MEETING REVIEW

Gene regulation by small RNAs

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The 5th annual international RNAi conference at Oxford, RNAi2010: Gene Regulation by Small RNAs, was held at St Hilda’s College, Oxford, UK (17-18th March). The meeting brought together experts from industry and academia and served as a forum to discuss new technological developments and interesting findings in the RNAi field. Research posters were displayed throughout the conference. Key sponsors of the conference included Thermo Scientific, PerkinElmer and Abcam.

RNAI: MOLECULAR AND CELLULAR BIOLOGY

Erik Miska (University of Cambridge, UK) discussed several aspects of small RNA biology in a wide-ranging presentation. (1) As many of the mechanistic details of microRNA (miRNA) biology are as yet unclear, the Miska group aimed to identify genes that are either required for or inhibitory to miRNA function. A Caenorhabditis elegans reporter system and a high throughput ‘worm sorter’ based on flow cytometry were used to show that, despite continuous expression, let-7 activity was developmentally controlled. LIN-28 was found to sequester pre-let-7 and target it for degradation through polyuridylation by PUP-2 (a poly(U) polymerase) thus identifying a novel means of miRNA post-transcriptional regulation (Lehrbach et al, 2009). (2) PIWI interacting RNAs (piRNAs) are known to silence transposable elements in the germline of many higher eukaryotes. However, in C. elegans only the Tc3 transposon was found to be suppressed in this manner. Instead, an alternate class of small RNAs (21U-RNAs) perform the function of piRNAs in the worm (Das et al, 2008). (3) Heritable RNAi effects in C. elegans detectable after as many as 70 generations post induction were also discussed.

George Sczakiel (University of Lübeck, Germany) described the translocation of Argonaute 2 (Ago2), a key component of the RNAi pathway, to stress granules in response to cellular stress. This translocation is associated with reduced small interfering RNA (siRNA) and miRNA functionalities. The stress conditions considered were heat, NaAsO2-induced oxidative stress and, notably, a typical lipoplex transfection protocol. The latter condition is important as lipofection is a widely used method for introducing RNAi effectors into cells in culture. It will be interesting if similar results are observed following viral transduction as this would have wide-ranging implications for the RNAi field.

Cell-to-cell spreading of RNAi effectors has been demonstrated in plants and invertebrates but is still controversial in mammals. Luc van der Laan (Erasmus MC-University Medical Centre, The Netherlands) presented evidence for the transmission of RNAi between human cells independent of cell contact. miR-122 is highly expressed in Huh7 (human hepatoma) cells. Co-culture of HepG2 or HEK293T cells with conditioned medium from Huh7 culture results in transfer of miR-122 to the recipient cell lines (which normally express the miRNA at very low levels). Similarly, lenti-expressed shRNAs targeting therapeutic anti-HCV targets could also be transferred in conditioned medium. The lack of direct contact between cells suggests a release and uptake mechanism, potentially involving exosomes.

Petr Svoboda (Institute of Molecular Genetics, Czech Republic) presented data showing that Ago2 and reporter-tagged mRNAs do not co-localise with P-bodies in the mouse germinal vesicle-intact (GV) oocyte prior to fertilisation, in contrast with somatic cells. Microinjection of reporter constructs containing either partially complementary or fully complementary endogenous miRNA target sites showed that while miRNA-mediated translational repression was greatly reduced, RNAi-like mRNA cleavage was much less affected (Ma et al, 2010).
These results are consistent with a recent report that deletion of Dcr8 results in relatively small transcriptome changes and normal oocyte development (Suh et al, 2010). Taken together, these observations suggest that during oocyte development endogenous siRNA regulation dominates and regulation by miRNAs is non-essential.

Inhibition of neovascularisation by targeting retinal VEGF with siRNA is a potential therapy for age-related macular degeneration. However, recent reports have demonstrated knockdown of VEGF using non-specific siRNAs suggesting innate immune system involvement (Kleinman et al, 2008). Glen Reid (University of Sydney, Australia) emphasised the need to confirm that in vivo knockdown of a target gene is due to RNAi and not an innate immune response. A novel real time PCR technique for detection of miRNA cleavage products; Molecular Beacon 5’ Rapid Amplification of cDNA Ends (MBRACE), was described to this effect (Lasham et al, 2010).

