Meeting Report

Keystone Symposia 40th Season: MicroRNAs and Noncoding RNAs in Cancer

Kaja A. Wasik and Clare A. Rebbeck

Abstract

This year's 40th season of Keystone symposia meetings was held in Banff, Alberta, Canada, on February 11–16 and sponsored by Astellas Pharma and Regulus Therapeutics. The meeting was organized by Gregory Hannon, Curtis Harris, and Martine Roussel and centered on microRNAs (miRNA), noncoding RNAs, and cancer. The meeting was grouped around the following topical areas: miRNA mechanisms, oncogenesis, immune response, angiogenesis and metastasis, cancer biomarkers, stem cells, and therapeutics. This report highlights findings and concepts presented during this meeting. Cancer Res; 71(19): 1–4. ©2011 AACR.

Introduction

This year's 40th season of Keystone symposia meetings was held in Banff, Alberta, Canada, on February 11–16 and sponsored by Astellas Pharma and Regulus Therapeutics. The meeting was organized by Gregory Hannon (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), Curtis Harris (National Cancer Institute, Bethesda, MD), and Martine Roussel (St. Jude Children's Research Hospital, Memphis, TN) and centered on microRNAs (miRNA), noncoding RNAs, and cancer.

This meeting was held simultaneously with the “MicroRNAs and Human Disease” meeting. The meetings opened on the same day, with keynote speeches delivered by Victor Ambros (University of Massachusetts, Worcester, MA) and Scott Lowe (Cold Spring Harbor Laboratory).

Since the discovery of the first miRNA, some 17 years ago, the field has advanced with an explosion of investigation into how these short, single-stranded RNA molecules can influence cellular states in both normal and disease conditions. With respect to the latter, they provide a new class of targets for cancer therapeutics and for disease prognosis. It seemed a natural choice, therefore, that the introductory talk was given by Victor Ambros, one of the discoverers of the first miRNAs. Starting out with an introduction into miRNA function and evolution, he highlighted the conservation of targets and functions across species. Moving onto his choice of model organism, Caenorhabditis elegans, he emphasized how very few miRNAs display a natural phenotype, and only under stress conditions (e.g., starvation and temperature fluctuations) do specific phenotypes become apparent. He then went on to describe his recent work in the developmental influences of Lin-4 and Let-7 and their involvement in the dauer diapause, a quiescent response to unfavorable environmental conditions. This work identified the daf12 gene and its role in regulating many downstream miRNAs, including Let-7, and how, in support of this, different allelic mutations in this hormone-binding receptor result in phenotypes similar to those seen with miRNA repression.

Scott Lowe discussed integration of mosaic mouse models, the ex vivo manipulation of stem and progenitor cells followed by transplantation into specific organs of syngeneic recipient mice, with RNA interference and cancer genomics, to identify new components of tumor suppressor gene networks and how these influence tumorigenesis and treatment response. Complex pools of short hairpin RNAs (shRNA) introduced into progenitor cells taken from the Eμ-Myc lymphoma mouse model resulted in strong selection for those shRNAs capable of promoting lymphomas in transplanted recipients. Two genes of significance were identified, AMD1 and EIF5A1, both thought to be involved in the polyamine biogenesis pathway. Lowe also presented his ongoing project to make doxycycline-inducible shRNA and green fluorescent protein sensor transgenic mice.

The meeting continued grouped around the following topical areas: miRNA mechanisms, oncogenesis, immune response, angiogenesis and metastasis, cancer biomarkers, stem cells, and therapeutics. This report highlights findings and concepts presented during this meeting.

miRNA Mechanisms

Despite many advances in the field of miRNAs, there is still much to learn about the mechanisms by which they exert their effects and how understanding these mechanisms can be achieved with the use of techniques designed to inhibit miRNA function. Stefan Ameres (University of Massachusetts Medical School, Worcester, MA) presented his work on Drosophila melanogaster small RNAs, proposing an evolutionary reason for the lack of complete complementarity between fly miRNAs and their targets. Extensive base pairing of Argonaute1 (AGO...
1–bound miRNAs to their targets triggers tailing and trimming of these miRNAs, favoring regulation of partially complementary targets. AGO2-bound siRNAs, however, cleave highly complementary RNAs while remaining stable, due to HEN1 enzymatic addition of a 2′-O-methyl group to their 3′ end. In accordance with this hypothesis, degradation of AGO2-associated siRNAs was observed in flies lacking HEN1. He proposed, therefore, that this mechanism allows for specialization of AGO proteins and suggested that this was also conserved in mammals. David Corey (University of Texas Southwestern Medical Center, Dallas, TX) also provided insights into the mechanisms of mammalian gene silencing, raising a potentially significant role of AGO2 in silencing via what he termed promoter-targeted antigen RNAs. Utilizing the well-characterized progesterone receptor gene, he described how AGO2 could be recruited to noncoding transcripts overlapping the promoter. He proposed that the antisense transcript is the target for antigene RNA binding, leading to a reduction of RNA polymerase II recruitment at the gene promoter, resulting in gene silencing. Corey went on to show that miR-423-5p was recruited by AGO2 to the gene promoter region and could silence the gene. He concluded his talk by stressing the importance of looking beyond the mRNA for targeting of miRNAs.

