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Control of imprinted expression by the Air macro ncRNA

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ABSTRACT: Non-coding RNAs with gene regulatory functions have the possibility of becoming a common feature of mammalian gene regulation, following the discovery that the majority of the genome comprises ncRNAs. The prototype has long been the Xist ncRNA that induces X-chromosome inactivation in female cells. However, a new paradigm is emerging - the silencing of imprinted gene clusters by macro (i.e., long) ncRNAs. The Igf2r imprinted cluster is an epigenetic silencing model in which expression of a macro ncRNA silences multiple genes in cis (Seidl et al, 2006 EMBO J.25:3565). We have recently used genome tiling arrays to map parental-specific epigenetic modifications in a 250kb region in mouse embryo fibroblast cells, to show that repressive histone modifications on the silent Igf2r gene are localized to discrete regions (Regha et al., 2007 Mol.Cell 27:353). This demonstrates that the Air ncRNA does not spread 'silence' in a manner similar to that proposed for the Xist ncRNA during X chromosome inactivation. Thus, the Air ncRNA may directly act to silence genes in the Igf2r imprinted gene cluster in a manner dependent only on its transcription (reviewed in Pauler et al., 2007 TIG 23:284). We have examined the kinetics of Air ncRNA mediated silencing in a new ES cell in vitro differentiation model that recapitulates the onset of Igf2r silencing seen in the early preimplantation embryo. The role of the Air ncRNA in directly silencing Igf2r is being investigated in this ES model system using targeted alleles carrying different lengths of the Air ncRNA.

Comparing non-viral methods for siRNA and shRNA delivery to the mouse neural stem cell niche

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ABSTRACT: Vectorisation *in vivo* remains one of the critical limitations to both realising the enormous therapeutic potentials opened up by RNA interference (RNAi) and fully exploiting it's capacity as a research tool. What is more, in the context of the brain one has also to address the added complexity of delivering RNAi to specific target cells, as the brain is the organ with the highest number of cell types and limited accessibility. We have been developing methods for vectorising RNAi to neural stem cells in the adult mammalian brain using either synthetic small interfering RNAs (siRNAs) or plasmid DNA containing short hairpin cassettes (shRNAs). In each case the identity of the cell types targeted is determined with immunocytochemistry. siRNA delivery has been optimised with a combination of a cationic lipid (Jetsi™) with a neutral lipid (DOPE). In contrast, optimal vectorisation of shRNA is obtained with a cationic polymer (polyethylenimine, PEI). In this latter case, the use of a hybrid CMV-H1 construct was found to give maximal silencing. These approaches permit effective and specific silencing of the expression of the gene targeted without affecting the expression of other genes.

These methods have been used to address the roles of thyroid hormone (TH) signalling in control of neural stem cell proliferation and led to the identification of TH target genes and the receptors implicated in their control.

Small RNAs in germline development

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ABSTRACT: Since the discovery of RNAi small RNA molecules have been under intense study. They have been shown to impact many different processes, ranging from development to organ function and carcinogenesis. Recently, it has become clear that many distinct small RNA families exist. However, all act through a member of the well-conserved Argonaute family of proteins. We try to understand how specificity of the different Argonaute proteins is achieved, and we are particularly interested in Argonautes that may contribute to the epigenetic marking of genomic DNA in animals. Piwi proteins are Argonaute proteins that in vertebrates are specifically expressed in germ cells, and have been implicated in modifying chromatin structures. We demonstrate that the zebrafish piwi proteins (Ziwi and Zili) are expressed in both the male and the female gonad. Both proteins are localized to the cytoplasm, but Zili is also found within the nucleus. Of the two Piwi's only Ziwi is maternally transmitted to the progeny, and loss of any of the two piwi proteins results in loss of germ cells due to apoptosis.

We have characterized small RNAs that bind to both piwi proteins (piRNAs) in both ovary and testis, and found that they play a role in the silencing of transposable elements. Both the biogenesis and function of Piwi proteins and associated piRNAs will be discussed.