Sandra Laufer (University of Lübeck, Germany) described the utility of HeLa S100 cell extract in vitro models for analysing RISC activity and a biotinylated mRNA pull-down method for identifying RISC components.

HIGH-THROUGHPUT RNAI SCREENING

Large-scale RNAi screening is a powerful reverse genetics method for identifying biological interactions and discovering potential therapeutic drug targets. Attila Seyhan (Pfizer-Wyeth, USA) utilised whole-genome pooled lentiviral RNAi screens and Ingenuity pathway analysis to identify novel chemosensitiser/chemoresistor genes in a breast cancer cell line. A number of target genes were found to influence the efficacy of the legacy Wyeth cancer drug. It was suggested that modulating the expression of these genes could serve as part of a combination therapy, or alternatively, their expression levels could be utilised as biomarkers for selecting suitable patients for treatment. Similarly, William Arthur (Merck & Co Inc, USA) performed RNAi screens to identify novel components of the Wnt/β-catenin pathway which is involved in tumor initiation and control of bone density. Multiple cell lines were studied in a systematic screening process and several candidate genes were identified as likely regulators of the pathway. Targeting these genes may form the basis of novel therapies for hepatocellular carcinoma, colorectal cancer and osteoporosis. Cecilia Lundin (University of Oxford, UK) showed how high-throughput RNAi screens are being used to understand homologous recombination DNA repair networks.

UNDERSTANDING MICRORNA FUNCTION

Specific miRNAs are often found to be abnormally expressed in disease conditions. In particular, many cancers exhibit unique miRNA expression profiles. Charles Lawrie (University of Oxford, UK) showed how miRNA expression profiling in 40 lymphoma cell lines has determined distinct miRNA profiles associated with both B and T cell lineages and with differing molecular subtypes of diffuse large B-cell lymphoma (DLBCL). Differential expression of certain miRNAs was also found to correlate with disease outcomes (Lawrie et al, 2009). The observation that disease-specific changes in miRNA expression can be detected in patient serum demonstrates the potential of miRNAs as biomarkers for non-invasive diagnostics and monitoring of disease progression.

Antisense oligonucleotide based strategies for inhibiting miRNAs have previously been demonstrated using 2'-O-methyl, 3'cholesterol modified phosphothioates (antagomiRs) and locked nucleic acid (LNA)/DNA mixmers. Martin Fabani (MRC Laboratory of Molecular Biology, Cambridge, UK) demonstrated inhibition of miR-155 in cultured B cells and in vivo by using peptide nucleic acids (PNAs), a DNA analogue chemistry. PNAs show promise in RNA targeting applications due to their specific binding and high biostability. Stephanie Urschel (Thermo Scientific Genomics) described a workflow for studying miRNA expression and identifying miRNA targets in the context of epithelial-mesenchymal transition. The utility of miRNA mimics and miRIDIAN miRNA inhibitors were highlighted as tools in miRNA research.

ADVANCES IN RNAI TECHNOLOGY

The ability to specifically target any gene and the short drug-development process relative to conventional pharmaceuticals means RNAi effectors show great potential as therapeutics. However, serum stability, renal clearance, off-target effects and problems with delivery remain obstacles to effective therapy. To address these challenges, Dmitry Samarsky (RXi Pharmaceuticals, USA) presented novel combinations of duplex architecture and chemical modification in order to confer drug-like properties on RNAi effectors. Notably, self-delivering RNAi effectors (sd-rxRNAT), comprising miniaturised single oligonucleotides, show enhanced spontaneous uptake, improved cell penetration and reduced passenger strand-mediated off-target effects in the absence of a gene transfer agent.

Jörg Vollmer (Pfizer-Coley, Germany) discussed novel ways to regulate immune modulation by DNA and RNA therapeutics. Specifically, the diverse immune profiles activated by oligonucleotides can be altered by modifying their nucleotide content and introducing LNA, 8-Oxo and 2′O-methyl modifications. Brian Schyth (Technical University of Denmark) used a high-throughput salmonid fish model infected with viral hemorrhagic septicemia (VHS) virus to investigate the non-specific effects of chemical modifications of siRNAs (Schyth et al, 2007), LNA modifications inside the duplex ablated the non-specific anti-viral response. Conversely, segmentation of the passenger strand (small internally segmented interfering RNA, sisiRNA) resulted in increased anti-viral response. These findings have implications for the design of future chemically modified siRNAs, as the response of the innate immune system must be carefully considered when introducing chemical modifications.

