accounts for 13% of cases of acute liver failure, and the etiology of liver injury in up to 50% of cases is unknown. This study examined miR-122-5p, as well as microRNAs (miR-378a-5p, -27b-3p and -320a) that regulate the Cytochrome P-450 isoforms (CYP2E1, CYP3A4 and CYP1A2), involved in APAP metabolism in HepaRG™ cells and in serum samples obtained from children with APAP overdose. miRNA expression was compared to ALT, LDH, and APAP protein adducts. HepaRG™ cells treated with 20 mM APAP had elevation of APAP protein adducts at 1 h and elevation of ALT and LDH at 12 and 24 h, respectively. CYP2E1, CYP1A2 and CYP3A4 mRNA expression levels were suppressed with 20 mM APAP treatment (p<0.05), while expression levels of secreted miRNA showed significant induction of miR-122-5p, -125b-5p, -378a-5p, and -27b-3p at 6 h, miR-125b-5p at 12 h, and miR-27b-3p at 24 h. Similar findings were observed in the serum samples of children with APAP toxicity (N=12, median age 15.79 y; median ALT levels of 1266.5 IU/L, median APAP protein adduct levels of 1.15 nmol/L) when compared to control subjects (N=15, median age: 7.42 y; median ALT of 16.0 IU/L; median APAP protein adducts 0.0053 nmol/L). A 140 fold increase in miR-122-5p was observed in children with APAP toxicity, compared to healthy controls (p<0.05). miR-378a-5p, a regulator of CYP2E1, was increased by 12.6 fold in APAP overdose subjects compared to healthy controls, while miRs -125b-5p, -320a and -27b-3p were increased by 8.4, 2.3 and 4.4 fold, respectively, compared to controls. In children with APAP overdose, APAP protein adducts correlated with miR-27b-3p (R=0.68), whereas ALT correlated with miR-122-5p (R=0.89) and miR-378a-5p (R=0.82) at p < 0.05. Overall, expression of miRNAs that regulate CYP2E1, CYP3A4 and CYP1A2 metabolism are increased in serum samples of children with APAP toxicity, in agreement with in vitro data using HepaRG™ cells exposed to APAP. miR-122 levels in children with APAP toxicity are comparable to levels previously observed in adults with APAP toxicity.
**Evaluation of miR-217 and miR-375 As Biomarkers of Pancreatic Injury**

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Pancreatic injury in preclinical toxicity studies is primarily detected through histopathological changes and conventional serum biomarkers such as amylase/lipase. However, amylase/lipase have a short half-life and are markers of pancreatic acinar not islet cell injury. We investigated whether circulating microRNA (miR) levels that are enriched in acinar cells (miR-217) or islet cells (miR-375) could serve as markers of pancreatic injury. Rats were treated with a single ip dose of either vehicle, the pancreatic acinar cell toxicant--caerulein (0.05 mg/kg), or the β-cell toxicant--streptozotocin (STZ) (150 mg/kg). Rats were necropsied at 4, 24, and 48 hrs and pancreas, liver, heart, kidney and skeletal muscle were collected and analyzed for histopathology. Blood was collected at necropsy and processed to serum for amylase/lipase enzymatic determinations and miR qPCR analysis. Caerulein induced degeneration/necrosis of acinar cells after 4 hrs that persisted after 24 and 48 hrs, and increased amylase/lipase levels at 4 hrs but not at 24 or 48 hrs. Increases in miR-217 (100-1000x controls) persisted for 48 hrs following caerulein and only occurred in rats with evidence of acinar cell damage. STZ induced islet cell necrosis after 4 hrs followed by atrophy at 24 and 48 hrs. STZ did not induce significant increases in either amylase or lipase but did induce increases in miR-375 levels at 4 (90x controls) and 24 hrs (5x controls). No significant increases in miR-375 were observed in caerulein-treated or miR-217 in STZ-treated rats. Our results indicate that used together, miR-217 and miR-375 represent promising biomarkers of exocrine and endocrine pancreatic injury in rats.
Poster No: 13

