RNAi2011: Gene Regulation by Small RNAs

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RNAi2011: Gene Regulation by Small RNAs, the 6th international Oxford RNAi conference, was held at St Hilda’s College, University of Oxford (UK), between 29 and 31 March, 2011. Sessions covered a variety of topics and provided an excellent opportunity to discuss and reflect upon recent developments in this fast-moving and promising field. A number of conference posters were also on display throughout the conference, and a number of trade stands gave conference participants the opportunity to learn about the latest product developments.

DELIVERY AND TARGETING

If the potential of small RNAs as therapeutic agents is to be realised, the development of powerful, efficient delivery methods are crucial. The conference opened with a series of talks describing novel techniques that show great promise as agents to deliver small interfering RNAs (siRNAs) and microRNAs (miRNAs) to target cells. Kostas Kostarelos (University of London, UK) described how carbon nanotubes, which are internalised by the cell without apparent damage to cellular structure, could be chemically functionalised and combined with siRNAs to form gene-silencing complexes that are effectively delivered to target cells. To illustrate this principle, he presented data showing effective in vivo delivery and knockdown in neurological conditions and xenografted cancer cells.

Polyethylenimine (PEI) is a transfection reagent used to package siRNAs and plasmids. The potential for PEI in the delivery of miRNAs was discussed by Achim Aigner (Philips-University Marburg, Germany). The miRNAs can be effectively packaged and delivered in vivo to tumour xenografts in mice. The effect of chemical modifications on the efficiency of PEI as a delivery vehicle is currently being investigated.

Professor Andrew Miller (King's College, London, UK) gave an engaging overview of "what we think we know about RNAi delivery". He described the use of synthetic ABCD nanoparticles in tumour imaging in vivo. The use of siRNA-ABC nanoparticles for in vivo delivery of siRNAs to tumours, along with an associated phenotypic reduction in tumour growth rate, was also presented. Roger Adami (Marina Biotech Inc, USA) presented a dialkylated amino acid-based (DiLA2) delivery system with accompanying data demonstrating efficient hepatocellular uptake and endosomal escape.

Exosomes are small vesicles secreted by cells that can transport small RNAs and proteins. Samira Lakhal-Littleton (University of Oxford, UK) described how exosomes can be harnessed for delivery of siRNA molecules to the mouse brain with the aim of knocking down expression of BACE1, a therapeutic target in Alzheimer's disease. Exosomes were obtained from dendritic cells engineered to produce the exosomal membrane protein Lamp2b fused to RVG, a neuron-specific peptide. After introduction of BACE1 siRNA to the exosome by electroporation and intravenous transfer of exosomes to wild-type mice, gene-specific knockdown was observed in target brain cells (Alvarez-Erviti et al, 2011).

RNAi MODULATION FOLLOWING DELIVERY

Georg Szczakiel (University of Lübeck, Germany) reminded us that efficient delivery of siRNAs to a cell is not the only hurdle to an efficient therapeutic application of RNAi. Once inside a cell only a minority of the siRNAs are actually free in cytosol and available for silencing of target RNAs. He described methods that affect the proportion of siRNAs released within cells. He also
described recent results showing the influence of cell stress on Ago2 localisation and RNAi efficiency (Detzer et al, 2011).

Mirko Ludwig (Friedrich-Schiller-University of Jena, Germany) described the development of ‘intelligent’ siRNAs and the formation of the spinoff company BianoScience. By coupling siRNAs to short peptides containing the target site of a cell type-specific peptidase, it is possible to prevent the RNA-induced silencing complex (RISC) from forming and thus prevent siRNAs from exerting their silencing effect. When these siRNA-peptide complexes are introduced to cells, cleavage of the peptide, RISC formation and siRNA-mediated silencing is observed only in cells containing the peptidase corresponding to the specific peptidase recognition sequence. This principle was demonstrated by binding a peptide containing the target sequence for caspase-4, which is expressed in Jeg-3 choriocarcinoma and MCF-7 mammary cancer cells but not HEK (human embryonic kidney) cells. Knockdown of Signal Transducer of Activation (STAT3) was only achieved in cells expressing the caspase-4 peptidase (Koehn et al, 2010).

