

MEETING REVIEW

RNAi at Oxford

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The 1st annual Oxford RNAi conference, RNAi2006: Advances in RNA Interference Research (22-23rd March 2006, St Annes College), drew together many influential figures from both academic and commercial backgrounds to showcase recent advances in mechanistic understanding of RNAi and its utilization in both research and therapy. There was particular emphasis on the emerging therapeutic applications of small RNAs, and the techniques that might be applied to dissecting their many endogenous cellular functions.

The conference was opened by the recent recipient of the Lasker Medical Research Award, Professor Sir Edwin Southern, who spoke with enthusiasm of the endogenous roles of RNA interference, highlighting the intrinsic biological questions that will ensure longevity for the field relative to other approaches to gene silencing. RNAi research has been greatly advantaged in that the field has emerged at a time of maturity in antisense research, and the two technologies complement each other well.

miRNA IN CANCER DIAGNOSIS

The relationship between miRNA and cancer is of great interest both in its potential diagnostic application, through observation of miRNA expression profiles that correlate with different clinical outcomes, and also the mechanistic relationship between miRNA dysregulation and tumorigenesis. Erik Wiemer (Erasmus Medical Center, The Netherlands) presented a novel Locked Nucleic Acid (LNA) microarray platform that showed enhanced sensitivity in distinguishing single base mismatches in miRNA targets relative to non-modified oligonucleotide arrays. Using RNA extracted from primary tissue samples of non-small cell lung carcinomas he identified eleven miRNA that were commonly down-regulated in tumour samples, and with a panel of six miRNAs showed that “good” and “poor” clinical prognosis breast cancer samples could be distinguished by miRNA expression profile. Sharon O’toole (Trinity Centre for Health Sciences, Ireland) described the use of a Taqman PCR based miRNA expression profiling

system to show common and specific downregulation of miRNAs in cervical cancer samples. Interestingly, the targets of many of the down-regulated miRNAs were components of the cell cycle regulatory machinery, such as P16 and E2F, indicating a potential link between miRNA and proliferative control. Discussions following these talks commented specifically upon the need for care in purifying and enriching RNA samples for analysis. A common observation in tumour cell lines is dysregulation of transcription, and reports of global miRNA down-regulation must distinguish specific down-regulation of miRNAs from a global elevation in RNA synthesis that might lower the ratio of miRNA:total RNA in the assayed sample.

miRNA AND DEVELOPMENT

The first keynote speech of the conference was delivered by Professor Ronald Plasterk (Hubrecht Laboratory, The Netherlands) and provided an engaging overview of the role of miRNAs in developmental regulation. Particularly he showcased *in situ* staining of miRNAs in zebrafish, using a novel LNA probe developed in conjunction with Exiqon pharmaceuticals (Denmark). The tissue specific distribution of miRNAs was strongly evident, and this work pointed towards a large potential for projects that identify the expression profiles of miRNAs, with a view to understanding how the partially overlapping expression of several miRNA and mRNA targets modulates the level of translation of the mRNAs in a specific tissue.

OPTIMISATION OF siRNA DELIVERY FOR GENE KNOCKDOWN

The meeting was well supported by commercial sponsorship and featured a refreshingly insightful selection of commercial speakers from the industry. Of particular note was work presented by Dr William Marshall, Vice President of Dharmacon (Colorado, USA), who detailed his company’s efforts to reduce the “off-target” effects of siRNA duplexes. By using a pool of siRNA duplexes targeted against an mRNA it was possible to reduce the

individual duplex concentration (and so lower their contribution to off-target silencing) whilst maintaining the effective concentration of duplexes against the specific target.

A number of reports focussed upon the delivery problem that must be overcome if siRNA based therapies are to advance to clinical trials. George Sczakiel (Universitat zu Lubeck, Germany) reported the novel observation that phosphorothioate oligonucleotides stimulate internalization of naked siRNA in cell culture, through a mechanism that may involve components of the caveolar pathway. Ilimaquinone-mediated disruption of the golgi increased target suppression (presumably through release of golgi-sequestered duplexes), suggesting a new/additional route for dsRNA delivery in cell culture.

Ian MacLachlan described a stabilised nucleic acid-lipid particle (SNALP) that enhanced endocytic uptake of dsRNA both *in vitro* and *in vivo*. SNALPs are PEG-lipid coated to enhance serum stability and extend circulation time; a 3mg/kg dose of SNALP encapsulated siRNA is >40 fold more active downregulating hepatitis B viral RNA in mouse models.

EMERGING SUCCESSES OF siRNA THERAPEUTICS *IN VIVO*

There is increasing confidence within the field that siRNA based therapeutics will prove commercially viable. Several investigators have reported success designing siRNAs against mouse liver disease models. Given that the liver is responsible for clearance of siRNA from the bloodstream, it is of little surprise that targeting siRNAs to the liver has proved relatively simple. Of greater challenge is the systemic delivery of siRNAs directed to specific cell types. Erwin Song (China) reported the successful application of a protamine-siRNA-fab ligand complex that binds to breast cancer cells expressing the Her2 surface protein implanted into nod/SCID mice. This siRNA complex successfully and specifically silences the BCL-2 mRNA in Her-2 expressing cells, and leads to a ~70% reduction in tumour volume 3 weeks post inoculation. Using a modified version of this protamine-antibody complex that targeted HIV-1 envelope glycoprotein gp120, Song further reported that 3x100 µg siRNA complex doses led to a ~40% reduction in HIV infected tumour cells implanted in a mouse model, relative to a non-complexed siRNA control.