Relative Tissue Distribution of miRNAs in the Cynomolgus Monkey

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MicroRNAs (miRs) are small non-coding RNAs that regulate gene expression. Several miRs are currently being evaluated as potential circulating markers of target organ toxicity due to their stability, tissue specific distribution, and conserved expression across multiple species. Only a few studies have reported the relative expression of miRs in normal tissues with most of these being conducted in rodents. Limited information exists for tissue distribution of miRs in other species commonly used in safety assessment. We examined the relative expression of miRs in normal cynomolgus monkey tissue using human TLDA card arrays. Tissues examined included the heart (left ventricle and aortic valve), skeletal muscle (soleus and quadricep femoris), pancreas, liver, kidney, and eye (retina and cornea). Examination of the heart tissue revealed enrichment of 4 miRs in cardiac valves (miRs-320, 197, 125b, 342-5p) and 8 in the left ventricle (miR-133a/b, 1, 208b, 302a, 499-5p, 27b, 142-5p) compared to the other tissues. MiR-208 was specifically expressed in the heart where it was detected in both the left ventricle and aortic valve. In the skeletal muscle, miR-208b, 302a and 499-5p were detected only in the soleus while the expression of miRs 133a/b, 1 and 342-5p were enriched in both the soleus and quadricep femoris muscles. In the pancreas, miRs-216a/b, 217, 375 and 148a were highly enriched compared to the other tissues. MiR-192, 194 and 20a were highly enriched in the liver but only miR-122 was specifically expressed in the liver. No retina or cornea specific miRs were detected. Three miRs (miR-192, 194 and 215) were enriched in the kidney compared to other tissues. In general, miR tissue distribution was similar in the cynomolgus monkey compared to the rat. Assessing such cross-species differences is an important step in establishing the translational value of miRNAs as biomarkers of target organ toxicity in relevant animal species used in safety assessment and in humans.
Evaluation of the Liver-Specific microRNA, miR-122, As a Marker of In Vitro Hepatocyte Toxicity in Liver Slices and Cultured Rat Hepatocytes

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MicroRNAs (miRNA) are small single stranded non-coding RNA molecules that regulate gene expression at the post-transcriptional level. MiR-122 is specifically expressed in the liver and has been postulated to be involved in maintaining hepatocyte differentiation. Recently, circulating miR-122 levels have been used as a biomarker of liver toxicity in several different species including rats, dogs, and humans. We examined whether miR-122 release into extracellular media could also be used as a measure of cytotoxicity in vitro with cultured cryopreserved rat hepatocytes and in rat liver slices. MiR-122 levels were easily detected in both rat liver slices and in cryopreserved rat hepatocytes using qPCR techniques. MiR-122 levels were highly expressed in cryopreserved rat hepatocytes but its levels significantly decreased after 2 days in culture. The decline of intracellular miR-122 level was associated with the decrease of some rat cytochrome 450 enzymes (cyp3a2, cyp2d2 and cyp2e1). In contrast miR-122 levels were maintained over time in liver slices. Upon treatment with the toxicant, precocene, an increase of miR-122 release and decrease of intracellular miR-122 were observed at both 24 hours and 48 hours post exposure in rat liver slice incubations. The effects were moderate at 24 hours but much more significant (> 10 fold) at 48 hours. Following incubations with precocene in rat liver slices, miR-122 levels in the extracellular media increased which correlated with increases in LDH release. Our results indicate that miR-122 release is a potentially sensitive marker for assessing hepatocyte toxicity in vitro.
Poster No: 15

MicroRNAs As Biomarker of Toxicity Effects of Chemical Dispersed Oil Pollution in Caenorhabditis elegans