SephaCe - A tool for accurate segmentation of biological cells from brightfield microscopy

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ABSTRACT: Brightfield microscopy of biological cells provides accurate anatomic information of cellular structure. However the transparent nature of cells results in a lack of intensity contrast in the brightfield images, making the cells difficult to visualise. A software tool, SephaCe, has been developed which allows the user to delineate accurate boundaries for biological cells using these images with minimal user input. It operates by harnessing several advanced techniques from the medical image processing domain which maximise the information content available in the images. SephaCe is also able to co-register misaligned brightfield and fluorescence images, and to use the computed cell boundaries to extract fluorescence timeseries from multiple fluorescence images. It is hoped that SephaCe will be a useful tool for the biological microscope imaging community.

In vivo electrodelivery of silencing RNA

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ABSTRACT: Delivery of silencing RNA into cells requires approaches allowing a free crossing of the plasma membrane, a physical barrier, to get access to their cytoplasmic targets. There is a need to get a safe method for such a transfer. New physical methods are under developments but electrodelivery appears as one of the most successful. Reversible membrane permeabilization resulting from electric field pulse application on cells was observed in the 70's. This approach brought a successful transfer of plasmid DNA. It was shown that direct pulse application on the skin tumors of patients brought an enhanced effect of IV injected bleomycin. More recently we showed that plasmid delivery was achievable in vivo on murine tumors. In vivo electropulsation appears as a promising tool for exogenous agents delivery. SiRNA can be easily delivered in muscles and/or in tumors by the applications of calibrated electric pulses on the skin covering the organ after the local injection of the polyelectrolyte. Biophysical predictions of the behaviour of cells and tissues were validated by in situ assays. Delivery is fast and geometrically targeted as the field effect is very local. Operating protocols are now well established. No need for invasive electrodes is present making the treatment very easy. No damaging effect due to the membrane permeabilization are present. Silencing was obtained during more than 2 weeks in muscles and only on a few days in tumors. Chemical modifications of the siRNA brought a significant increase in the duration of silencing. Delivery of plasmids coding for shRNA gave silencing during more than 2 months.

This work was supported by grants from the AFM, the region Midi Pyrenees, the EU "Cliniporator" project, the ANR and the ITAV.

Increased RNAi is related to intracellular release of siRNA after phosphorothioate-stimulated cellular delivery: Sub-cellular localization and biological efficacy

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ABSTRACT: The recently identified phosphorothioate (PS)-stimulated cellular uptake pathway of siRNA represents a promising alternative as it makes use of a caveosomal rather than an endosomal pathway which is

used by the majority of known delivery systems. Further, this PS-stimulated mode delivers large amounts of physical naked siRNA primarily into the perinuclear space. This is related to measurable though moderate target suppression. The observed limited efficacy seems to be related to intracellular trapping of siRNA. In this system, we studied the sub-cellular localization of siRNA and Argonaute 2 (Ago2) by density gradient centrifugation and fluorescence microscopy after PS-stimulated delivery and in the use of lipofectamin2000 as a transfectant. The established ECV-304 cell system was used as well as SKRC-35 cells that internalize even larger amounts of siRNA than ECV-304 cell but show no measurable target suppression.

This study provides experimental evidence fort the view that co-localization of siRNA and Ago2 in the vicinity of the rough ER occurs when target inhibition is observed. Further, we describe a cell system that may be used to identify steps of intracellular trafficking of siRNA after PS-mediated delivery that are crucial for target suppression.

Enrichment of hexamer and heptamer 'seed' sequences in siRNAs scoring highly in a functional screen

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ABSTRACT: TNF related apoptosis inducing ligand (TRAIL) induces apoptosis in many transformed, but not normal cells and shows potential as a possible anti-cancer agent. The mechanisms by which sensitivity is determined are not fully understood. RNAi screening was used to attempt to identify novel factors involved in the determination of sensitivity of transformed cells to TRAIL. Exploratory experiments showed that knockdown of 50% of a selection of genes previously associated with the TRAIL pathway, including 5/5 members of the apoptotic machinery, reduced the sensitivity of HeLa cells to TRAIL induced apoptosis. A library of siRNAs targeting ~6000 genes in the druggable genome was screened for novel genes in the pathway. Genes targeted by the 20 siRNAs that scored highest in the screen were selected for confirmation. Confirmation experiments allowed the initial classification of 6 of these genes as 'hits'. In a further 6 cases, the original screen result could be shown to be due to off-target effects.