CHARACTERISATION OF miRNAs: ANNOTATION, FUNCTION AND TARGET IDENTIFICATION

miRNAs regulate gene expression by binding to target regions within messenger RNA (mRNA) molecules. This can lead to degradation of the mRNA target or to translational repression via mechanisms that have not yet been fully elucidated (Huntzinger et al, 2011). Although it is predicted that up to 45,000 conserved miRNA target sites exist in the 3'UTR of genes across the human genome, the overwhelming majority of these target genes have not been validated. Caspar Robinson (Sigma-Aldrich Life Sciences, USA) described how MISSION® 3'UTR Lenti GoClones and human miRNA mimics can be used for this purpose and illustrated his talk with data demonstrating validation of known and conserved miRNA targets.

One common method of investigating the role played by miRNAs is to block their action and examine the resulting effects on gene expression. Anti-miRNA Oligonucleotides (AMOs) can achieve this via a steric blocking mechanism. Mark Behlke (Integrated DNA Technologies Inc, USA) described a novel chemical modifying group that has been shown to improve potency and nuclease resistance whilst maintaining a high level of specificity in an in vitro environment, with in vivo action currently under investigation.

Sam Griffiths-Jones (University of Manchester, UK), creator and curator of miRBase, described recent changes to the miRNA database, including the incorporation of publicly available sequencing data. Users will now also be able to search for miRNA species expressed in a tissue or at a developmental stage of interest. Intriguing data from deep sequencing concerning arm-switching mechanisms in miRNA were also presented, with the predominant mature miRNA product formed by miR-10 hairpin precursors shown to vary dramatically between species. The preferred product was shown to be dictated by sequences within the pri-miRNA sequence but not within the mature miRNA molecule (Griffiths-Jones et al, 2011).

SMALL RNAs AND BIOLOGY

In the first of the conference's two keynote speeches, Professor Sir David Baulcombe (University of Cambridge, UK) presented work carried out in Arabidopsis that demonstrates the remarkable mobility of siRNAs and the role this might play in virus resistance.

Until recently, it was believed that as well as lacking nuclei and ribosomes, erythrocytes completely lacked all species of RNA. Andrew Hamilton (University of Glasgow, UK) presented his recent data showing that red blood cells in fact contain a considerable quantity of small RNA that is stable upon cell lysis, and most of which represent known miRNAs. He is currently investigating this intriguing phenomenon and the biological function that erythrocyte miRNAs may play.

Johannes Grillari (University of Natural Resources and Applied Life Sciences, Vienna, Austria) presented data on the miRNAs released when cells become senescent. Interestingly, the primary miRNA identified is found in elevated levels in the serum of elderly volunteers compared to younger ones. He also described potential downstream consequences in cells that take up these circulating miRNAs.

Natalia Botchkareva (University of Bradford, UK) described work exploring the role of miRNAs in hair follicle development. She presented evidence that miR-31 is required for correct hair follicle formation and that it regulates bone morphogenetic protein (BMP) signalling.

RNAi AS A HIGH-THROUGHPUT TOOL

Steve Brown (University of Sheffield, UK) discussed the facilities and services available at the University of Sheffield RNAi Screening Facility (SRSF) and how these can benefit researchers seeking to carry out genome-wide screens in Drosophila cells. He also described the application of high content microscopy to genome-wide RNAi screens in Drosophila, with the aim of uncovering the functions of genes.

David Horn (London School of Hygiene and Tropical Medicine, UK) presented RNAi target sequencing (RIT-seq), a high-throughput phenotyping technique that uses Illumina sequencing to examine changes in expression levels associated with >90,000 targets within the African trypanosome genome after RNAi induction. As well as determining functions for a large number of previously-uncharacterised genes, these data also provided a number of new drug targets and suggested putative mechanisms for drug resistance (Alsford et al, 2011).