Stem Cells and Regulators of Cellular Differentiation

Recent work on stem cells and induced pluripotent stem (iPS) cells has identified many important transcriptional regulators involved in cell fate. Varda Rotter (The Weizmann Institute of Science, Rehovot, Israel) described how a p53 knockout enhances the reprogramming of mouse embryonic fibroblasts by Oct4/Sox2/Klf4 to produce iP cells. However, these same cells generated aggressive malignant tumors when differentiated in vivo. She therefore proposed that p53 controls the efficiency of reprogramming. In her model, p53 was somehow involved in selecting for “good” cells to become true iPS cells, capable of giving rise to stable differentiated cells, and thereby providing a critical role in preventing malignant tumors.

John Rinn (The Broad Institute of Harvard and MIT, Cambridge, MA) presented his work identifying large intergenic noncoding RNAs (LincRNA), many with tissue specificity. Using a “guilt by association” model (whereby the function of a LincRNA can be predicted on the basis of the protein-coding pathway to which they correlate), he found that a majority of these associate with various chromatin-remodeling complexes to assist in the regulation of the epigenetic design in pluripotent cells. Discussing more recent work, he presented studies showing the involvement of LincRNAs in adipogenesis and identifying 8 LincRNAs that modulate key regulatory pathways during this process. Robert Blelloch (University of California San Francisco, San Francisco, CA) expanded on the topics of pluripotency and how miRNAs play an important role in embryonic stem (ES) cell self-renewal. He discussed work on the DCCrib-knockout mouse, identifying multiple miRNAs (including miR-290 cluster, miR-30 cluster, miR-20, and miR-93) that promote ES cell proliferation by suppressing the G1 checkpoint. These miRNAs, which he coined ESCC (for ES cell-cycle–regulating miRNAs), shared a common seed sequence, suggesting that they regulate common targets.

Deepak Srivastava (University of California San Francisco) focused his talk on miR-1, showing how this miRNA works with miR-133 to promote differentiation of cardiac stem cells by regulating the cardiac transcription factor, Hand2. Loss of miR-1 results in an increase in the protein level of Hand2. Emily Pugach (Harvard Medical School, Cambridge, MA) contributed her work on the role of miR-205 in embryonic skin and hair follicle stem cells. miR-205 was shown to colocalize with primary hair germ cells and rest in the hair follicle stem cell niche. Experiments in which miR-205 was disrupted resulted in a loss of the follicle stem cell compartment. Targets of miR-205 were identified to be involved in cell-cycle control and migration. Closing the session on stem cells was Edward Morrissey (University of Pennsylvania, Philadelphia, PA), highlighting how the miR-302/367 cluster was involved in pluripotency. He described how expression of miR-302/367 led to efficient reprogramming of fibroblasts to create iPS cells. He proposed that these miRNAs had a greater reprogramming efficiency than the typical Oct4/Sox2/Klf4/Myc genes by up to 2 orders of magnitude.

miRNAs as Oncogenes and Tumor Suppressors

This meeting was specifically designed to focus on the roles of miRNAs in cancer and provided opportunities to highlight new work on the function of small RNAs as both oncogenes and tumor suppressors.

Andrea Ventura (Memorial Sloan-Kettering Cancer Center, New York, NY), Martine Roussel and Pieter Mestdagh (Ghent University Hospital, Ghent, Belgium), and Jean-Christophe Marine (Katholieke Universiteit Leuven, Leuven, Belgium), all observed a miR-17-92 upregulation in various types of cancer. Andrea Ventura concluded that this cluster was a direct transcriptional target of c-Myc and n-Myc, consistent with prior results published by O’Donnell and colleagues (1). A prominent role for the miR-19 seed family suggested it to be the driving force for oncogenic properties of the cluster. Martine Roussel also confirmed the oncogenic properties of this cluster of miRNAs. Overexpression of this miRNA cluster in granule neuron progenitors was shown to promote medulloblastoma formation, most likely by collaborating with Sonic hedgehog signaling. Breaking down this cluster, Roussel’s results mimicked those shown by Andrea Ventura, providing a critical role for the miR-19 seed family; in addition, they also implicated the miR-17/20 seed family. Jean-Christophe Marine presented his work, suggesting that this cluster cooperates with p53 in the formation of retinoblastoma. Using a different approach, namely, the use of high-throughput proteomics, Pieter Mestdagh drew similar conclusions in his short talk, presenting the downstream targets of the miR-17-92 cluster. He identified multiple components of the TGF-β pathway that were regulated by this miRNA cluster, providing yet more support for the miR-17-92 cluster being involved with
oncogenic signaling pathways. With its involvement in so many cancers, it is clear that the miR-17-92 cluster could provide a strong case for therapeutic targeting.

