

MEETING REVIEW**Non-coding RNAs: Bridging Biology and Therapy**

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The 4th annual international RNAi conference at Oxford, RNAi2009: Bridging Biology and Therapy, was held at St Anne's college, Oxford, UK (18-19th March). The meeting attracted a wide range of participants from both academic and industrial backgrounds to assemble and disseminate, discuss and debate advances within the rapidly moving field of RNAi. There was a spectrum of experts, both national and international, who covered a range of stimulating RNAi topics. A selection of research posters was also on display for the duration of the conference. Conference participants were given the opportunity to learn about the latest commercial products at the trade exhibition, which featured Thermo Scientific, Qiagen, Eurogentec, Integrated DNA Technologies, Pfizer-Coley, Exiqon, Agilent Technologies and Oxford University Press.

miRNAS IN IMMUNITY, DISEASE AND DEVELOPMENT

When the immune system is activated by pathogens the Toll-like and IL-1 receptors effect rapid stimulation of a variety of genes. Research is underway to delineate the role of miRNAs in this process. Mark Lindsay (University of Manchester, UK) presented results on the role of miR-146a and miR-155 during an IL-1 β -induced inflammatory response in alveolar cells.

Marcus Peter (University of Chicago, US) addressed the role of miRNAs in tumour progression using a panel of 60 cancer cell lines. Genetic analysis shows the lines can be broadly divided into two groups, representing early and late stages of tumour development. Work in Dr Peter's laboratory has identified key differences in miRNA expression that may underpin the phenotypic differences between the two cell types, and thus cancer progression. For example, the miRNA let-7 was shown to correlate with cell types bearing an epithelial gene expression profile. A second miRNA family, miR-200, was identified

that regulates Vimentin and E-Cadherin, two genes whose expression is often lost during cancer progression. The miR-200 family, which is up-regulated in the epithelial-like cancer lines, appears to inhibit Vimentin and E-Cadherin expression by targeting two transcription factors involved in their normal activation, ZEB1 and ZEB2 (Park et al, 2008).

Enrico De Smaele (University of Rome, Italy) also described how miRNA expression analysis has emerged as a powerful tool for identifying new important molecules involved in malignancies. Application of this technique to medulloblastoma carcinomas identified patterns of miRNA expression that correlate with cancer histotypes (anaplastic, classic and desmoplastic) and with molecular features (such as over-expression of ErbB2 or c-Myc) (Ferretti et al, 2009). Identification of deregulated miRNAs in cancer progression should provide diagnostic biomarkers and potential targets for new therapeutic strategies.

The mechanisms that regulate activity of specific miRNAs during development remain largely unexplored. Youn-Bok Lee (University of Bristol, UK) analysed the interaction of transcription factors with miRNAs during mouse development. The results showed that Twist-1, a basic helix-loop-helix transcription factor, activates transcription of miR-199a and miR-214 in neural cells.

IN VITRO AND IN VIVO DELIVERY OF NUCLEIC ACID THERAPEUTICS

Despite the power and potential of RNAi as a therapeutic technique, a therapy is only as good as its delivery system. There are two key considerations with a delivery system, the identification of an optimal route of administration, and establishing efficient cellular uptake and intracellular release of the nucleic acids.

Dmitry Samarsky (RXi Pharmaceuticals, US) presented potential systems for delivery of rxRNA silencing compounds, that exhibit reduced immune stimulation and high potency. Jörg Vollmer (Pfizer Coley, Germany) and Achim Algner (Phillips-University School of Medicine, Germany) also presented their work on delivery systems that reduce toxicity *in vivo*. Cy Stein (Albert Einstein College of Medicine, US) described a remarkable system for the insertion of antisense oligonucleotides into cells, termed Gymnotic delivery. Cells were thought to be impermeable to polyanions such as oligonucleotides, necessitating the use of chemical carriers. Efficient Gymnotic delivery of an antisense oligo against Bcl-2 depends on the cell plating density used and prolonged treatment over six days, and results in more than 90% silencing at the mRNA and protein level. Georg Sczakiel (Lübeck University, Germany) also demonstrated the uptake of naked oligonucleotides into human cells stimulated with phosphorothioate. This mechanism delivered a large amount of siRNA and made use of a caveosomal instead of an endosomal pathway.

For siRNAs to be effective they not only require efficient uptake into cells, but must also be released from the endosomes that internalise them. Raymond Schiffelers (Utrecht University, the Netherlands) described a technique called photochemical internalisation (PCI) for the *in vivo* release of siRNA from endosomes. A431 tumour cells were injected with a photosensitive chemical and siRNA lipoplexes against EGFR. Exposure of these cells to light increased EGFR silencing efficiency by 150% (compared to cells not exposed to light), demonstrating the potential of this technique in cancer therapy (Oliveira et al, 2007).

RNAi AND VIRUSES

A wide range of miRNAs have been identified in plants, mammals and viruses. Viral miRNAs appear to modify viral and host gene expression. Venugopal Nair (Institute for Animal Health, UK) highlighted this in strains of herpesviruses that cause Marek's disease, a rapid-onset T-cell lymphoma, in poultry. Dr Nair's team identified a group of highly expressed viral miRNAs that appear to play a role in pathogenesis (Yao et al, 2008).

Lars Dölken (Ludwig Maximilians-University Munich, Germany) described the identification of 18 distinct miRNAs from the murine cytomegalovirus (MCMV). Analysis of RNA levels during infection of fibroblast cells suggests a highly regulated program of viral miRNA expression (Dölken et al, 2007). Nikolaus Machuy (Max-Planck-Institute, Germany) demonstrated the power of RNAi to identify genes involved in the influenza virus life-cycle.