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Background: The BP oil spill is among the most severe environmental disasters in US history. The nematode Caenorhabditis elegans (C. elegans) has been a useful tool for environmental toxicity studies. In previous study, we found the crude oil and/or dispersant induce growth inhibition and offspring reduction in C. elegans. At the molecular level, microRNA-mediated gene silencing has emerged as a fundamental regulatory mechanism of gene expression in many biological processes. However, miRNAs-mediated gene regulation in response to major pollution events is poorly understood. Methods: Here we systematically investigated a total of 231 microRNA expression by using qRT-PCR and analyzed the expression profile of miRNAs in C. elegans in response to oil-alone, dispersant-alone and the mixture of oil and dispersant. TargetScan 6.2 and Miranda were applied to predict the targets of differentially expressed miRNA genes in each treatment individually. Then, a further target function analysis was performed based on the KEGG pathway database. Results: The aberrant expressions of miRNAs were induced and KEGG pathway enrichment analyses indicated that those significantly changed miRNA expression affect many biological processes in C. elegans. Many affected pathways are related to environmental information processing, such as ABC transporters, MAPK signaling pathway, Erbb signaling pathway, JAK-STAT signaling pathway, MTOR signaling pathway and calcium-signaling pathway. Some pathways are related to oil uptake and metabolism, such as endocytosis, fatty acid biosynthesis and phosphatidylinositol signaling system. Conclusion: Based on the genome-wide investigation of microRNA profile, it suggests that C. elegans may respond to environmental stimuli, like oil, dispersant and oil-dis mixture and activate many pathways. These pathways are related to many important biological processes, such as cell cycle, cell proliferation, differentiation and apoptosis. Since the currently identified proteins and microRNAs in C. elegans show remarkable conservation with mammals including humans, the oil/dispersant may also induce similar change in microRNAs expression and affect many biological processes that lead to reproduction toxicity.
Environmental Neurotoxicant Manganese Alters Exosomal miRNAs and Autophagic Regulation in Cell Culture Model of Parkinson’s Disease

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Many age-related neurodegenerative disorders share a common pathogenic mechanism involving the aggregation and deposition of misfolded proteins. In Parkinson’s disease (PD), the accumulation of misfolded and aggregated α-synuclein (αSyn) is considered a key pathophysiological feature. These misfolded proteins can be degraded by autophagy via the lysosomal pathway. However, the lysosomal degradation pathway is impaired in the disease state, leading to a significant accumulation of autophagic vesicles in the neuronal body. Given the role of the divalent metal manganese (Mn) in PD-like neurological disorders, we characterized the effect of Mn on misfolding and its role in autophagic/lysosomal dysfunction using an MN9D dopaminergic cell model of PD, which stably expresses wild-type human αSyn. Western blot analysis revealed that Mn increased the expression of the autophagosomal markers LC3-II and Beclin-1, whereas the lysosomal marker LAMP2 was downregulated in αSyn-expressing cells relative to vector control cells, suggesting that Mn treatment impairs the autophagic/lysosomal degradation pathway. Interestingly, Mn treatment also induced the release of αSyn-containing exosomes into the extracellular media, as determined by NanoSight particle analysis and electron microscopy. Furthermore, we found these αSyn-containing exosomes are bioactive and able to induce neuroinflammatory response and neurodegeneration in cell culture models of PD. To further elucidate the molecular mechanisms underlying Mn-induced autophagic/lysosomal dysregulation, we performed next-generation miRNA sequence analysis of manganese- and vehicle-stimulated exosomes. We identified 43 miRNAs differentially expressed in Mn-stimulated αSyn exosomes as compared to control exosomes. Among them, 12 mRNAs were associated with regulation of autophagic/lysosomal degradation pathway. Three miRNAs (miRNAs-124, 320a and 325) were previously reported to control autophagic regulation by targeting Bim, Hsc70 and E2F1 in experimental models of PD. Collectively, our results suggest a novel paradigm in which dysregulation of exosomal miRNA pertaining to autophagic degradative machinery may play a role in cell-to-cell transmission of misfolded αSyn protein.(NIH ES19267, ES10586, and NS088206)
ABSTRACT
Invited Speaker Abstracts are listed with poster numbers in the 100 series and will not have poster presentations.

