MicroRNAs Europe 2010 Meeting
MicroRNAs: Biology to Development & Disease

November 1-2, 2010, Peterhouse, University of Cambridge,
Cambridge, United Kingdom

Krishnarao Appasani

GeneExpression Systems, Inc.

PO Box 540170

Waltham, Massachusetts 02454 USA

Corresponding Author:

Krishnarao Appasani, PhD., MBA.

GeneExpression Systems, Inc.

PO Box 540170, Waltham, Massachusetts 02454 USA

Tel: 781-891-8181; Fax: 781-891-8234

E-mail: DrAppasani@expressgenes.com,

Introduction:

MicroRNAs are the *"biology's Big Bang"* and this is the fifth annual focused theme conference igniting the spirit of microRNomics in order to catch the emerging microRNA revolution. We hope that the emerging microRNA field will be followed in the same path of RNA interference to bring an impact in the scientific and commercial enterprise for the development of new diagnostics, and therapeutics for several human diseases. Therefore, GeneExpression Systems identified a *'market-niche'* to organize this cutting-edge technology meeting every year in order to educate and keep attendees updated on the emerging developments. This sense of collaboration was prominent at the Fifth International '*MicroRNAs Europe- 2010 Meeting'* held by GeneExpression Systems in Cambridge in November which recognized the promise of microRNAs and profiled key advancements in various areas of microRNAs research. The two-day intensive single track meeting was arranged in six scientific sessions. The meeting brought together industry leaders and entrepreneurs, renowned international scientists from high caliber academic institutions, contributing seminars, posters, and product presentations displaying the latest tools in microRNAs research. There were about 100 participants around the world attended this focus theme conference and 10 posters were presented.

MicroRNAs (miRNAs) are a novel class of endogenous, non-coding, single-stranded RNAs that regulate gene expression post-transcriptionally by base pairing to a complementary sequence in their specific target mRNAs. It is estimated that about 30% of all human genes may be regulated by miRNAs and to date about 1,000 human microRNAs have been identified and characterized. These miRNAs regulate biological processes by inhibiting the translation of messenger RNAs. In mammalian cells, the miRNA-RISC targets mRNAs by forming a short region of complementarity between the miRNA sequence and the 3′ upstream translating region of its target sequence, resulting in translational repression or and/or deadenylation and degradation of the mRNA target. 4,361 miRNAs have been identified in multiple species and substantially larger numbers are predicted. For majority of individual miRNAs the function remains unknown. The potential of microRNA research is seemingly unlimited. With this tool scientists and clinicians are able to focus not just on finding better treatments but also on finding cures for many of the diseases ailing the world today, including viral, cancer, cardiovascular, neurodegenerative, inflammatory, and metabolic diseases. Modern biomedical science is finally bringing together the intellectual forces of international academic researchers, industry scientists, and clinicians. Such collaborations are of high relevance for emerging science such as microRNAs research, which hold such great potential for therapeutics and understanding of development and diseases. This report covers few representative talks from academia and biotech industry those were presented in the meeting.

MicroRNAs in Virology and Delivery:

The conference was inaugurated by **Chris Boshoff** (University College London Cancer Institute, London, UK), who summarized the progress made so far in the discovery of microRNAs in the field of virology and presented his group's findings towards the identification of microRNAs in Kaposi sarcoma and detailed the role of both cellular and viral microRNAs. MicroRNA (miRNA) mimics and inhibitors are frequently employed to better understand the contributions of non-coding RNAi to cell physiology. Currently, the predominant method for over-expressing mature miRNAs involves delivery of synthetic mimics into target cells that can be readily transfected. To further expand the collection of tools available to researchers, and expand into cell lines that are difficult to transfect **Stephanie Urschel** (Thermo Scientific Genomics, Germany) presented a new lentiviral-based miRNA expression platform for delivery applications. Obtaining reliable miRNA expression profiles from high-throughput array data is an essential concern in many studies. One crucial step is the raw data normalization that allows unbiased between-array comparisons in the downstream analyses. Therefore, **Alain Sewer** (Philip Morris International, Neuchatel, Switzerland) developed a novel evaluation miRNA-specific normalization method followed by validation with qRT-PCR, while comparing both Affymetrix and Exiqon platforms.