RNAi has also been proposed as a potential therapy against viruses, including HIV. Ben Berkhout (University of Amsterdam, the Netherlands) demonstrated this *in vitro* using RNAi to effectively silence essential genes in the HIV genome, resulting in abolished viral proliferation (ter Brake et al, 2008). However, the virus shows a remarkable ability to escape the inhibition, often through mutations

that alter DNA sequence without altering amino acid usage. Dr Berkhout's exciting results show that inhibition escape can be prevented if at least four different regions are simultaneously targeted by RNAi, offering hope for a new HIV therapy.

TECHNOLOGY FOCUS

The fast pace within the field of RNAi means that the technology involved has to be dynamic, adaptable and up to date. Speakers covered a range of advancements in the currently available technology. These included synthetic miRNA inhibitors, mimics and improvements in biological activity and nuclease resistance.

Martin Kreutz (Qiagen, Germany) presented information on the miScript system, which enables the detection and quantification of small non-coding RNAs, including miRNAs and piRNAs. This was used in combination with a range of miRNA mimics to study the expression pattern and function of miRNAs during T-cell activation in Jurkat cells. Stephanie Urschel (Thermo Scientific, Germany) also described a range of products to analyse miRNA expression and function during osteoblast differentiation.

Natal'ya Kruglova (Institute of Chemical Biological and Fundamental Medicine, Russia) used 2'-O-methyl analogues at sites in siRNAs predicted to suffer from nuclease sensitivity. Modification at these sites increased nuclease resistance, resulting in a prolonged silencing effect compared to unmodified controls (Volkov et al, 2009). Tatiana Kabilova (Institute of Chemical Biological and Fundamental Medicine, Russia) described how siRNA structure can influence non-specific biological effects, such as immune stimulation.

Mark Behlke (Integrated DNA technologies, USA) presented a novel method to silence gene expression which does not rely on RNAi or antisense oligos. The technique involves the use of bimodular oligonucleotides, known as U1 adaptors, which tether the U1 small nuclear ribonucleoprotein splicing factor to the terminal exon of the target gene. This results in impaired polyadenylation of the target and subsequent RNA degradation (Goraczniak et al, 2009). The synthetic U1 adaptors direct specific down-regulation of target genes with few off-target effects, and when combined with siRNAs result in even greater levels of gene knock-down.

INTERFERING RNAS: BIOLOGY, DISEASE AND DEVELOPMENT

A broad range of potential RNAi applications were presented, ranging from the identification of drug biomarkers to the role of RNAi in the autoregulation of heterochromatin. Zubair Ahmed (University Birmingham, UK) used RNAi to dissect the pathways involved in apoptosis following optic nerve damage. The results suggest a potential therapy to prevent nerve cell loss and promote cell regeneration following injury to optic nerves.

Leukaemia progression often involves chromosomal translocations that remove oncogenes from their normal

chromatin environment, leading to inappropriate activation. In certain cases the translocation results in a fusion of oncogenes. Olaf Heidenreich (Newcastle University, UK) proposed that these fusion proteins provide unique therapeutic targets, and showed evidence that siRNAs designed to straddle the fusion site can specifically knock down the aberrant proteins (Thomas et al, 2006).

Nick Proudfoot (University of Oxford, UK) described the auto-regulation of convergent genes by transient heterochromatin formation in *S. pombe*. During the G1 phase of the cell cycle double-stranded RNA is generated that leads to RNAi-mediated heterochromatin formation across convergent genes. Cohesin, a protein involved in chromosome dynamics, binds chromatin between the convergent genes in G2 phase, preventing dsRNA formation (and the subsequent heterochromatin formation) and relieving the gene repression (Gullerova and Proudfoot, 2008). This remarkable mechanism of transient heterochromatin formation further demonstrates the breadth and power of RNAi.

Cancerous growths within any given tissue can arise through a variety of mechanisms. This heterogeneity hinders our efforts to develop appropriate treatments and means that patients respond differently to specific drugs. Identifying biomarkers that correlate with drug resistance should lead to improvements in patient management. René Bernards (Netherlands Cancer institute, the Netherlands) used RNAi to knock down over 8,000 human genes in breast cancer cells and identify genes conferring resistance

to Herceptin treatment. This loss-of-function assay implicated genes of the PI3 Kinase pathway in the resistance to this breast cancer drug (Berns et al, 2007). The technique is now being extended to study the mechanistic basis of resistance to a range of drugs.

CONCLUDING REMARKS

The conference gave the RNAi community the opportunity to present and discuss advances that have occurred over last twelve months. Research presented at this conference highlighted the increasing momentum that RNAi is gaining; a field which was in its infancy at the beginning of the decade. Whilst a number of technical challenges remain to be conquered, we surely stand at the cusp of a revolution in the way that diseases like cancer and HIV are treated. The next decade, and indeed the next twelve months, should be very exciting for the field of RNAi.

REFERENCES

- Berns K et al. 2007. *Cancer Cell*, 12, 395-402.
- Dölken L et al. 2007. *J Virol*, 81, 13771-13782.
- Ferretti E et al. 2009. *Int J Cancer*, 124, 568-577.
- Goracznik R et al. 2009. *Nat Biotechnol*, 27, 257-263.
- Gullerova M and Proudfoot N. 2008. *Cell*, 132, 983-995.
- Oliveira S et al. 2007. *Biochim Biophys Acta*, 1768, 1211-1217.
- Park S et al. 2008. *Genes Dev* 22, 894-907.
- ter Brake O et al. 2008. *Mol Ther*, 16, 557-564.
- Thomas M et al. 2006. *Acta Pharmacol Sin*, 27, 273-281.
- Volkov A et al. 2009. *Oligonucleotides*. 19, 191-202.
- Yao Y et al. 2008. *J Virol*, 82, 4007-4015.