

MEETING REVIEW

RNAi2015 – Ten years of RNAi Oxford

Findlay Bewicke-Copley†, Priya Samuel† and David RF Carter*

Oxford Brookes University, Faculty of Health and Life Sciences, Department of Biological and Medical Sciences, Gypsy Lane, Oxford, OX3 0BP, UK

†These authors contributed equally to this publication

*Correspondence to: David Carter, Email: dcarter@brookes.ac.uk, Tel: +44 1865 484216; Fax: +44 1865 483242

Received: 02 June 2015; Revised: 21 July 2015; Accepted: 21 July 2015; Published: 21 July 2015

© Copyright The Author(s). First Published by Library Publishing Media. This is an open access article, published under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0>). This license permits non-commercial use, distribution and reproduction of the article, provided the original work is appropriately acknowledged with correct citation details.

The tenth RNAi conference was held at St. Hilda's College Oxford on the 24–26 March 2015. The conference offered researchers from all over the world the chance to present, discuss and discover work pertaining to the field of RNAi. RNAi has become an essential technique in genomic research for functional validation as well as an exciting avenue to explore in therapeutic medicine. Emerging techniques such as CRISPR as well as improvements in efficiency of existing techniques and expansions in libraries have cemented the importance of RNAi at the cutting edge of research. Featured presentations and posters showcased recent research in the field ranging from RNA detection in bio fluids through to potential oligonucleotide therapies.

KEYNOTE ADDRESS

Dr Greg Hannon from Cold Spring Harbour laboratory and Cancer research UK Cambridge Institute was the keynote speaker at the conference. In the keynote speech, Dr Hannon described the development and use of modern RNAi tools to probe the mechanism of metastatic progression. A big development has been the creation of shERWOOD, a computer algorithm that identified positional and sequence determinants that maximise shRNA gene knockdown efficiency (Knott et al, 2014). Development of the algorithm involved analysing data generated by 'Sensor Assays' which use fluorescent reporters and flow assays to identify the most potent shRNAs. The RNAi library developed using predictions made by shERWOOD could have up to 99% repression of target mRNA. This new library can be used in many applications, such as the identification of drivers of tumour behaviour. Dr Hannon reported the results of a study in which the shRNA library was used to identify genes that mediate metastatic behaviour within a heterogeneous collection of breast cancer cells. By analysing the behaviour of different clones the involvement of two candidate genes

in entering the vasculature of mice. Cells expressing these genes, SERPINE2 and SLP1, are sufficient to confer vascular mimicry, allowing the clone to enter the vasculature and spread to other organs (Wagenblast et al, 2015). These data elegantly demonstrate the potential for large shRNA libraries to uncover novel mediators of tumour progression.

ncRNAs AS BIOMARKERS OF DISEASE

Non-coding RNAs (ncRNAs) have the potential to be used as biomarkers to diagnose disease or to non-invasively evaluate the response to treatments. Dr Anna Zampetaki (King's College London, UK) showed how certain circulating miRNAs can be used to assess disease severity and risk. She identified a number of miRNAs that can inform on the likelihood of diabetic complications (Zampetaki et al, 2010) and myocardial infarction. Dr Iris Lavon (Hadassah Hebrew University Medical Centre, Israel) spoke about using circulating miRNAs to assess tumour treatment of gliomas with bevacizumab. Measuring the levels of these miRNAs was also able to give information on tumour progression. This is particularly useful as brain tumours are generally inaccessible and can be difficult to visualise.

ncRNAs AS THERAPEUTIC TARGETS

It is becoming clear that ncRNAs play an important role in normal cellular processes. As such it is no surprise that a range of ncRNAs are being uncovered whose expression is de-regulated in various diseases. Many of these RNA molecules therefore represent potential therapeutic targets.

Dr Mark Perry (Imperial College London, UK) spoke about the role of ncRNAs in respiratory disease. miR-221 was shown to be increased in airway smooth muscle cells of severe asthmatics, leading to decreased p21(WAF) and

p27 (kip1) levels and concomitant increases in proliferation, which may account for the increased smooth muscle mass in the airways of asthmatic patients (Perry et al, 2014a). A network of miRNAs and long ncRNAs was also found to be affected during inflammatory response and following application of drugs used to treat asthmatics (Perry et al, 2014b) targeting these ncRNAs could yield novel therapeutics for the treatment of respiratory disorders like asthma.

