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Cell stress-induced translocation of human Argonaute 2 protein is related to decreased RNA interference

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Abstract
The Argonaute proteins constitute a highly conserved family of nucleic acid-binding proteins whose members have been implicated in RNA interference (RNAi) and related phenomena in several organisms. In particular, Argonaute 2 (Ago2) represents one of the key players in the RNA induced silencing complex (RISC). However, recently published literature describes that Ago2 itself is regulated under certain cellular conditions by post-translational modifications. In this work, we investigated the activity of human Ago2 under various conditions of cell stress. Under such conditions, the sub-cellular localization of Ago2 was altered which was coincident with its decreased function in the RNAi pathway. This work implies that specific cellular stress conditions induce an intracellular translocation of Ago2 protein excluding it from sites of action of the RNAi machinery. Details will be presented and discussed.

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Workflow for identifying miRNA function and targets in EMT

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Abstract
Although microRNAs (miRNAs) have been shown to regulate genes that play roles in important biological processes such as development, differentiation and disease, identifying miRNAs of interest and characterizing their mechanism of action remains a challenge. The miR-200 family has been shown to regulate epithelial to mesenchymal transitions (EMT), a process that is critical for normal development and tumor metastasis. Furthermore, recent publications indicate that the miR-200 family regulates EMT by targeting ZEB1 and ZEB2, transcription factors that repress E-cadherin. Here, we use breast cancer cell lines as an EMT model system to characterize the miR-200 family's role in these biological processes. miRIDIAN microRNA microarray profiling was use to characterize cell lines followed by introduction of miRIDIAN miRNA mimics and hairpin inhibitors to modulate miRNA expression in these cell lines. Based on these studies we have devised a workflow for studying miRNA expression and identifying miRNA targets.

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In vivo screening of backbone modified siRNAs for their ability to induce interferon based off-target effects

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Abstract
Small interfering RNAs (siRNAs) are promising new active compounds in gene medicine. But one serious problem with delivering siRNAs as treatment is the well-established stimulation of innate immune reactions by some RNA duplexes and lack of effective delivery systems. Innate immune reactions towards double stranded RNAs include the 2´-5 oligoadenylate (OAS) system, the protein kinase R (PKR), RIG-I and Toll-like receptor activated pathways all resulting in activation of antiviral defence mechanism. We have previously described a high throughput animal screening model in which the level of stimulation of interferon-related anti viral effects is measured as increased resistance of siRNA-injected small fish to a pathogenic virus. Here we show how this fish model can be used to make a fast in vivo analysis of the effect of chemical base modification in the siRNAs on the level of antiviral off-target effects.

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RNAi screens to determine homologous recombination networks

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Abstract
Defects in DNA repair and damage response can drive tumour progression and results in genomic instability in tumour cells. Whereas these DNA repair defects can be exploited in cancer therapy where unrepaired lesions induced by anti-cancer agents increase tumour killing, these DNA repair defects can also cause resistance to anti-cancer treatments. Defects in DNA repair pathways may also render tumour cells dependent on other complementary repair pathways as seen with the synthetic lethal effect of PARP inhibitors in BRCA-defective tumours. Altogether, there is a potential use of DNA repair inhibitors in anti-cancer treatment, either in combination therapy to increase the efficacy of e.g. radiation or as mono-therapy to target essential compensatory DNA repair pathways. Homologous recombination (HR) is a repair pathway involved in repairing double-strand breaks and replication-associated lesions, the main toxic lesions induces by anti-cancer drugs. Depletion of the key protein involved in HR, the RAD51 protein, is lethal but loss of other proteins involved in HR is compatible with survival. To understand the wider network of HR proteins and with the aim to find novel proteins that can be used as targets for HR-inhibition, we have used a RNAi screen approach to create functional genetic network maps describing proteins involved in homologous recombination. Foci formation of the RAD51 protein has been studied in U2OS cells subjected to protein knock-down using a RNAi library in co-treatment with either irradiation or camptothecin. This identifies proteins involved in the HR response to different types of lesions. In addition, a GFP-reporter-based HR assay has been used to identify proteins involved in HR repair of a site-specific double-strand break. With these experiments we hope to increase our understanding of the complex network of homologous recombinational repair as well as find potential targets for anti-cancer treatments.