It has been proposed that siRNAs may act as miRNAs to knock-down unintended targets. Key to the specificity of miRNA-like effects is the hexamer or heptamer seed sequence at the 5' end of the siRNA guide strand. The seed sequences of the 20 highest scoring siRNAs from the screen were examined. Five hexamer and five heptamer seed sequences appear in multiple siRNAs, including siRNAs targeting 'confirmed hits'. Gene set enrichment analysis was used to show that a further 17 hexamer and 13 heptamer seed sequences tended to occur in siRNAs that scored highly in the screen, suggesting siRNAs containing these seeds scored highly due to miRNA like off-target effects. Frequency analysis was used to identify seed sequences that were common in either all human 3'UTRs or 3' UTRs of genes previously associated with the pathway, and therefore mark siRNAs containing these seeds as potentially promiscuous. 80% of all heptamer seed sequences found in the library were found in the 3'UTR of one or more of the genes previously associated with the TRAIL pathway. Genes were identified whose 3'UTRs were enriched in matches to seed sequences also enriched in highly scoring siRNAs. Thus novel candidates for genes involved in the TRAIL pathway were identified that may have been missed in the primary screen.

This shows that the screening process enriches for siRNAs with relevant off-target effects. However, off-target effects can also be used to identify novel candidate genes from screening data.

Analysis of gene function in trypanosomes

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ABSTRACT: Trypanosomatids of the order Kinetoplastida are major contributors to global disease and morbidity, and understanding their basic biology coupled with the development of new drug targets represents a critical need. Additionally, trypanosomes are among the more accessible divergent eukaryote experimental systems. The genome of Trypanosoma brucei contains >8,000 predicted open reading frames (ORFs), of which over half have no known homologues beyond trypanosomatids. Thus, a major challenge following completion of the *T. brucei* genome sequence is to obtain functional data for trypanosome ORFs. We are developing Tet-on conditional RNAi for functional analysis. In the first phase, we established systematic 'knock-down' followed by phenotype screening and online data dissemination (See the TrypanoFAN website.) representing the first systematic analysis of gene function in a parasitic organism. In total, >200 genes have been targeted in the bloodstream form parasite. Current and future objectives include further vector optimisation and use in high-throughput drug target validation.

RNAi opens new possibilities for functional gene analysis during development of the vertebrate nervous system

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ABSTRACT: Developmental processes are regulated with tight temporal control. Thus, for functional gene analysis during embryogenesis time has to be taken into account. However, classical genetic tools do not provide tight temporal control of loss of gene function during vertebrate embryonic development. In contrast, RNAi in combination with the accessibility of oviparous animal models provides both the temporal and the spatial control that is required. We have established in ovo and ex ovo RNAi as efficient tools for gene silencing during the development of the vertebrate nervous system. These approaches allow for the analysis of gene function in both spatially and temporally controlled manner. Therefore, genes that are embryonic lethal when knocked out with classical genetic tools can be studied during neural development as well as gene functions during later windows of activity that are normally inaccessible for analysis due to aberrant development caused by the absence of a gene's function during early stages of embryogenesis.

The power of temporal control of gene silencing is best demonstrated by our finding that the morphogen Sonic Hedgehog (SHH) acts as an axon guidance cue for postcommissural axons (Bourikas et al., Nat. Neurosci. 8(2005)297-304). During early stages of neural tube development, Shh activity mediated by Patched and Smoothened is involved in cell differentiation and patterning of the dorso-ventral axis of the spinal cord. Slightly later, Shh acts as a chemoattractant in parallel to Netrin-1, attracting dorsal commissural axons toward the floor plate, the ventral midline of the spinal cord (Charron and Tessier-Lavigne, Development 132(2005)2251-2262). This activity is still mediated by Patched and Smoothened. As soon as axons have crossed the midline, they are repelled by Shh. This rapid change in activity from attractant to repellent is mediated by a receptor switch from Patched to Hip (Hedgehog-interacting protein).