Poster No: 17

Quantification of Plasma miRNAs in a Group of Healthy Smokers, Ex-Smokers and Non-Smokers and Correlation to Biomarkers of Tobacco Exposure

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Changes in miRNA expression can be an early biological event in disease development. Furthermore, a toxicological stress can damage tissues and lead to the leakage of biological material in the bloodstream, including miRNAs. Plasma miRNA have been used as markers for early diagnosis of lung cancer and show differential expression in other tobacco-related diseases such as COPD and cardiovascular diseases. Therefore we asked whether miRNAs have the potential to be used as early biomarker of biological effect in healthy smokers and if miRNAs candidates were correlated with biomarkers of tobacco smoke exposure. We profiled by QRT-PCR a panel of 84 disease-associated plasma miRNAs in 30 smokers, 20 non-smokers and 20 ex-smokers. A robust statistical strategy was applied with replicate samples to account for reproducibility of the results. We identified differential expression of miR-124 (fold change>2, Bonferroni adjusted p-value<0.05) between the smoking and control groups. The Storey method also revealed that let-7a was a potential miRNA associated with smoking. We investigated for the first time the dose correlation between the miRNA biomarkers (miR-124 & let-7a) and two biomarkers of tobacco exposure with a long half-life (CeVal, blood marker for exposure to acrylonitrile) and a short half-life (TNEQ, total nicotine in urine). miR-124 was correlated with the biomarker of acrylonitrile exposure (p<0.01) but not with the biomarker of nicotine exposure whilst let-7a was correlated with nicotine exposure (p<0.05). Although miR-124 and let-7a show a correlation with biomarkers of tobacco exposure, we found that the relationship is dependent on other confounding factors. In future, it might be worth investigating the correlation in a larger group of subjects with a broader biomarker of exposure panel representing different chemical families.
Poster No: 18

The Role of miRNA in the Interplay between Genes and Environment in Parkinson’s Disease 3d In Vitro Model

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MiRNA play a significant role in brain development and homeostasis and as well are associated with neurological diseases such as neurodevelopmental disorders, neurodegeneration and cancer. Parkinson’s disease, affecting one million people in the US, is a neurodegeneration of dopaminergic neurons in the Substantia nigra. Although there are several genes known to be associated with the familial forms of the disease, sporadic onset is suggested to result from the interplay between genetics and environment. miRNA are genetically and functionally redundant molecules and are involved in the control of organism robustness. This led us to hypothesize that they may have neuroprotective function in the interplay between genes and environment. Thus, the dysregulation of miRNA may contribute to disease pathogenesis. In this project we made use of our recently developed 3D human in vitro dopaminergic model (3D LUHMES) to study gene/environment interactions with a focus on miRNA. Dopaminergic organoids were exposed for 12 and 24h to sub-toxic concentrations of two mitochondrial toxciants, MPP+ or rotenone. Expression of mitochondria and neural-specific miRNA along with genes involved in transsulfuration pathway (donor of antioxidant glutathione) was analyzed immediately after the hit and after compound withdrawal and 7-day recovery. miR-7, enriched in dopaminergic neurons, was significantly down-regulated 12h after hit and went back to control levels after recovery, while pro-apoptotic mir-16 was up-regulated only after 7 days recovery period in the 24h-exposed group. Mitochondria respiration repressor, miR-210, remained unchanged. These results suggest that mir-7 downregulation may be an early event in the mitochondria-induced neurodegeneration. mir-7 recovery after compound withdrawal and over-expression experiments suggest a neuroprotective role of this miRNA. Further mechanistic studies of the role of this and other miRNA in neuroprotection against environmental stress may significantly contribute to PD early diagnostis and drug development.
**Poster No:** 19

**Differential miRNA and mRNA Expression in Immortalized Human Keratinocytes (HaCaT) after Low Arsenic Exposure Suggest Changes in Cell Proliferation, Cell Migration, Cytoskeleton Remodeling, and Carcinogenesis Pathways**