SMALL RNAs, CANCER AND CELLULAR STRESS

The importance of miRNAs in cancer is well documented, and several presentations focused on advancements in our
understanding of miRNA deregulation during tumour progression. Cervical cancer can be triggered by the human papillomavirus (HPV). John O'Leary (University of Dublin, Ireland) presented an intriguing account of how knocking down a specific HPV gene leads to an altered transcriptional profile in cervical cells, findings which may have therapeutic implications. In the second part of his talk he described the role of miR-21 in mediating chemoresistance in ovarian cancer. This microRNA appears to regulate the innate immune TLR4/MyD88 pathway (Sheedy et al, 2010).

In the second keynote presentation, Adrian Harris (University of Oxford, UK) discussed new data relating to a mechanistic role for miR-210 in tumour growth and survival in vitro and in the adaptive response to hypoxic conditions. miR-210 has previously been associated both with hypoxia and, independently, with an aggressive phenotype and lower chances of survival in breast and head and neck cancers. Recent work has suggested several targets for miR-210; these include crucial genes required for mitochondrial chaperone function, for example iron sulphur complexes necessary for enzymes, such as aconitase and members of the electron transport chain, and DNA repair pathways (Favaro et al, 2010). Data relating to miRNAs that are down-regulated under hypoxic conditions were also presented.

In addition to their function as regulators of gene expression, there exists the possibility that circulating miRNAs may act as biomarkers for specific conditions. Yaping Tian (Chinese PLA General Hospital, China) presented details of how specific serum miRNAs were investigated as potential biomarkers for liver pathologies. miR-885-5p was found to be significantly elevated in the sera of patients with hepatocellular carcinoma (HCC), liver cirrhosis (LC) and chronic hepatitis B (CHB) as compared to healthy controls and therefore has the potential to act as a biomarker for detection of liver pathologies (Gui et al, 2011).

THERAPEUTIC APPLICATIONS OF SMALL RNAs

Short activating RNAs (saRNAs) are designed to up-regulate expression of their target genes. Nagy Habib (Imperial College, UK) described the potential of saRNAs in manipulating the transcriptional identity of stem cells. This could have therapeutic potential to treat a range of diseases. Patrick Lu (Siranomics, USA) presented remarkable data on the use of siRNAs to inhibit scar formation following mechanical wounds or burns. The approach involves knocking down genes involved in inflammation by topical application of siRNA nanoparticles. This demonstrates the potential of RNAi as a therapeutic tool in the near future.

TECHNOLOGY FOCUS

Two afternoon workshops provided the opportunity for delegates to learn about the latest developments in image analysis from Molecular Devices and in RNAi and gene knock-out techniques from Sigma-Aldrich. MetaXpress application modules and PowerCore distributed image analysis software were demonstrated by Christian Holz (Molecular Devices, USA) using the results of a micronuclei assay. Caspar Robinson (Sigma-Aldrich, USA) and colleagues described a number of recently-developed technologies that provide new opportunities for gene silencing. These included the generation of SAGE animals and CompoZr cell lines, the latter of which involves custom zinc-finger nucleases (ZFNs) that are pre-designed and pre-validated.

CONCLUDING REMARKS

The conference’s attendees bore witness to a number of exciting breakthroughs, particularly in the areas of RNAi delivery. Andrew Miller (King’s College, London, UK), who presented lifetime achievement awards to Professor Sir David Baulcombe (University of Cambridge, UK) and Professor John O’Leary (University of Dublin, Ireland), accurately summed up the challenges and opportunities faced by all who wish to use RNAi as a therapeutic tool. Progress is slow but steady, but we must continue to invest in the potential of RNAi and have faith that our efforts will bear fruit in the years to come. We will return in 2012 to review our progress.

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