More recently, we have extended the applicability of *in ovo* RNAi to older stages of embryonic development to study the role of morphogens in cerebellar development, as aberrant Shh signaling in the cerebellum has been identified as a cause of medulloblastoma, one of the major forms of childhood tumors.

siRNA therapeutic target genes in Atherosclerosis: Selection and validation

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ABSTRACT: Genomic, proteomic, and metabolomic studies of complex cardiovascular diseases have helped in the identification of genes involved in pathophysiology of atherosclerosis. Chemical inhibitors of these new therapeutic targets are presently at various stages of development. However siRNA antagonists of these genes present an opportunity for development of novel therapeutics based on this natural phenomenon reducing the possibility of cytotoxicity. The main therapeutic approaches to atherosclerosis may be broadly classified as those pertaining to lowering cholesterol (LDL-C), raising HDL-C, anti-inflammatory and inhibitory to cellular adhesion. The most successful cholesterol-lowering drug for the past 20 years in the market has been the 5-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitory statins. The risks associated with statins, like liver toxicity and muscle inflammation make replacement of statins with siRNA approach an attractive alternative. In order to raise HDL cholesterol the strategy involves blocking the cholesteryl ester transfer protein (CETP) that transfers cholesterol from HDL to LDL. Recently the CETP inhibitory molecule Torcetrapib failed at phase III clinical trial, as it was found to be toxic resulting in increased Blood pressure. The toxicity may be attributable to the particular drug moiety rather than a failure of strategy itself that now is amenable to siRNA development. An anti inflammatory approach involves the recently identified *ALOX5AP* gene that encodes the enzyme 5-lipoxygenase activating protein (FLAP) as a drug target, since it participates in leukotriene synthesis. The leukotriene, especially leukotriene B4 (LTB4), mediates inflammation within the vasculature and has been observed to participate in murine atherosclerosis. Proprotein convertase subtilisin/kexin type 9 serine protease (*PCSK9*) inhibitors seem a ripe target for adjunct therapy in patients in whom LDL targets are not achieved on statins either as monotherapy or combined with cholesterol absorption inhibitors, or who cannot tolerate high-dose statins. The role of vascular cell-adhesion molecule (VCAM-1) and ICAM-1 in mediating permanent attachment of monocytes to the endothelium surface, makes them rational targets for the anti adhesion approach to atherosclerosis drug discovery. The expression of monocyte chemoattractant protein-1 (MCP-1) in human atherosclerotic lesions may be a future target for anti-adhesion therapy. Dietary cholesterol absorption inhibitor based on interference is also an attractive target. These targets can expand the range of existing drugs that provide effective treatment to atherosclerosis. The presentation discuses the designing and validation of siRNA molecules for some of these targets.

Stability and biodistribution of ^{32}P-labeled siRNA duplexes in mice

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ABSTRACT: For the potential therapeutic application of siRNA, it is important to improve the potency and efficacy of siRNA *in vivo* by achieving better biodistribution and pharmacokinetic properties. In the present study, we compared the effect of chemical modification on siRNA duplex integrity, blood clearance, and biodistribution in mice. Six duplexes were investigated, including unmodified, light and heavy LNA modified, phosphorothioate modified, lipophilic siRNA and sisiRNA (small internally segmented interfering RNA). Antisense strands were labeled with [γ-^{32}P] ATP. siRNA duplex at a total dose of 400 µg/kg body weight was tail vein injected, blood samples were collected at 1, 5, 15, 30 minutes and 24 hours post-injection and tissues were collected from kidney, liver, heart, spleen and lung at either 30 minute or 24 hours endpoints. Both tissue and blood samples radioactivity were analysed by scintillation counting and RNA integrity by denatured gel electrophoresis. Our results showed rapid clearance of siRNA from blood, 60~80% at 5 minutes and >95% at 30 minutes, comparing to the level at 1 minute. Intact RNA could be detected in the blood only for modified siRNA duplexes up to 30 minutes, whereas naked siRNA duplex was degraded 1 minute after injection. For scintillation counting of tissue samples, radio activity reached the highest level in kidneys, except for lipophlic siRNA, that appeared to be present in relative equal level for all five organs at 30 minutes; a level that was significantly decreased up to 5-fold in kidneys, but not in other organs at 24 hours. Intact siRNA could be detected in different organs at 30 minutes after injection. The highest level was found in lungs and kidneys for light LNA modified siRNA, sisiRNA and phosphorothioate siRNA; a relative equal level was found in heavy LNA modified siRNA and the lipophilic siRNA. All signals disappeared at 24 hours, except in lungs of mice administered with heavy LNA modified siRNA which was still detectable. Our results, supported by intact siRNA analysis, underscore the importance of chemical modification to improve the stability and biodistribution for siRNA *in vivo*. Moreover, intact siRNA levels do not necessarily correlate with the total ^{32}P-radioactivity levels. Importantly, for therapeutic application, we also found that even a relative low dose of modified siRNA can be used to modulate biodistribution.