MicroRNAs in Development:

MicroRNAs are essential for development, and for the control of cell proliferation/differentiation in various organisms including plants. It is well recognized that short interfering RNA (siRNA) sequences play an important role in gene expression. These are a class of short double-stranded RNA molecules involved in the RNA interference (RNAi) pathway, which regulates the expression of specific genes. **Nagy Habib** (Imperial College of London, London, United Kingdom) demonstrated the *'proof-of-principle'* that short activating RNA (saRNA) could be designed to up-regulate specific genes such as Kruppel-like factor 4 (KLF4). The KLF4 gene is a transcription factor necessary for maintaining embryonic and somatic stem cells that control the expression of pluripotency genes including POU5F1, Sox2, c-Myc, and Nanog. His group has designed and tested several saRNAs to up-regulate the KLF4 gene, and demonstrated that some of the saRNAs can up-regulate KLF4 in addition to Sox2, c-Myc, and Nanog expressions in hematopoietic and mesenchymal stem cells. Human mesenchymal stem cells (hMSCs) represent a population of multipotent stem cells, easily expandable in culture and able to differentiate into many lineages. **Maria Roubelakis's group** (Academy of Athens, Athens, Greece) has isolated MSCs from amniotic fluid and compared with the transcriptional profiles from the MSCs isolated from bone marrow (BM) and umbilical cord blood (UCB) cells. During the course of these studies she also validated the miRNA expression levels using Real Time PCR and *in silico* detection methods. On the other hand, **Christina Roberts** (Uppsala University, Uppsala, Sweden) used *Arabidopsis thaliana* root as a model system to analyze

pattern organization and cell fate. Class III HOMEODOMAIN LEUCINE ZIPPER (HD-ZIP III) proteins are transcription factors, involved in shoot dorso-ventral specification that are post-transcriptionally regulated by miR165/166.

MicroRNAs in Neurological Diseases:

Matthew Wood (University of Oxford, Oxford, United Kingdom) presented an overview of non coding RNAs in neurodegenerative diseases. According to **Iris Lavon** (Hadassah Hebrew University Medical Centre Jerusalem, Israel) gliomas (brain tumours) exhibit a miRNA expression profile reminiscent of neural precursor cells. About half of the miRNAs expressed in the shared profile, cluster in seven genomic regions susceptible to genetic/epigenetic alterations in cancers. Her results provided the first evidence for association between mir17-92 clusters and gliomas, further these were localized on chromosome 14q32.31. Recent evidence suggests that MicroRNAs (miRs), including MiR-26a, miR-221/2 and a battery of differentially expressed miRs across tumors and prognosis may be key regulators of Glioblastoma multiforme (GBM) tumorigenesis. To characterize their regulatory role, **Pavel Sumazin** (Columbia University, New York, USA) constructed an integrated gene-miR regulatory network that is being used to identify GBM master regulators. He also presented results on the identification of miR regulators and targets in GBM tumors. In the brain, amyloid precursor protein (APP) and Aβ are critical in the pathogenesis of Alzheimer's Disease (AD), which is the most common form of dementia in the elderly. **Francesca Ruberti (**CNR-Institute of Neurobiology and Molecular Medicine, Rome, Italy) showed by *'gain and loss of function'* experiments, that miR-101 are a negative regulator of APP expression and Aβ load in primary rat hippocampal neurons.

Beate Niesler (University of Heidelberg, Heidelberg, Germany) found that the *HTR3E* SNP was associated with diarrhea predominant irritable bowel syndrome in women. It was also observed that the SNP affected binding of miR-510 to the *HTR3E* 3´UTR which caused elevated expression. This is the first example indicating microRNA related expression regulation of a serotonin receptor gene with a cis-regulatory variant affecting this regulation and appearing to be associated with female diarrhea predominant irritable bowel syndrome.