Professor Chris Murphy (University of Oxford, UK) gave a talk on the regulation of cartilage matrix production by several miRNAs. A regulatory network controlling the level of SOX9 transcription factor was proposed. SOX9 is known to be an important regulator of cartilage production, and Professor Murphy's studies have revealed roles for ncRNAs including miR-675, H19 (a long ncRNA), and miR-145 (Martinez-Sanchez et al, 2012).

Dr Sandor Batkai (Hannover Medical School, Germany) discussed the therapeutic potential of miRNAs in cardiac disorders. Dr Batkai showed that miR-132 is a pro-hypertrophic miRNA, increasing the proliferation of cardiomyocytes by repressing the FoxO3 transcription factor (Ucar et al, 2012). This leads to increased mortality in a mouse model of heart pressure-overload. Inhibition using a miR-132 antagomir protected against heart failure in mice, thus providing exciting evidence for the potential therapeutic benefit of using miRNA-inhibitors in a clinical setting.

Dr David Carter (Oxford Brookes University, UK) described the role of miRNAs in the development of drug resistance in ovarian cancer. A functional role in causing resistance was shown for the passenger strand, miR-21* (Pink et al, 2015). Interestingly the mature miR-21 was found to be associated with drug-sensitive tumours, whereas the star-strand, miR-21* drives drug-resistance by down-regulating the product of the NAV3 gene. Dr Carter also described the potential for extracellular ncRNAs in mediating the radiation-induced bystander effect in breast cancer cells (Al-Mayah et al, 2012).

RNAi TOOLS AND TECHNIQUES

As the field of RNAi and ncRNAs grows the range of tools available to researchers also expands. A number of interesting technologies were described, with detection and specific knockdown of RNA being a particular theme during the meeting.

Dr Graeme Doran (Firefly BioWorks Inc) presented the Firefly system for miRNA detection in biofluids. Normally detection of miRNA in biofluids is complicated by loss of sample during extraction. Firefly is a new technology for miRNA detection that uses hydrogel particles. The Firefly system was shown to detect miRNA from plasma, serum whole blood and exosomes containing less than 100pg of total RNA.

RNAi screens allow rapid identification of genes and gene networks. However, off-target effects, where phenotypes may be observed due to knockdown of non-targeted mRNAs, remain a big problem. Dr Ryan Raver (Sigma Aldrich) described the tools available for testing the func-

tion of genes, including RNAi screens, zinc finger nucleases, and CRISPR-Cas9. A suggested method was to use multiple siRNAs for the predicted gene target which is then validated by other tools, thus increasing the confidence of any observed phenotype being caused by loss of function of the targeted gene. Similarly, Dr Annaleen Vermeulen (GE Healthcare/Dharmacon) described a screening system to reduce off-target effects. First a high-throughput siRNA screen is undertaken to find possible gene targets. High-confidence predicted targets are then validated by gene editing with CRISPR/Cas9. Dr Vermeulen used this combinatorial approach to identify genes involved in the proteasome with high confidence.

Dr Mark Behlke (Integrated DNA Technologies) spoke about the differences in levels of lncRNA-knockdown when using siRNA and antisense oligonucleotides (ASO). Dr Behlke used combinations of ASOs and siRNAs to ascertain the conditions under which these different tools work most effectively.

When studying interactions between miRNAs and mRNAs many researchers often use techniques such as miRNA over-expression (for example using mimics) or inhibition (such as with antagomirs). However, a limitation of this approach is that the interaction of the miRNA with many of its targets may be affected, complicating the interpretation of results of specific miRNA-mRNA interactions. Professor Tudor Fulga (University Of Oxford, UK) described the use of CRISPR/Cas9 and transcription activator-like effector nuclease (TALEN) technologies to more specifically probe the interactions between miRNAs and their putative miRNA-response elements (MREs) in target mRNAs. By disrupting the MRE of the target mRNA, the miRNA is only blocked from acting on that specific mRNA. Professor Fulga utilised this approach to test the interactions of several miRNA-mRNA pairs in *Drosophila*, zebrafish and human cells (Bassett et al, 2014).

RNAi AND ncRNA BIOLOGY

Professor Georg Sczakiel (University of Lübeck, Germany) has a long-standing interest in the mechanics and efficiency of the RNAi machinery in cells. At the meeting he presented details of an elegant new study in which the relationship between Ago2, siRNA strands and the target mRNA were explored. His work gives new insight into the role of all three in the assembly of a functional silencing complex.