Evaluating the potential of RNAi in the biological control of insect pests

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ABSTRACT: Insect pests cause crop losses of 18% worldwide. Currently crop protection is heavily reliant on synthetic insecticides, with their associated resistance and environmental problems. This project involves assessing the potential of using RNA interference (RNAi) as a control agent for insect pests. Due to the sequence-specificity of RNAi, this approach is likely to have fewer negative environmental effects than using chemical pesticides. Working with Manduca sexta, the tobacco hornworm, RNAi will be used to knock down gut genes (to cause mortality and reduce feeding) and immune genes (to increase susceptibility to other biocontrol agents such as *Bacillus thuringiensis*).

MicroRNA expression profile changes between glucose responsive and non-responsive Min-6 pancreatic beta cells

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ABSTRACT: Min6 cells exhibit glucose stimulated insulin secretion (GSIS) at physiological levels, similar to that observed with freshly isolated islets, this indicates that Min-6 is an appropriate model for studying the mechanisms of GSIS in pancreatic beta cells. However, ourselves and others have observed that with increasing time in culture, the GSIS functionality of these cells is lost or severely diminished. Three microRNAs (miRNAs) mir-375, mir-9 and mir-124a have recently been shown to be involved in GSIS and associated pathways of insulin signalling and secretion. However, the full extent of miRNA association and involvement in regulating this fundamental process has yet to be determined.

This study aimed to identify microRNAs differentially expressed in glucose responsive (L- low passage) and non-responsive (H- high passage) Min6 cells and associated with loss of GSIS.

TaqMan low density human miRNA arrays (TLDAs), representing 384 miRNAs, were performed on biological triplicate samples of Min-6 L and Min-6 H cells. Differentially expressed miRNAs were selected following the criteria of P-value < 0.05 and fold change ≥ 1.2. Of the 384 miRNA detectors per array, homology comparisons indicated 47% of these are known to be present in the mouse genome. Following normalisation with an endogenous control 12 of the miRNAs detected were shown to be significantly down regulated in Min-6 H compared to Min-6 L. These differentially expressed miRNA targets were then validated using qRT-PCR, to verify TLDA results.

In conclusion, this study represents the first ever global analysis of miRNAs in beta cells. Further analyses of these target miRNAs will provide a more in depth description of the molecular mechanisms associated with loss of GSIS and enable us determine their potential to be exploited as therapeutic targets in diabetes.

siRNA delivery enhancement by intracellular study of vector unpacking: Results and indications