MicroRNA Profiling:

Regulatory T-cells (Tregs) play a key role in immune system homeostasis and tolerance to antigens, thereby preventing autoimmunity, and may be partly responsible for the lack of an appropriate immune response against tumor cells. Recent reports have shown that HDAC inhibitors modulated microRNA expression profiles in various cell types. Hence, **Bassam Badran's** group (Lebanese University Hadath Beirut, Lebanon) investigated the effect of valproate on microRNA expression profile in CD4+CD25- T cells purified from cord blood, and found that, valproate treatment induced the acquisition of the miRs Tregs signature. These results were further confirmed by using FOXP3 lentiviral expression vector system, and found no

changes in miRs expression. He concluded that the modification in the miRs expression profile is not due to an increased expression of FOXP3, but directly results from HDAC inhibition.

Growing body of evidence implicates deregulated microRNA expression in various aspects of human disease, including cancer; insights in global microRNA function remain limited. In order to develop microRNA body map, an interactive online compendium and mining tools were developed by **Pieter Mestdagh** (Ghent University Hospital, Ghent, Belgium). The developed microRNA body map enabled them to prioritize microRNAs candidates based on their expression profile across tissue or disease subgroup. This microRNA body map project has great potential to become a community resource. Despite the considerable progress made in the understanding of Polycystic Kidney Disease (PKD) pathogenesis the exact mechanism of cyst formation is still unknown. In order to understand microRNA gene regulation, **Kyriacos Felekkis** (University of Cyprus, Cyprus) identified 27 differentially expressed miRNAs that play a crucial role in the development of PKD. by using two different PKD animal models immediately after birth (0 days) and identified miRNAs whose expression is deregulated in rats' kidneys by miRNA profiling and bioinformatic methods. Among these 27 deregulated miRNAs only mir-99b and miR 672 appear to be common among the two differentt models.

MicroRNAs as Biomarkers and Therapeutics:

Expression profiling of microRNAs has been demonstrated to be a more accurate method of classifying cancer subtypes than expression profiles of protein coding mRNAs. Due to the high stability of microRNAs in clinical source material (FFPE blocks, plasma, serum, urine, saliva etc.), microRNAs have great potential to be used as diagnostic and prognostic markers for cancer and other diseases. Due to their small size, accurate and sensitive detection of microRNAs is highly challenging.

In recent years, Locked Nucleic Acid (LNA) modified oligonucleotides that bind to and sequester specific microRNAs (miRs) have been widely used to characterize miR functions. Exiqon has developed a series of unique research tools based on Locked Nucleic Acid (LNA™) technology to detect microRNAs and uncover their functions. **Hazel Pinheiro** (Exiqon A/S, Vedbaek, Denmark) presented the miRCURY LNA™ Universal RT microRNA PCR System, which was used to profile over 700 microRNAs with just 40 ng of total RNA (or RNA isolated from just 70µL blood serum or plasma) without the need for pre-amplification. Additionally, LNA™ microRNA RT-qPCR technology was used to discover novel microRNA biomarkers for early detection of colorectal cancer from patient blood plasma samples.

Colorectal Cancer (CRC) screening with the faecal occult blood testing, although effective, is generally considered to lack convenience, sensitivity and specificity for use as a general screening test. This explains why the search for an improved, non invasive and more accurate screening test has continued despite a recent commencement of National Bowel Screening Programme in the UK. **Muhammad Aslam** (Leicester Royal Infirmary, Leicester,

United Kingdom) identified cancer related microRNAs in the circulating blood at levels sufficient to be measurable for the detection of tumours. This feasibility study investigated the potential use of circulating microRNAs for the early detection of colorectal cancers. **Thomas Brefort** (Febit Biomed GmbH, Heidelberg, Germany) used his company's proprietary *'automated Geniom® microarray platform'*, and analyzed blood-derived miRNAs from respective patient and control groups and observed to be deregulated miRNAs. Most importantly, his presentation emphasized the potential of blood-derived miRNA signatures for non-invasive diagnostics, improved surveillance of disease progression and response to treatment.

Arthur A. Levin (Santaris Pharma, Hørsholm Denmark and San Diego, CA, USA) presented clinical data on Miravirsen (SPC3649), an LNA modified oligonucleotide complementary to miR-122, a critical factor in hepatitis C viral replication. Phase 1 studies in healthy volunteers produced results consistent with those predicted by laboratory studies and miravirsen has now entered Phase 2 clinical trials in patients infected with HCV. Results of non-clinical safety studies and initial clinical results demonstrated that targeting miRs for therapeutic use in humans is possible and as the first miR-targeting therapeutic in clinical trials, miravirsen is leading the way for a new field of miR-targeting therapeutics.