**miRNAs and Prognosis and Therapeutics**

The strong correlation between certain diseases and specific miRNAs had led some to propose that miRNAs can serve as important prognostic and diagnostic markers. Carlo Croce (Ohio State University, Columbus, OH) discussed the prognostic implications of miRNAs, coupling them to chromosomal abnormalities in chronic lymphocytic leukemia. His work on the miR-15a/16-1 cluster led to the proposition that these miRNAs, p53, and miR-34b/34c cluster are linked in a molecular pathway that explains the pathogenic and prognostic implications (indolent vs. aggressive form) of recurrent deletions in lymphocytic leukemia. He concluded that expression levels of Zap-70, influenced through this miRNA/p53 pathway, also provide a significant prognostic marker for lymphocytic leukemia and that in the near future many markers controlled by miRNAs, and miRNAs themselves, will be revealed. Danilo Allegra (University Hospital of Ulm, Ulm, Germany) and Desire Bonci (Istituto Superiore di Sanitá, Rome, Italy) both provided additional data on the miR-15a/16-1 cluster. Allegra focused on the ratio between pri- and pre-miRNAs, suggesting a defect in Drosha processing contributes to the downregulation of this miRNA cluster in lymphocytic leukemia. Bonci discussed how downregulation of miR-15/16 in cancer fibroblasts promotes tumor growth via repression of Fgf2 and its receptor Fgfr1.

Sohail Tavazoie (Rockefeller University, New York, NY) discussed the correlation between genes regulated by miR-126 and patient survival from metastatic relapses. miR-126 was shown to repress endothelial cell recruitment to metastatic sites, and knockdown of this miRNA lead to a reduction in lung metastasis and systemic metastasis. Furthermore, investigation into tumor nodule size led to the conclusion that this miRNA is involved in the regulation of metastasis initiation, rather than the complete process, as larger nodules with increased vessel density and vessel function were seen in the miR-126 knockdowns despite a reduction in metastatic tumors. Microarray analysis found 8 genes thought to be regulated by miR-126, and patients with a lower metastasis survival rate were seen to have increased expression of these genes. Of these, PITPNM1 was found to regulate IGFβ2 secretion and promote migration, and MERTK was thought to inhibit chemotaxis.

miR-21 was a particular focus during this meeting. This miRNA is upregulated in at least 13 major types of cancer. Curtis Harris highlighted the role of this miRNA in both lung cancer and colon cancer and showed that by pairing miR-21 expression with an inflammatory risk score, a better estimate of patient outcome could be obtained. He also discussed the downstream mechanism underpinning miR-21 in human cancers, proposing that both K-ras and STAT6 pathways resulted in positive regulation of miR-21 whereas genotoxic stress and FOXP3A inhibited expression of miR-21. Mark Hatley (University of Texas Southwestern Medical Center) supported this work by presenting data using a K-ras/miR-21 model, showing the Ras/MEK/ERK pathway to be a positive regulator of miR-21, which, in turn, inhibits negative regulators of that pathway (Spry1, Spry2, Btg2, and Pdcd4). Overexpression of miR-21 also saw a decrease in apoptosis, suggesting an additional role of this miRNA. Frank Slack (Yale University, New Haven, CT) followed on from this, implicating miR-21 in the formation of pre-B-cell lymphoblastic lymphoma. Inactivating miR-21 led to a regression of transplanted mouse tumors within the lymph nodes and recovery of the hematopoietic system. He proposed that miR-21 acted as an oncogene, with tumors being “addicted” to its overexpression, and could therefore be a target of anticancer therapy. Eric Marcusson (ISIS Pharmaceuticals) went further with this idea, presenting data from experiments using antagonists against miR-21. Utilizing an H-ras–driven model of hepatocellular carcinoma, he was able to show that anti-miR-21 extends the life of an affected mouse. He discussed the use of chemically modified antagonists as a tool for in vivo inhibition.