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ABSTRACT: The flexibility and easy modifiability of polymers is responsible for their being considered extremely viable and potentially successful candidates for gene delivery. These considerations are ultimately with a view to the prospects for the usage of polymers in gene therapy. Advances in this field have of late been rapid, with numerous new techniques being proposed and tested. Principal among emergent technologies is siRNA. The author attempts to evaluate the sub-techniques and sub-processes used for polymer-based siRNA delivery in an analysis that involves extensive lab work corroborating the theoretical analysis at every step, to attempt to offer solutions that may aid in process improvements for the sub-processes for the purpose of enhancing polymer-based siRNA delivery. The author performed a specific analogous study with siRNA, which, to the best of his knowledge, is one of the first *experimental* ventures in the specific field area. This was done assuming the polymer and the siRNA encapsulated within, as a generic poly-cation and poly-anion. An early pioneering study by Tsuchida and Osada (Macromol Chem Phys, 1974, 175, 593) found that the poly-ion complex stability constants demonstrate an exponential increase with poly-cation length. The principal finding necessary to further investigation, confirming earlier studies was that as the nucleic acid length of a cationic polymer increases, its complex's strength increase is in direct proportion(owing to increased charge availability) (Schaffer, et al, Biotechnol Bioengin, 2000, 67, 598). siRNA, having a lower molecular weight than DNA and thereby creating relatively unstable complexes with the same poly-cation, siRNA-polymer dissociation is on the favorable side of the energetics balance, as cited by Gary et al (J Cont Release, 2007, 121, 64). The author, in what is his chief experimental advancement at this stage, performed an *intracellular* study, the results of which primarily suggest that vector unpacking is a lesser threat to siRNA delivery than it is to DNA as per data from *C. Plank et al.*, though still worth taking into account as a participatory force that may affect the process substantially and in ways that may be quantifiable. These issues have mostly already been deliberated on theoretically with some prominent reviews [3] having provided a proper perspective and context that assisted the planning of the author's experimental work, which the author estimates to be one of the first. The results and their implications which attempt to address the interplay of the parameters stated above are of some significance, and, the author hopes, will be of interest to those in the field.

Optimised non-viral vectorisation of siRNA into the sub-ventricular zone of mouse brain

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ABSTRACT: The mechanisms involved in the genetic regulation of neural stem cells (NSC) maintenance, division, and differentiation remain unclear. RNA interference is a powerful method of gene expression silencing that opens up enormous perspectives to functional genomic investigation. However as for any nuclei acid, delivery of small interfering RNA (siRNA) to target cells *in vivo* remains a crucial problem. Non-viral vectorisation methods provide stable, safe and efficient formulation for siRNA delivery. We have developed and efficient cationic lipoplex method to deliver siRNA into the newborn mouse brain. To determine the identity of target cells transfected within the neurogenic niche, we transfected a random sequence of siRNA labeled with alexa fluo. Then we performed an immunostaining on newborn mice brain sections using specific antibodies against glial fibrillary acidic protein (GFAP), expressed in slowly proliferative NSC (Type B). We first showed that labeled siRNA are transfected into the cells around the lateral ventricle. Immunohistology reveals colocalization of labeled siRNA and GFAP in the subventricular zone. This demonstration of siRNA delivery to NSC of lateral ventricle thus validates the use of gene interference to study regulatory mechanisms of NSC in newborn mice. However, a better understanding of adult neurogenesis is also needed as modulating the process in adult and ageing brains could open up enormous perspectives for therapeuthical strategies. Unfortunately gene transfer to the adut population presents more constraints than the newborn brain. A new delivery reagent based on novel cationic lipids (INTERFERin^{TM}) provides a high silencing efficiency *in vitro*. We are now evaluating the vectorisation of siRNA with INTERFERin^{TM} *in vivo* with the overriding aim of delivering siRNA to the neurogenic zone of the adult mouse.

Cotranscriptional processing of intronic miRNAs

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ABSTRACT: Several studies suggest that exons of nascent pre-mRNA are tethered to the elongating RNA polymerase II (Pol II), so that the continuity of the nascent intronic sequences is not essential for efficient splicing. Recent studies report that more than half of all known mammalian miRNAs are located within introns of either protein-coding or non-coding transcriptional units. We have therefore

investigated whether the introns containing functional pre-miRNAs are cotranscriptionally processed without affecting the splicing reaction.

MiRNAs biogenesis begins in the nucleus where the Microprocessor complex containing Drosha, an RNase III like enzyme, and its cofactor DGCR8, generates a pre-miRNA hairpin product ~70 nt long. We specifically localise the Microprocessor complex to introns containing miRNAs by ChIP analysis. Furthermore RNase treatment of chromatin shows that this interaction is mediated by the nascent intron transcript. These data indicate that the Microprocessor complex is recruited to nascent intronic miRNA as soon as it is transcribed by elongating polymerase.