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Identification of new regulators of the Wnt-β-catenin pathway via RNAi screening

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Abstract
The canonical Wnt/β-Catenin pathway controls myriad fundamental cellular processes, such as differentiation and proliferation. Aberrant regulation of this signaling cascade can result in several disease phenotypes and is a hallmark of colorectal cancer and hepatocellular carcinoma. Genetic evidence links Wnt/β-Catenin signaling to the control of bone density. The identification of new components of this pathway may lead to the development of novel therapies targeting a variety of cancers and osteoporosis. Accordingly, we have conducted genome scale RNAi screens in multiple cell contexts to discover new pathway regulators. We developed a screening process that included steps for initial hit identification, mitigation of off target effects, elimination of cell line-specific effects, and measurement of Wnt/β-Catenin signature regulation. We further validated a number of these new pathway mediators in vitro for physical association with canonical pathway members and in vivo by use of zebrafish models. These studies have characterized AGGF1, BTK, and DHFR as new modulators or the Wnt/β-Catenin pathway.

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Development of small interfering RNAs selectively activated in target cells

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Abstract
SiRNA molecules have a high potential for therapeutic applications. Here we present a mechanism of cell-specific, peptide-blocked siRNA. SiRNA is bound to short peptides preventing RISC formation. Peptides contain target sequence of peptidases, exclusively active in target cells. After intracellular delivery, only in target cells, peptidases cleave peptides leading to siRNA activation. Used peptide sequence is the target sequence for caspase-4, expressed in Jeg-3 choriocarcinoma and MCF-7 mammalian cancer cells, but not in human embryonic kidney (HEK) cells. We aimed to silence Signal Transducer of Activation (STAT3) expression by such modified siRNA specifically in Jeg-3 as well as MCF-7 in contrast to HEK cells. Western blot was performed to detect STAT3 protein. Furthermore, proliferation of STAT3 silenced cells was analysed. The peptide was bound to the siRNA antisense strand via amino-C6-linker based on Fmoc chemistry. Correct binding was analysed by PAGE and Maldi-MS. In Jeg-3 and MCF-7 modified siRNA became activated and reduced STAT3 expression in contrast to HEK cells lacking caspase-4. Moreover, proliferation was significantly reduced in STAT3 silenced cells. In conclusion, STAT3, which is responsible for increased proliferation and invasive properties of various cancer cells, can be cell-specifically silenced using the presented novel technology. Preliminary experiments indicate that the presented mechanism of peptide-inhibited; peptidase-activated siRNA can be transferred to a variety of cell specific active peptidases and related disease models.

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Cell-to-cell transmission of small RNAs extends the therapeutic reach of RNAi

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Abstract
Background: RNA interference (RNAi), the degradation of cognate mRNA by small interfering RNA (siRNA), has emerged as a promising therapeutic entity for viral infections, including hepatitis C virus (HCV) HCV. In plants and

invertebrates, RNAi-mediated protection can spread to neighboring cells; however, such a phenomenon has not been described in mammalian cells. In this study, we investigated whether endogenous expressed liver-specific microRNA and vector-delivered siRNA can transfer between cells and whether this exchange could extend the therapeutic effect of RNAi against HCV infection. Methods: Human hepatoma cell lines Huh7 and HepG2, Huh7-ET HCV replicon cells, and renal epithelial line 293T were co-cultured with conditioned medium or cells stably transduced with integrating lentiviral vectors expressing green fluorescent protein (GFP) and small hairpin RNAs targeting the HCV NS5b (LV-shNS5b), CD81 (LV-shCD81) or non-targeted control (LV-shCon). Liver-specific microRNA, miR-122, was quantified by real-time RT-PCR. Results: MiR-122 is not only highly expressed in Huh7 cells but also detectable in Huh7-CM, suggesting release of miR-122 by the cells. Upon incubation of HepG2 or 293T cells, which are 200 and 50,000-fold lower in miR-122 expression, with Huh7-CM the miR-122 level in these cells was increased by 4-20 fold, indicating uptake of miR-122 from the medium. To further investigate whether small interfering RNA delivered by vectors can be transferred between cells, Huh7-ET was co-cultured with stably transfected Huh7 cells expressing shNS5b or shCon. A significant reduction of viral replication was observed at 1:1 ratio of shNS5b cells (52±12% $p<0.01$) but not with shCon cells. Similarly, at suboptimal transduction of Huh7 with LV-shCD81, CD81 expression of non-transduced cells was also significantly down-regulated (30±12.9%, $P<0.001$). Moreover, conditioned medium of transduced cells could convey RNAi-mediated silencing of HCV (39%±12, $P<0.01$) and CD81 (23.5%±5.1, $P<0.01$) without transfer of GFP, suggesting a similar mechanism of transfer as for microRNA. Conclusion: It was shown that transmission of small RNA is an innate function of human cells which can both mediate the exchange of microRNA and vector delivered RNAi, thereby extending the therapeutic effects of RNAi on HCV.