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**Background:** Arsenic is a toxic natural element and widely distributed in the environment. Epidemiological studies have linked chronic arsenic ingestion to several cancers, especially skin cancer. Arsenic-induced skin cancer mechanisms are not well defined, but several studies indicate that mutation is not the driving force as it is for UV-induced skin cancers. Environmentally-driven epigenetic alterations including microRNA dysregulation influence disease development. Preliminary data showed changes in miRNA expression profiles of arsenic induced premalignant hyperkeratosis and squamous cell carcinomas suggesting miRNA dysregulation in malignant progression of arsenic-induced skin lesions. Chronic exposure to low levels of arsenite also malignantly transforms immortalized human keratinocytes (HaCaT). Hypothesis: Differential expression of miRNAs at early stages of arsenite exposure in HaCaT will reveal early changes in the transformation process. Methods: HaCaT were maintained with 0 or 100 nM sodium arsenite for 3 and 7 weeks. Total RNA was purified and miRNA and mRNA expression was assayed using Affymetrix microarrays. Targets of differentially expressed miRNAs were overlapped with the mRNA expression data using Partek Genomic Suite™ software then mapped to their pathways using MetaCore™ software. Results: Several miRNAs and mRNAs, including those encoding oncogenes and tumor suppressors, were differentially expressed at 3 and 7 weeks. The data suggest that genes involved in cell proliferation, cell migration, cytoskeleton remodeling and carcinogenesis pathways were induced at 7 weeks. Conclusions: Our data provide strong evidence of early changes in miRNA profiles and their target genes in human keratinocytes contributing to arsenic-induced carcinogenesis. Supported in part by NIH grant R21ES 023627.
ABSTRACT

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Poster No: 20

Air Pollution Induced Placental Epigenetic Alterations in Early Life: A Candidate miRNA Approach

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Background: Particulate matter (PM) exposure during in utero life may entail adverse health outcomes later in life. Epidemiological studies in adults have linked air pollution’s adverse effects to alterations in gene expression profiles, which can be regulated by epigenetic mechanisms including microRNAs (miRNAs). Objectives: We investigate the potential influence of air pollution exposure in early life on placental miRNA expression. Methods: Within the framework of the ENVIRONAGE birth cohort, we measured the expression of miR-16, miR-20a, miR-21, miR-34, miR-146a and miR-222 by qRT-PCR in placental tissue from 211 mother-newborn pairs. Multiple regression models were used to study miRNA expression and in utero exposure to particulate matter over various time windows during pregnancy. In silico analysis was performed to predict genes and pathways targeted by the studied miRNAs. Results: Four out of six measured miRNAs were associated with air pollution exposure in early life. For each 5 μg/m³ increase in PM2.5 exposure during the 2nd trimester of pregnancy, the expression of miR-20a, miR-21, miR-146a and miR-222 at birth was reduced by 26.2% (95% CI: -45.2, -0.1, p=0.051), 33.8% (95% CI: -53.2, -6.4, p=0.021), 31.1% (95% CI: -48.1, -8.6, p=0.011) and 25.2% (95% CI: -47.2, -2.2, p=0.035). miR16 and miR-34a were not significantly associated with either PM2.5 or NO2 exposure over the different time windows analyzed. Pathway analysis revealed immune responses as the core pathways targeted by the studied miRNAs that were significantly associated with in utero air pollution exposure. Conclusions: Environmental exposure to particulate air pollution in early life can modify the expression of miR-20a, miR-21, miR-146a and miR-222 in human placental tissue. These miRNAs might be relevant targets involved in PM-induced effects in fetal programming and could potentially influence health outcomes later in life.
Polychlorinated Biphenyl Exposure Alters the Expression Profile of microRNAs Associated with Vascular Diseases