We next addressed the timing of Drosha processing in the context of Pol II transcription using different experimental approaches. Firstly we isolated Drosha cleaved transcripts using a hybrid selection circular RACE assay and we show that the major substrate for this processing event is an unspliced mRNA transcript. We also show that Drosha cleavage occurs on nascent unprocessed transcripts still attached to the elongating polymerase. Overall these results, together with the localization of the Exosome and Xrn2 exonuclease activities on introns contaning miRNAs, provide strong evidence for cotranscriptional cleavage and degradation of the host miRNA introns

Baculoviruses as novel gene therapy vectors for breast cancer

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ABSTRACT: Breast cancer is the most frequently occurring cancer in women. Cumulative evidence suggests that abnormal glycosylation of cell surface proteins of breast cancer cells enhances their invasive potential and increases the risk to develop metastatic disease or relapse. RNA interference (RNAi) has been used to reduce breast tumour growth in nude mice and gene inhibition of these aberrant glycoproteins may offer a therapeutic strategy against this malignancy. Unfortunately, the successful application of this therapy has been hampered by the low efficiency of gene delivery offered by conventional viral and non-viral vectors. *Autographa californica* multinucleopolyhedrovirus (AcMNPV) can efficiently transduce breast cancer cell lines, with a low cytopathic effect and a high degree of safety. In addition, they are easier, faster and relatively cheaper to produce than conventional viral vectors, highlighting their potential as delivery vectors for gene therapy in breast cancer.

We aimed to address whether an AcMNPV-based vector carrying RNAi was able to block or reduce the expression of aberrant surface glycoproteins in an *in vitro* model of human breast cancer. We transduced MCF-7 cells with recombinant baculoviruses carrying either i) a fluorescent marker (DsRed), ii) RNAi scrambled sequence as negative control or iii) RNAi against *N*-acetylgalactosaminyl transferase-3 (GalNac-T3). To study the efficacy of RNAi delivery within the cells, serial time points were analysed by fluorescence microscopy and expression of GalNac-T3 was_monitored by western blot. Baculovirus vectors were able to transduce MCF-7 cells, delivering small interfering RNAs (siRNA) into their nucleus with no apparent cytotoxicity. In addition, baculovirus-mediated delivery of siRNA against GalNac-T3 reduced the expression of this protein in MCF-7 cells compared with cells transduced with DsRed or a scrambled non-target sequence.

In conclusion, we compare the merits of using Baculovirus, to mammalian virus-based vectors, and suggest it is a safer and more efficient system for gene delivery in breast cancer cells.

Biological effects of HPV 18 E6 siRNA in human cervical cancer cells

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ABSTRACT: Cervical cancer is the most common genital tract malignancy in Hong Kong women. Human papillomavirus (HPV) DNA is almost found in all cervical tumors and pre-tumors, with HPV16 and HPV18 being the two most prevalent types. It has been shown that the expression of HPV E6 and E7 genes is necessary and sufficient for immortalization of the primary human keratinocytes. RNA interference (RNAi) is an occurring phenomenon that is highly potent in silencing gene expression. The key intracellular intermediates in RNAi are siRNAs that are composed of a 19bp duplex with two nucleotide 3' overhangs. Synthetic siRNAs exhibiting similar features have been shown to silence specific genes in human cells and are being tested as potential therapeutic agents. This study was to test the effects of siRNA sequences of HPV18 E6 gene on apoptosis and cell proliferation in two HPV18- positive human cervical carcinoma cell line, HeLa and C-4I cells. Cervical cancer cell line HT-3 is negative for HPV DNA to be as control. Three siRNAs targeting HPV18 E6 gene and one control siRNA were designed and synthesized. Cells were transfected with siRNAs in serum-free medium using Oligofectamine. Cell growth curves were determined by cell counting. Apoptotic cells were identified using annexin-V-Fluos kit. The results showed that the growth of HPV positive cervical cancer cell line cells was inhibited by one designed HPV 18 E6 siRNA. The apoptosis increased in HPV positive cancer cells after treatment with 2 HPV 18 E6 siRNAs. These preliminary observations suggest that HPV 18 E6 siRNA might have potential for the treatment and possibly prevention of human cervical cancer in women.