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Boranophosphate-modified RNA as potential anticancer therapies: Aptamers and RNA interference

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Abstract

Borane (–BH3) chemistry offers some unique chemical characteristics that make these compounds promising for enhancing the potential of three anticancer strategies; (a) RNA interference (siRNA) (b) the selection of tumor specific aptamers and (c) Boron Neutron Capture Therapy, a highly selective type of radiation therapy. Borane oligonucleotides are nuclease resistant and have increased lipophilicity compared to natural oligonucleotides, yet the modified borane nucleotide triphosphates (NTPαBs) still are efficiently recognized and utilized by RNA polymerase enzymes which enable the enzymatic synthesis of RNA (siRNA and aptamers). The novel properties of boranophosphate RNA molecules could lead to an increase in affinity and specificity of the siRNA and aptamers, as well impart stability of the nucleic acids to cellular nucleases. We hypothesize that borane-RNA molecules will interact a new diverse array of ligand sites in proteins (RISC siRNA carriers or ErbB2 therapeutic target) because of the distinct hydrophobicity, shape, and polarity properties imparted by the phosphorus-boron (P-B) chemical bond compared to the natural phosphorus-oxygen (P-O) bond. (a) A major cause of chemotherapeutic treatment failure against human cancers is the aberrant regulation of genes such as MDR1 in cancer cells. Controlling the expression of cancer genes with antisense technology is a possible cancer therapy. MDR1 codes for a p-glycoprotein (Pgp) that is overexpressed in multidrug resistant cancer cells. Specifically, using modified Small interfering RNAs (siRNAs) that target and degrade the p-glycoprotein mRNA produced by the MDR1 gene can be used to correct the overexpression of p-glycoprotein. (b) The selection of boranophosphate modified RNA aptamers by the SELEX (Systematic Evolution of Ligands by EXponential enrichment) against ErbB2. It is likely that targeting a more specific cancer membrane target such as the ErbB2 receptor with a borane RNA aptamer may also be an effective two-prong strategy in breast cancer therapy. Like the antibody protein, Herceptin, an aptamer may assume a distinct shape and block the receptor. (c) Further, borane aptamers can be used as specific carriers of 10B in Boron Neutron Capture Therapy (BNCT). BNCT specifically destroys tumor cells near boron molecules. The selection of α-P-borano-modified RNA aptamers against receptors highly over-expressed in breast cancer should provide the opportunity of testing the efficacy of a target specific method for delivering boron to the cancer cells. Such boron molecules could be potent and unique anti-tumor surrogates for antibodies because of their ease in selection, smaller size, nuclease resistance, susceptibility to BNCT, and versatility in terms of adding chemical modifications to increase potency.

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Modifying p19 viral suppressors of RNA silencing: Developing FRET-based probes for detecting and quantifying protein-small RNA interactions

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Abstract
The RNA silencing pathway is engaged during the cellular innate immune response to double-stranded RNA. Particularly in plants, this serves as an anti-viral defense mechanism and results in the degradation of homologous viral RNA. Several viruses have evolved mechanisms to undermine the RNA silencing pathway. Tombusviruses, such as the Carnation Italian Ringspot virus (CIRV), express a 19 kDa protein (p19) which acts as a suppressor of the RNA-silencing pathway. As a dimer, p19 binds double stranded small RNAs in a size-selective and relatively sequence-independent manner, thereby preventing their incorporation into RISC. The p19 protein binds 21 nucleotide double-stranded small RNAs with nanomolar affinity. These unique selective binding features allow p19 to be used as a molecular ruler that can be used to detect small RNAs. We have further increased the potential of the p19 protein through engineering several recombinant p19 proteins which allow us to detect protein-RNA interactions in solution. By constructing linked versions of the CIRV-p19 dimer we have improved the stability and binding affinity of the p19 dimer while still maintaining its ability to discriminate RNA according to length. A recombinant p19-CFP fusion protein allows us to use fluorescence-based techniques to detect and quantify protein-RNA interactions based on Förster Resonance Energy Transfer (FRET). In this method, when the linked recombinant p19-CFP dimer binds Cy3-labelled dsRNA, intermolecular FRET occurs between the CFP donor and the Cy3 acceptor giving a quantifiable signal. In order to extend this methodology to *in vivo* detection of small RNA, we have developed an intramolecular FRET probe which incorporates YFP, another GFP analog, into the 2x-p19-CFP recombinant protein. Progress towards using this 'chameleon' recombinant protein, CFP-2x-p19-YFP, as a FRET-based probe for live-cell imaging of small RNA production and localization will be presented.