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Exposure to persistent organic pollutants, including polychlorinated biphenyls (PCBs) is correlated with multiple vascular complications including endothelial cell dysfunction, myocardial infarction and atherosclerosis. PCB-induced activation of the vasculature subsequently leads to oxidative stress and induction of pro-inflammatory cytokines and adhesion proteins (VCAM-1, ICAM-1). These proteins are also regulated by small, endogenous oligonucleotides known as microRNAs that interact with messenger RNA. MicroRNAs are involved in the pathogenesis of various cardiovascular diseases and have been increasingly implicated for disease diagnosis and therapeutic intervention. Critically, microRNAs are an acknowledged component of the epigenome, but the role of environmentally-driven epigenetic changes such as toxicant-induced changes in microRNA profiles are currently understudied. Therefore, the objective of this study is to determine the effects of PCB exposure on microRNA expression profile in primary human endothelial cells using two commercial PCB mixtures (Aroclor 1260 and 1254) at physiological relevant concentrations. Samples were analyzed using Affymetrix GeneChip® miRNA 4.0 arrays for high throughput detection and relative expression of selected microRNA genes was validated (RT-PCR). Microarray analysis identified 557 microRNAs that were changed with PCB exposure ($p < 0.05$). In silico analysis using MetaCore database identified 21 of these microRNAs to be associated with vascular diseases. Further validation showed that the Aroclors increased miR-21, miR-31 and miR-126 expression levels. Upregulated miR-21 has been reported in cardiac injury while miR-126 and miR-31 modulate leukocyte trafficking in inflammation. Our results demonstrated evidence of altered microRNA expression with PCB exposure, thus providing novel insights into mechanisms of PCB toxicity. Moreover, the current study implicates the relevance of microRNAs as potential biomarkers for environmental toxicant-induced diseases. Importantly, this is an applicable approach for disease prognosis and diagnosis in PCB-exposed human cohorts with cardiovascular complications. (Supported by NIH/NIEHS P42ES007380).
Poster No: 22

An Adaptive Method for Normalization of microRNA Array Data

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MicroRNAs (miRNAs) are known to play important regulatory roles in many cellular processes. However, there is currently no consensus in optimal normalization strategy for RT-qPCR miRNA quantification analysis. Despite this fact, rigorous normalization of miRNA data may be critical since relatively small changes in miRNA expression may be biologically and clinically significant. We propose miRAdnorm (MiR-Adaptive normalization method) to construct an adaptive set of housekeeping miRNAs that are stable in expression level and capture the systematic patterns induced by sample preparation and other technical artifacts. We illustrate the proposed method across a variety of scenarios through several case studies. These include preclinical and clinical samples obtained for the detection of miR-122, a tissue-specific miRNA that is transcriptionally regulated in the liver and has been associated with drug-induced liver injury, viral hepatitis, and autoimmune hepatitis.
Plenary Session Abstracts

Session I: MiRNAs As Biomarkers of Tissue Injury or Disease across Organ Systems

Poster No: 100

The Rat microRNA Body Atlas: MiRNA Discovery and Biomarker Validation

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Lilly Research Laboratories, Indianapolis, IN

MicroRNAs (miRs) have many attributes which have elicited considerable interest in their use as serum based biomarkers of toxicity. In order to identify tissue specific/enriched miRs and to understand serum miR changes, we have constructed a rat miR body atlas using Illumina miR sequencing of 22 rat tissues of toxicologic interest. We have evaluated miRs conserved between rat and dog in pancreas toxicity studies and demonstrate that miRs can be used as species translatable biomarkers of pancreas toxicity. We have identified ~1,200 rat miRs in addition to the current number in miRBase V21, clarified the tissue specificity and enrichment profile of previously identified miRs and have discovered additional candidate biomarkers of organ injury. These data serve as a valuable resource to identify conserved biomarkers with preclinical and potentially clinical application.

Poster No: 101

Discovery of Glomerular Versus Tubular Injury Specific Urinary miRNA Biomarkers in Rodents

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The most efficient way to manage nephrotoxicity (glomerular and tubular injury) in early drug development is to have sensitive, specific, detectable, and translatable biomarkers. Traditional blood (creatinine, urea nitrogen) and urine (protein, albumin, and creatinine) markers of kidney injury are fairly insensitive and nonspecific for localization of the site of injury. Recent studies have reported miRNA alterations in a variety of body fluids, raising the possibility that microRNAs could serve as useful biomarkers. Here we describe urinary microRNA expression alterations in a rodent model of renal tubular injury (gentamicin) using 2 different bioanalytical platforms: next generation sequencing (NGS) and qRT-PCR. Although our examination of the concordance between miRNA-seq and qRT-PCR in urine specimen suggests minimal agreement between platforms (probably due to the differences in sensitivity), the microRNAs identified by each of these profiling platforms may be promising urinary biomarkers for drug induced tubular injury. Additionally, we employed two different modes of glomerular insult, oxidative stress (Puromycin) and immune mediated mechanism (Heymann Nephritis), and identified miRNA changes (by qRT-PCR) in both isolated glomeruli as well as urine specimens that enabled identification of urinary miRNA biomarkers that are specific to the glomerulus.
Subsets of glomerular urinary miRNAs associated with these different modes of glomerular toxicity seem to be dependent on the mechanism of the induced injury, while nine miRNAs were common to both studies. We further show that the miRNAs identified as mechanism specific, early glomerular injury biomarkers target key pathways and transcripts relevant to the type of insult, while the insult independent changes might serve as universal glomerular injury biomarkers. The presentation will highlight the strengths and limitations of two technologies available for miRNA proofing as well as promising site specific urinary biomarkers of drug induced kidney injury.