Sakari Kauppinen (Sanitaris Pharma) presented his data using locked nucleic acids (LNA)—bicycle high-affinity RNA analogues. Working with a mouse model of lupus, in which an upregulation of miR-21 in B cells is correlated with disease, he showed that delivery of 8-mer LNAs complimentary to the seed sequence (termed tiny LNAs) efficiently reduced levels of Fas receptor protein and led to a reduced spleen size compared with the control. Inhibiting miR-122, he also found that these tiny LNAs were more efficient at lowering cholesterol levels than the 15-mer LNAs currently in clinical trials. Kauppinen also presented an update on the therapeutic use of LNA–anti-miR-122 against hepatitis C virus infection in chimps and discussed the progress of clinical trials into cholesterol reduction in humans with miraviren, the compound name for the 15-mer LNA against miR-122.

Introducing an alternative method of miRNA therapeutics, Sarah-Louise Gill (Royal College of Surgeons in Ireland, Dublin, Ireland) discussed her work on the use of ultrasound-responsive microbubbles to deliver miRNA mimics to cardiomyocytes. She found that encapsulation of the miRNA mimetics resulted in effective delivery, and combining this with the use of ultrasound to disrupt the cell wall, allowing the microbubble to enter the cell, she succeeded in increasing delivery efficiency.

David Patrick (University of Texas Southwestern Medical Center) presented promising data on the treatment of polycythemia vera, a disease characterized by extremely high proportion of red blood cells. Working on a mouse model of this disease, he showed that antagonists to miR-451 successfully reduced hematocrit levels to normal proportions and based on similar conclusions drawn from the use of human CD34+ cells, he proposed that antagonists to miR-451 could be used to treat patients with this disease. Muthiah Manoharan (Alnylam Pharmaceuticals) commented on the medicinal chemistry of siRNAs and their delivery systems. He talked about the key chemical modifications of siRNAs in their sugars, backbones, or conjugates, including the effects of a 2’-fluoro modification, as in the case of factor VII siRNA, and a
2'-fluoro addition at all pyrimidines, the later having a 2-fold better activity than unmodified siRNAs. He touched briefly on the use of targeted lipid nanoparticles (LNP) conjugated to a multivalent N-acetylgalactosamine (GalNAc), both with and without the addition of cholesterol, to target both the asialoglycoprotein receptor in hepatocytes and to treat the genetic disease transthyretin amyloidosis (currently in phase I clinical trials). He closed by summarizing the key aspects of recent LNP publications, the ongoing work on their use to target various liver cells, immune cells, and other tissues, and highlighting how novel lipids have allowed a much reduced delivery dosage compared with previous work.

Additional Talks of Interest

Stepping outside of the miRNA and cancer box, Gregory Hannon presented his group’s latest work on the piRNA pathway and methylation. Whole-genome shotgun bisulfate conversion data from mouse primordial germ cells (PGC) and secondary spermatocytes showed that only 4% of CpGs were methylated in PGCs compared with 78% in spermatocytes, indicating that the de novo genome-wide methylation events occurring during germ cell and zygotic development differ substantially, with perhaps a more stringent mechanism operating in somatic cells. They also observed hypomethylation of promoters and satellite repeats. Removing the piRNA pathway, they saw approximately 20% drop in methylation, mainly in young and active repeats targeted by the pathway. This led to the hypothesis that most methylation is by default, with genes and young elements able to protect themselves. Moving on to a species comparison, they compared data from human and chimp sperm. Interestingly, they observed differences in the level of conservation of the genome and epigenome, consistent with their ability to evolve independently.

Robert Darnell (Rockefeller University) discussed his recently developed methodology, HITS-CLIP, and its use in the study of AGO–miRNA–RNA ternary interactions to identify miRNA-binding sites on a genome-wide scale. Cross-linking miRNA and mRNA to AGO proteins and digesting to leave an approximately 50 bp long fragment of mRNA suitable for high-throughput sequencing, termed the AGO footprint, he showed in one example on activated and nonactivated T lymphocytes that they could detect substantial changes in footprints and abundance of reads. He proposed that these data could be useful in understanding miRNA abundance relative to the extent of silencing.

Summary

As discussed, many miRNAs have been implicated in cancers and other diseases and have been shown to be useful indicators for disease prognosis. However, with varying mechanisms of processing and targets, it is still an ongoing project to use these for reliable therapeutics. The talks presented at this meeting highlighted current and potential uses of miRNAs and also discussed in detail the advances that have been made in the detection tools and in the delivery systems to both increase and decrease particular miRNA expression levels to influence disease outcome.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Reference

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