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Mechanistic studies of siRNA-mediated RNA interference in HeLa cell extract

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Abstract
RNA interference (RNAi) is a highly evolutionary conserved RNA-dependent gene silencing process initiated by short double-stranded RNA molecules. These include micro RNA and short interfering RNA (siRNA), the latter causing sequence-specific degradation of homologous mRNA sequences. Soon after the groundbreaking discovery of siRNA-mediated regulation of gene expression, the RNA induced silencing complex (RISC) was found to be the effector complex of RNAi with argonaute proteins as the catalytic component. Here, HeLa cell cytoplasmic S100 extract was established as a minimal mechanistic *in vitro* model to study this process in more detail. Cleavage reactions of a radiolabeled *in vitro* synthesized target RNA by double-stranded siRNA were performed and cleavage products as well as turnover rates were measured as a function of Mg^{2+} concentration, nucleotide sequence and time. Despite a lot of advances in the understanding of RISC activity, different composition sizes and associated factors of this complex have been reported in the literature. The above described HeLa cell extract was used to isolate truly active RISCs by purification of complexes via the target mRNA component such that involved protein and nucleic acid components can be further characterized. In summary, HeLa cell extract was shown to be a valuable tool for kinetic and mechanistic studies of siRNA-mediated RNA interference.

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Useless miRNAs in mammalian oocytes

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Abstract
MicroRNAs (miRNAs) are small endogenous RNAs, which typically imperfectly base-pair with 3'UTRs and mediate translational repression and mRNA degradation. Dicer, an RNase III generating small RNAs in the miRNA and RNAi pathways, is essential for meiotic maturation of mouse oocytes. We found that 3'UTRs of transcripts up-regulated in Dicer1/oocytes are not enriched in miRNA binding sites implicating a weak impact of miRNAs on the maternal transcriptome. Therefore, we tested the ability of endogenous miRNAs to mediate RNA-like cleavage or translational repression of reporter mRNAs. In contrast to somatic cells, endogenous miRNAs in fully-grown GV oocytes poorly repressed reporter translation while their RNAi-like activity was much less affected. In addition, reporter mRNA carrying let-7-binding sites failed to localize to P-bodies, cytoplasmic foci containing proteins involved in RNA repression and degradation where miRNA-repressed transcripts typically localize. In fact, P-bodies are not found in fully-grown mouse oocytes but disappear early during oocyte growth. In fully-grown oocytes, several P-body components and other RNA binding proteins including DDX6, CPEB, MSY2, and the exon junction complex form transient, subcortical RNA-containing aggregates, which disperse during oocyte maturation, consistent with recruitment of maternal mRNAs that occurs during this time. In contrast, levels of P-body component DCP1A are low during oocyte growth and DCP1A does not co-localize with DDX6 in the sub-cortical aggregates. The amount of DCP1A markedly increases during meiosis, which correlates with the first wave of destabilization of maternal mRNAs. Our data suggest that normal miRNA function is down-regulated during oocyte development and this idea is further supported by normal meiotic maturation of oocytes lacking Dgcr8, which is required for miRNA but not RNAi pathway. We propose that suppression of miRNA function during oocyte growth is an early event in reprogramming gene expression during the transition of a differentiated oocyte into pluripotent blastomeres of the embryo. Furthermore, the cortex of growing oocytes serves an mRNA storage compartment, which contains a novel type of RNA granule related to P-bodies.