**Poster No: 102**

**MiRNA-206 As a Useful Biomarker of Skeletal Muscle Injury**

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Circulating miR-206 has been reported as a biomarker (BM) to detect skeletal muscle injury (SMI). To evaluate the utility of miR-206 as a BM for nonclinical toxicity studies, we examined the sensitivity and of miR-206 compared with those of conventional BMs such as creatine kinase, lactate dehydrogenase in rats treated with tetra-methyl phenylene diamine or in-house compounds which caused SMI. As a result, the increases in miR-206 levels were more marked than those in other BMs in some compounds, indicating that miR-206 would be more sensitive to detect SMI than other BMs. The results of our studies suggested that measuring miR-206 in nonclinical toxicity studies would help to detect SMI with higher sensitivity and screen compounds which have no/low potential of SMI in early stage of drug development. This presentation will provide an overview of study outcomes with experiences in measuring microRNAs as a biomarker in the blood.

**Poster No: 103**

**Plasma miRNAs As Potential Early Biomarkers for Acute Cardiac Injury**

A. da Costa  
UCB Biopharma, Braine L'Alleud, Belgium

Drug-induced cardiac injury (DiCI) detection remains a major safety issue in drug development. While circulating microRNAs (miRs) have emerged as promising translational biomarkers, novel early detection biomarkers of cardiotoxicity are needed. This presentation will focus on the development and deployment of miRNA biomarkers in acute toxicity studies after confirmation of cardiac specific injuries. The association between the miRNA changes found in the periphery and at a tissular level will be discussed as well as the performance of a selected list of miRNAs compared to other widely used cardiac biomarkers. During the presentation, the application of such strategies will be contextualized to drug development and a particular focus will be present on both forward and back-translational potential of MiRNAs.
**Poster No: 104**

**MiRNAs Signatures of Prostate Cancer in Urinary Exosomes**

P. Mouritzen  
Exiqon A/S, Vedbaek, Denmark

MicroRNA are promising new biomarkers for application in diagnosis of different diseases and in toxicology. We are aiming to exploit this potential and have identified a number of differentially regulated microRNAs in urinary exosomes from prostate cancer bearing individuals. Prostate cancer specific signatures have been obtained by different combinations of these differentially regulated microRNAs, which allows constructing receiver operating characteristic curves with areas under the curve well above 0.8. Results hold promise that a non-invasive test can be developed which outperforms currently available tests on specificity and sensitivity. Development of thorough sample and data quality assurance procedures together with new technologies have been central for achieving these results in a biofluid like urine where microRNA levels are extremely low. Key technologies developed include a highly sensitive LNA™-based qPCR platform and a simple exosome precipitation system which enable low speed centrifugation to harvest exosomes from urine.

**Session II: Technical Considerations for Measurement and Analysis of Small Non-Coding RNA in Biofluids**

**Poster No: 105**

**Measurement Methodology and Experimental Considerations for miRNA Quantification**

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Advances in our understanding of the biodistribution of miRNA and other small non-coding RNAs has led to significant interest in their use as biomarkers; with specific applications including: disease diagnosis, therapy selection & modification, and molecular toxicology. Of particular interest has been the development of highly sensitive assays for the detection and quantification of cell-free circulating miRNA. This seminar will focus on critical technical considerations for the development of quantitative assays specific to circulating miRNA biomarkers. Central to this discussion will be considerations for the development of accurate quantitative standards, which we have shown can be reproducibly developed using digital PCR.
**Poster No: 106**