MicroRNAs in Cancer Biology:

Michael J. Kerin (National University Hospital of Ireland, Galway, Ireland) presented microRNA profiling data from human breast cancer. MicroRNA-21 is up-regulated in different cancers and modulates cancer-relevant processes. Therefore, to identify genes involved in the regulation of miR-21, **Cindy Horwedel** (German Cancer Research Centre-DKFZ, Heidelberg, Germany) have screened a large-scale siRNA-library, and identified 100 genes that significantly regulated miR-21 activity. These candidates were followed up in different assays to analyze the regulation in transcription as well as their modulation in miRNA biogenesis. She concluded that using the large scale siRNA screenings her group successfully identified miR-21 specific and general regulators of miR-processing.

Paolo Ceppi (University of Turin at San Luigi Hospital, Orbassano, Italy) investigated the role of miR-200c, previously shown to control epithelial-to-mesenchymal transition (EMT), a process by which cancer cells acquire dedifferentiated/invasive characteristics, in non–small cell lung cancer (NSCLC). MiR-200c forced over-expression resulted in a reversal of EMT, reduced invasion and metastasis formation, and in enhanced sensitivity to chemotherapeutic agents. The hypermethylation of the promoter region was found responsible for the loss of miR-200c in invasive cells. Moreover, lower miR-200c levels were associated with higher lymph-nodal invasion, poor grade of differentiation, and lower E-cadherin expression in NSCLC patients. These results highlighted a pivotal role of miR-200c in NSCLC progression.

MicroRNAs are small non-coding RNAs that regulate important cellular processes including proliferation and apoptosis. *MiR-34a* and *miR-15a/16* are functionally related, share common targets and control similar processes including cell cycle progression and apoptosis.

Erik Vassella (University of Berne, Bern, Switzerland) showed that *miR-34a* and *miR-15a/16* act synergistically in inducing cell cycle arrest in a Rb-dependent manner. Consistent with a functional relatedness, *miR-34a* and miR-*15a/16* significantly correlated in their expression in adenocarcinomas of the lung. Co-regulation of these miRNAs is not directly linked nor is it due to defects in miRNA processing. He concluded that this is the first example of miRNAs in human acting in a synergistic manner and likely to contribute to the understanding of the integrated network of miRNAs involved in gene expression and may also have therapeutic implications. Osteosarcoma (OS) is the most common primary malignant bone tumor in children and young adults. Conventional therapy for OS has reached a plateau of 60-70%, a 5-year survival rate that has changed little in two decades, highlighting the need for new therapeutic approaches by the research of new targets. **Laura Pazzaglia** (Itituto Ortopedico Rizzoli, Bologna, Italy) performed different high-throughput analysis on OS cell lines and samples and identified several differentially expressed miRNA especially, mir-196a, mir-1, and mir-133b. In vitro functional studies are being developed to better investigate the role of these miRNAs as potential prognostic marker(s) and drug target(s).

Outstanding Accomplishments On behalf of the scientific committee Krishnarao Appasani (GeneExpression Systems, Inc, USA) honoured Professor Chris Boshoff (UCL Cancer Institute, London, UK), the keynote speaker on the first day with *"MicroRNAs Innovator Award"* for the discovery of *"identification of microRNAs in Kaposi sarcoma virus."*

The best poster award was given to ????, for *'????????.'*

Final thoughts MicroRNAs are becoming as *'master gene switches'* and regulate gene expression. Last year most of the presentations were in the fields of developmental biology and cardiology, but this year agenda included microRNAs discovery in neurobiology, virology, and cancer biology was well appreciated by all attendees. The message from the meeting is there are lot of issues has to be addressed before we take microRNAs into the clinic to treat human diseases. Most of the attendees felt that this was a *"unique, coherent, well-organized, target meeting for learning cutting-edge technology and meeting authorities in the microRNAs field*.

These opinions are exclusively of the authors and do not reflect those of GeneExpression Systems, Inc.