Coxackievirus B3 (CVB-3) infection is one cause of dilated cardiomyopathy. Jens Kurreck (University of Berlin, Germany) presented a number of promising RNAi-based strategies for inhibiting CVB-3 replication. These
included LNA/UNA (unlocked nucleic acid) modified siRNAs (Werk et al, 2010), self-complementary pseudotyped adeno-associated virus (AAV9) delivering double siRNA expression cassettes to murine cardiomyocytes and a potent synergistic strategy combining RNAi and a protein-based (soluble virus trap) approach. Tobias Poelman (Friedrich-Schiller-Universität Jena, Germany) presented a novel peptide-inactivated siRNA (PI-siRNA) technology. siRNA function is inhibited by a short peptide covalently bound to the siRNA backbone. Introduction of the PI-siRNA into cells expressing high levels of caspase-4 (i.e. Jeg-3 and MCF-7 cancer cell models) leads to cleavage of the inhibiting peptide and siRNA functionality is restored. Selective activation of PI-siRNA shows great potential in cell-type specific therapeutic gene silencing. Mariam Sharaf (Duke University, USA) presented data showing gene knockdown by siRNAs carrying boranophosphate-modified backbones which exhibit increased nuclease resistance, higher lipophilicity and improved potency relative to phosphothiorate or unmodified oligonucleotides.

NANOPARTICLE DELIVERY

As with other nucleic acid-based therapies, a major challenge in the development of therapeutic RNAi is delivery of effector molecules to target cells. Given the inherent safety issues associated with viral vectors there is a demand for safer non-viral alternatives. Nanoparticles are a diverse class of synthetic, non-viral vectors that offer a potential mode of effective siRNA delivery to target sites. Zdravka Medarova (Massachusetts General Hospital, USA) demonstrated the utility of iron oxide magnetic nanoparticles conjugated to siRNA (MN-siRNA) and Cy5.5 dye allowing non-invasive in vivo imaging of siRNA delivery by magnetic resonance and near-infrared optical imaging. Magnetic nanoparticles were found to accumulate in tumours in vivo and effectively deliver siRNAs targeting both reporter and endogenous genes (Medarova et al, 2007). In addition, this approach was also used to deliver siRNAs targeting graft rejection-associated genes in pancreatic islands in vitro prior to transplantation.

Patrick Lu (Sirnaomics Inc, USA) showed how pooled siRNA cocktails and polymer-/liposome-based nanoparticles are being developed as treatments for a number of conditions. Several treatments based on local or topical delivery are in the late preclinical stage of development for the treatment of H5N1/H1N1 influenza infection, ocular neovascularisation disease and a formulation for reducing scarring at skin wounds through inhibition of fibrosis. Roger Adami (MDRNA Inc, USA) described some of the beneficial properties of siRNAs containing UNA modifications (UsiRNAs) such as reduced passenger strand RISC loading, reduced guide strand miRNA-type off-target effects, and reduced innate immune system induction. These chemically modified siRNAs are being combined with a di-alkylated amino acid based nanoparticle delivery system (DiLA2) for potential therapies for bladder cancer and hepatocellular carcinoma. Andrew Miller (Imperial College London, UK) outlined the ABCD nanoparticle paradigm of synthetic, self-assembly nanoparticles for in vivo delivery of siRNA and plasmid DNA. This four component system consists of an enveloped or complexed nucleic acid/cationic lipid core, a stealth/biocompatibility polymer coat (e.g. polyethylene glycol) and a biological ligand to confer active targeting properties (Kostarelos et al, 2005). This approach has been utilised in the development of targeted delivery of siRNAs to human xenograft tumours in vivo.

CONCLUDING REMARKS

Studies presented at RNAi2010 demonstrated that gene silencing is an invaluable tool for understanding gene function and that RNAi-based therapeutics show much promise. Exciting technological developments are gradually overcoming the obstacles facing the translation of preclinical research into safe and effective treatments through the reduction of off-target effects, increased bioavailability and improved targeted delivery strategies. The potential for a short developmental timescale of tailored nucleic acid therapies, relative to conventional pharmaceuticals, continues to drive efforts to make therapeutic RNAi a reality.

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