The mechanisms by which miRNAs are able to repress gene expression are not fully known. One question that still courts controversy is whether miRNAs work primarily by repressing translation or degrading mRNA. Professor Martin Bushell (University of Leicester, UK) described compelling data that support a role for translational repression (Meijer et al, 2013). He showed that the RNA helicase eIF4A2 is required for miRNA-induced translational repression, and that this disrupts the structure of eIF4F-mRNA complex. He revealed a fascinating correlation between conserved miRNA binding sites in the 3'UTR and highly structured 5'UTR regions on the same mRNA, suggesting that factors bound in both regions are required to mediate the effects of miRNAs. He proposes a model in which miRNAs initially

disrupt translation which subsequently leads to the degradation of the target mRNA.

Dr Nicholas Dibb (Imperial College London) spoke about isomiRs; miRNAs with added bases at the 5' or 3' ends. They are found complexed with Argonaute proteins and show activity in the luciferase assay, suggesting active roles in cells. Analysis of the miRgator and miRBase databases suggests that canonical miRNA and isomiRs can switch during evolution. Dr Dibb's work shows that the proportion of different isomiRs is tissue-specific and that the addition of bases to a miRNA can alter its targeting, suggesting that they may play a role in controlling the functional effects of expressing a given miRNA (Tan et al, 2014).

DELIVERY METHODS

'Delivery, Delivery, Delivery', as mentioned by Dr Dmitry Samarsky (RiboBio Co, China), is still the biggest challenge to overcome for successful therapeutic applications of oligo-based drugs. Dr Samarsky gave an informative overview of the field, describing the history of oligonucleotides as therapeutics, the challenges of scaling up production to an industrial scale, and the problems of delivering them to the correct cells at therapeutic doses. He also showed data on the use of Arg-Gly-Asp (RGD)-conjugated siRNAs to effectively knock down expression of the VEGFR2 gene *in vivo* as a potential anti-tumour therapy (Liu et al, 2014).

Professor Stephen Hart (University College London, UK) spoke about the problems of nanoparticle carriers for oligonucleotide delivery. Most nanoparticles end up in the liver where they are cleared. However, a novel PEG coated nanoparticle was presented that was able to selectively deliver siRNA to a tumour with minimal siRNA found in the liver.

Professor Raymond Schiffelers (Utrecht University, The Netherlands) talked about an RGD-conjugated PEG-coated nanovesicle that showed promising gene knockdown. He also discussed extracellular vesicle based systems for oligo-delivery and the pitfalls associated with loading them with a specific oligonucleotide (Kooijmans et al, 2013). Professor Schiffelers also described a model for testing the transfer of exosomes *in vivo*. The system involves the transfer of Cre recombinase in exosomes from one cell type into cells bearing LoxP sites and expressing a fluorescent protein; such transfer events in a mouse xenograft model lead to a change in colour of the fluorescent cells and an increase in the migratory and metastatic potential of the cells as observed during intravital imaging (Zomer et al, 2015).

Dr Muhammad Danish (Nottingham University, UK) reported a system based on cross-linked polyplexes, previously used for delivery of plasmid DNA. He presented findings noting that the complexes were stable, not toxic and also achieved efficient silencing.

Professor Andrew Miller (King's College London, UK, GlobalAcorn Ltd) outlined the challenge of targeting RNAi therapeutics with special mention of the triggerability of lipid based nanoparticles. Modifying the shielding layer of the nanoparticles produce complexes that could effectively be targeted to different microenvironments. Potential trig-

gers for oligonucleotide release include pH, redox potential and enzymatic triggers.

Professor Ian Blagbrough (University of Bath, UK) discussed the use of lipoplexes with symmetric or asymmetric conjugates of spermine. He described that these can be non-toxic and show not only successful delivery but also effective EGFP silencing (Metwally et al, 2012).

PANEL DISCUSSION

Dr Dmitry Samarsky chaired the panel discussion which revolved around oligonucleotides as therapeutics. Dr Samarsky gave an introduction regarding siRNA delivery. He then posed a question to the panel: "If you had \$5 million and the task of filing IND (investigational new drug) with oligo therapeutics in 4-5 years, what would you do?"

The other members of the panel, Professor Andrew Miller, Dr Mark Behlke, Dr Raymond Schiffelers and Professor Georg Sczakiel then considered this question during a stimulating discussion.