A practical concern in the development of siRNA based therapeutics is producing siRNA active at a clinically feasible dose (<1 mg/kg). Dr Ian MacLachlan (Protiva) reported a SNALP-based siRNA treatment for ebola virus infection; a 4x0.75 mg/kg regimen beginning 1hr post lethal infection allows 80-100% survival of guinea pigs. It is of note that a scrambled duplex control provides some level of protection also, indicating a potential upregulation of the immune system by the siRNA dose.

Dr Ekambar Kandimalla (Idera Pharmaceuticals, USA) presented the result of work that was designed to understand the similar up-regulation of the immune response

to doses of antisense oligonucleotides. The toll-like receptor 3 (TLR) recognises dsRNA and leads to activation of the immune response, whereas TLR9 recognises the unmethylated CG sequence of antisense oligonucleotides. Idera have designed “immunomer” immunostimulatory compounds containing DNA and RNA recognition motifs for TLRs that induce production of cytokines and a “priming” of the immune system. A 2.5mg/kg immunomer dose regimen more than doubles the survival time after implantation of tumour cells in a mouse model, and can prevent tumour implantation on re-challenge. Whilst exciting in terms of therapy, these results also indicate that care must be taken when designing controls for animal studies, so as to distinguish the specific gene silencing effects from immune system stimulation.

VIRAL EVASION OF siRNA REPRESSION

HIV-1 remains a major focus for many therapeutic approaches, and the flexibility of siRNA synthesis offers an attractive means to quickly respond to genetic evasion by viruses. Two talks, from Jens Kurreck (Free University, Berlin) and Ben Berkhout (University of Amsterdam) illustrated potential approaches to targeting viruses, and the means by which they might escape siRNA mediated silencing.

Kurreck presented studies into the targeting of coxsackievirus, a (+) strand RNA virus with clinical relevance to myocarditis, meningoencephalitis and pancreatitis. He demonstrated that target site thermodynamic stability negatively correlated with siRNA activity, suggesting a role for secondary structure in obscuring a target site from siRNA binding. Ben Berkhout described an approach to designing shRNAs against HIV-1; shRNA targeted to highly conserved regions of the viral genome proved poorly effective, as escape mutants appeared after a 4-5 days in forced evolution culture. Analysis of the escape mutants indicated that the shRNA target site was either deleted or modified to overcome inhibition. Notably, one escape mutant contained a single nucleotide substitution outside of the target site which stabilised an alternate RNA secondary structure which occluded shRNA binding, re-inforcing the evidence that secondary structure plays some role in RNAi target site recognition.

Both Kurreck and Berkhout demonstrated that vectors expressing multiple shRNA prevented escape mutants from forming in their cell culture models. Such additive inhibition can be readily tailored to virus sub-types, indicating the wide ranging anti-viral potential of RNAi.

NEW ROLES FOR SMALL RNAs IN PLANTS

The important and prescient RNAi discoveries in plants are sometimes overlooked by researchers focussed on developing human drug therapies. Professor David Baulcombe (John Innes Centre, Cambridge) delivered the second keynote lecture of the conference during a plant RNAi session, and highlighted the importance of studies in plants to understanding the origin of RNAi and its

many potential roles in cellular organisation. Professor Baulcombe began by detailing the role of RNAi in defence against invasive nucleic acids and in its role in plant development. Of particular interest was the identification of ~24nt small RNAs that form part of a novel heterochromatin associated RNAi pathway in plants. With the observation that larger “small RNAs” exist in vertebrates also, it would not be unexpected if plant studies had again led to the finding of a similarly conserved mechanism in animal cells. Continuing the theme of RNAi and epigenetics, Professor Hugh Dickinson (Plant Sciences, Oxford) spoke about the role and regulation of RNAi and imprinting in the plant germline, suggesting a link between the ALY transcription factors, Rb (retinoblastoma protein) and imprinting in the endosperm. Dr Margis Rogerio (UFRGS, Brazil) continued to expand the evidence for yet-to-be characterised RNAi pathways in plants, examining the many dicer-related and dsRNA binding proteins that indicate that an RNAi-related mechanism may have evolved in some unicellular organisms also.

CONCLUDING REMARKS

The conference itself was well attended and efficiently scheduled to provide a wide range of speakers, covering the recent advances in core RNAi research - but also stimulating interest in the wider applications of the technique and creating a forum for attendees to discuss new research opportunities. There was a clearly structured presentation of information, highlighting the contribution of plant biology and simple animal studies to developing a promising and powerful therapeutic for human disease. Exciting reports of low side-effect systemic delivery and significant clinical benefits in mouse models suggest that many of the technical hurdles for generating RNAi-based therapeutics may be overcome. The increasing importance of RNA in basic cellular functions offers encouragement to for commercial and academic researchers alike; RNAi2006 perhaps revealed that the two often disparately focused groups might be equally keen to collaborate to unravel the potential of small RNAs.