**Extracellular Vesicle Enrichment of miRNA in Biofluids**

K. Witwer  
Johns Hopkins School of Medicine, Baltimore, MD

Extracellular vesicles (EVs) are a variety of double-membrane entities that are diverse in their size, biogenesis, cargo, and functionality, and include the variously defined exosomes and microvesicles. To the extent that EVs and their cargo reflect the cell of origin and can be isolated reliably from biological fluids, EVs add an important element of specificity to liquid biopsy approaches. Here, we present our findings on EV enrichment from biofluids and analysis of miRNAs in unfractionated and EV-enriched biofluids by sequencing, array, qPCR, and digital PCR. Illustrations are drawn from our research into cerebrospinal fluid and plasma biomarkers of the HIV-associated neurocognitive disorders (HAND). We conclude by assessing the broad potential of EVs and their miRNA cargo as useful markers of disease.

**Poster No: 107**

**Classes of Small Non-Coding RNA in Biofluids**

K. van Keuren-Jensen  
TGen, Phoenix, AZ

We are currently investigating the use of extracellular RNA (exRNA) expression in biofluids (plasma, urine, cerebrospinal fluid and saliva) to serve as biomarkers. ExRNAs, released from all cells in the body are typically carried within vesicles or bound to RNA-binding proteins in biofluids. Their dynamic expression profile in response to cellular stresses can be measured. We use next generation sequencing technologies, allowing us to detect thousands of RNAs at the same time. There are a variety of small RNAs detectable in biofluids (tRNA, piRNA, miRNA, etc). Our laboratory has examined isolation methods (cell-free RNA, vesicle-associated RNA, RNA-binding protein) and used several different sequencing approaches across several different biofluids. We have tested several analysis pipelines and small RNA databases. Each biofluid and each isolation method has a unique RNA profile. We report the RNA species identified through sequencing, as well as pros and cons for each method.
Poster No: 108

Reference Samples for miRNA Measurements

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National Institute of Standards and Technology, Stanford, CA

A variety of technologies and protocols can be used for profiling of microRNA gene expression to identify potential biomarkers of disease or toxicity. Reference samples with designed-in differences within or between samples provide laboratories with benchmarks for assessing or optimizing performance. We tested two types of reference samples in multiple miRNA measurement systems. One set of reference samples was composed of defined mixes of total RNA derived from diverse tissue types, providing a source of material with similar complexity to experimental samples. These samples were tested at several sites over multiple rounds. We also tested less complex samples composed of synthetic mimics for known miRNAs, which can be selected to control for specific parameters such as sequence similarity or secondary structure that potentially affect assay sensitivity or specificity. The pros and cons of different reference sample paradigms will be described.

Poster No: 109

Bioinformatics Challenges for Next-Generation Sequencing Analysis of microRNA Expression

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MicroRNAs (miRNAs) are key post-transcriptional regulators which play a major role in disease pathogenesis and toxicity. Knowing the landscape of miRNAs in various model systems and animal organs is one of the main objectives of next-generation sequencing based miRNA profiling. This presentation will discuss the impact of the choice of bioinformatics approaches for identification and detection of both known and novel miRNAs using RNA-seq. We investigated 384 representative pipelines that consist of four tools (mirDeep2, mirExpress, miRNAkey, sRNAbench), 24 profiling choices, and four normalization options on miRNA-seq data generated from thioacetamide-treated rat liver samples in four time points and three dose levels. The variability in the number of differentially expressed miRNAs (DEMs) was observed for each tool, particularly for miRNAkey and mirExpress. An extensive evaluation of mirDeep2 and sRNAbench was performed, which revealed that sRNAbench detected more miRNAs than mirDeep2 but the number of DEMs were almost the same under the same normalization method. Mapping options were found to be effective in the variability of detection and DEMs. However, in the DEM variability, effect of all profiling elements did not exceed 8% when treatment effect is considered. Although normalization choice caused low level of concordance, the trend for time-dose response is sustained. Our analysis showed that being less sensitive to parameter changes; mirDeep2 can be preferred over pipelines when mismatches are allowed up to 3 nts from both ends in the windowing option.