They discussed the pros and cons of antisense oligonucleotide (ASO) as opposed to siRNA-based RNAi. On the question of what disease and organ they thought was most promising, the panel differed. Some thought that cancer was the most obvious target in terms of therapeutic potential. Others felt that cancer represented a 'moving target' and that a more clearly characterised and accessible target would be more appropriate, such as a dermatological condition which could be treated via topical application. Many of the current oligonucleotide-based therapeutics are targeted against the liver, where most injected nanoparticles rapidly end up in prior to being processed and excreted. To treat other conditions we need to overcome the issues of avoiding systematic clearance via the liver and kidneys and find a way to target specific organs. However, other delivery systems such as topic application to the skin, aerosolised solutions in the lung and injections into the eye represent easier short term options for the development of 'targeted' delivery.

This vibrant discussion was fascinating to watch and gave delegates a flavour of the therapeutic potential of ncRNAs in treating disease.

CONCLUDING REMARKS

The various talks and discussions at the RNAi 2015 podium tabled advances in research in the wide ranging field of RNAi diagnostics and therapeutics. These continue to provide hope that *in vivo* delivery of siRNA and personalised medicine is nearer to being a reality. While exciting new advances continue to be made in diagnostics, the efficiency and targeting of oligonucleotide-based therapies is improving. Researchers can be encouraged to continue their research in this field as there is scope for their research to make a real difference in the lives of patients.

COMPETING INTERESTS

None declared.

REFERENCES

- Al-Mayah AH, Irons SL, Pink RC, Carter DR and Kadhim MA. 2012. Possible Role of Exosomes Containing RNA in Mediating Nontargeted Effect of Ionizing Radiation. *Radiation Res*, 177, 539–545.
- Bassett AR, Azzam G, Wheatley L et al. 2014. Understanding functional miRNA-target interactions in vivo by site-specific genome engineering. *Nature Communications*, 5, 4640.
- Kooijmans SA, Stremersch S, Braeckmans K et al. 2013. Electroporation-induced siRNA precipitation obscures the efficiency of siRNA loading into extracellular vesicles. *J Control Release*, 172, 229–238.
- Knott SR, Maceli AR, Erard N et al. 2014. A computational algorithm to predict shRNA potency. *Mol Cell*, 56, 796–807.
- Liu X, Wang W, Samarsky D et al. 2014. Tumor-targeted in vivo gene silencing via systemic delivery of cRGD-conjugated siRNA. *Nucleic Acids Res*, 42, 11805–11817.
- Martinez-Sanchez A, Dudek KA and Murphy CL. 2012. Regulation of human chondrocyte function through direct inhibition of cartilage master regulator SOX9 by microRNA-145 (miRNA-145). *J Biol Chem*, 287, 916–924.
- Meijer HA, Kong YW, Lu WT et al. 2013. Translational repression and eIF4A2 activity are critical for microRNA-mediated gene regulation. *Science*, 340, 82–85.
- Metwally AA, Blagbrough IS and Mantell JM. 2012. Quantitative silencing of EGFP reporter gene by self-assembled siRNA lipoplexes of LinOS and cholesterol. *Mol Pharma*, 9, 3384–3395.
- Perry MM, Baker JE, Gibeon DS, Adcock IM and Chung KF. 2014. Airway smooth muscle hyperproliferation is regulated by microRNA-221 in severe asthma. *American J Respiratory Cell Mol Biol*, 50, 7–17.
- Perry MM, Tsitsiou E, Austin PJ et al. 2014. Role of non-coding RNAs in maintaining primary airway smooth muscle cells. *Respiratory Res*, 15, 58.
- Pink RC, Samuel P, Massa D, Caley DP, Brooks SA and Carter DR. 2015. The passenger strand, miR-21–3p, plays a role in mediating cisplatin resistance in ovarian cancer cells. *Gynecol Oncol*, 137, 143–151.
- Tan GC, Chan E, Molnar A et al. 2014. 5' isomiR variation is of functional and evolutionary importance. *Nucleic Acids Res*, 42, 9424–9435.
- Ucar A, Gupta SK, Fiedler J et al. 2012. The miRNA-212/132 family regulates both cardiac hypertrophy and cardiomyocyte autophagy. *Nature Communications*, 3, 1078.
- Wagenblast E, Soto M, Gutiérrez-Ángel S et al. 2015. A model of breast cancer heterogeneity reveals vascular mimicry as a driver of metastasis. *Nature*, 520, 358–362.
- Zampetaki A, Kiechl S, Drozdov I et al. 2010. Plasma microRNA profiling reveals loss of endothelial miR-126 and other microRNAs in type 2 diabetes. *Circulation Res*, 107, 810–817.
- Zomer A, Maynard C, Verweij FJ et al. 2015. In Vivo imaging reveals extracellular vesicle-mediated phenocopying of metastatic behavior. *Cell*, 161, 1046–1057.