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RNA interference approaches to inhibit cardiotropic viruses

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Abstract
Being a member of the Picornavirus family, Coxsackievirus B3 (CVB-3) is one of the major pathogens that may lead to dilated cardiomyopathy. It is a small, non-enveloped RNA virus. Because of the plus-stranded RNA genome it is qualified for the application of RNA interference. We developed highly efficient small interfering RNAs (siRNAs) against the viral 3D RNA dependent RNA polymerase ($3D^{pol}$). Hence we were able to inhibit CVB-3 proliferation up to 90% in a plaque reduction assay. Recent experiments revealed the potential to use siRNAs modified with locked nucleic acids (LNA) or unlocked nucleic acids (UNA) to inhibit virus replication. For *in vivo* applications, vector delivery of shRNA expression cassettes was found to be a suitable approach to treat mice with virus-induced myocarditis. An AAV-vector expressing two shRNAs against CVB-3 was found to improve the heart parameters in a mouse model for an enterovirus myocarditis. Finally, a synergistic and potent antiviral effect in persistently infected human myocardial fibroblasts was obtained, when the siRNA was combined with a soluble variant of the coxsackievirus-adenovirus receptor, which acts as a virus trap. Taken together, these studies demonstrate that RNA interference is a powerful tool to inhibit cardiotropic viruses.

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Image-guided siRNA delivery

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Abstract
Our studies have focused on the application of imaging-capable nanoparticulate agents for the delivery of siRNA to target tissues. One example includes magnetic nanoparticles (MN), which have traditionally been utilized as contrast agents for magnetic resonance imaging. MN typically consist of a dextran-coated superparamagnetic iron oxide core (for magnetic resonance imaging), labeled with Cy5.5 dye (for near-infrared in vivo optical imaging), and conjugated to synthetic siRNA molecules targeting model or therapeutic genes. We have explored the potential of these nanoparticles as delivery modules for small interfering RNA to tumors and pancreatic islets. Furthermore, we have investigated the feasibility of combining the imaging and delivery capabilities of these nanoparticles for the tracking of siRNA bioavailability. The versatile functionalization potential of MN has allowed us to control properties of the agents, such as uptake mechanism and target organ distribution. The tumoral accumulation of MN-siRNA results in a remarkable level of target-gene down-regulation. Repeated treatment with MN-si*BIRC5*, targeting the tumor-specific anti-apoptotic gene, *birc5*, leads to the induction of apoptosis in the tumors and an overall reduction in tumor growth rate. Another application of MN-siRNA extends from the fact that these nanoparticles are also taken up by pancreatic islets following in vitro incubation. This uptake can be visualized by magnetic resonance and near-infrared fluorescence optical imaging and results in down-regulation of target genes. This approach has relevance in the context of pancreatic islet transplantation, which is a promising new treatment of type 1 diabetes. A potential application of this method would involve the selective knock-down of genes implicated in islet graft dysfunction, such as pro-apoptotic genes and genes involved in immuno-recognition. More recent studies that will be discussed briefly have focused on the delivery of knock-down LNA probes targeting specific miRNA pathways.

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Multi-targeted siRNA therapeutics with three generations of nanoparticle delivery

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Abstract
Development of siRNA therapeutics has already demonstrated clinical benefits in more than a dozen human trials. Using siRNA cocktail to silence multiple disease genes is truly realizing the advantage of small interfering RNA (siRNA)-based drugs. We have developed a set of siRNA cocktails using our proprietary algorithm and "Tri-Blocker™" platform, as the active pharmaceutical ingredient (API). Those siRNA cocktails were further tested and validated in the disease relevant animal models using a series of polymer- and liposome-based nanoparticles. The therapeutic programs in the late preclinical development stage, STP705 for improving skin scarless wound healing, STP702 for treatment of influenza H5N1/H1N1 infections and STP601 for treatment of ocular neovascularization diseases, are all based on the local and topical siRNA delivery using the self-assembled first generation nanoparticles. We further developed the second generation nanoparticles with peptide ligands and monoclonal antibodies for targeted cancer siRNA therapeutics which were tested in mouse xenograft tumor models. In addition, we are currently developing the third generation siRNA delivery vehicles, using Infrared-activated silica-coated upconversion nanoparticle (SC-UPNP) and oral-delivered nano-microspheres (OD-NMS). When the siRNA cocktail is applied with other drug modalities, such as monoclonal antibody, the therapeutic benefit was even further improved.

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A novel amino acid-based delivery platform for therapeutic applications in bladder cancer and hepatocellular carcinoma

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Abstract
A UsiRNA directed against mRNA for the human survivin gene was formulated into liposomes formed with a novel Di-Alkylated Amino Acid (DiLA2) resulting in well encapsulated (>85%) small (100-130 nm) particles. UsiRNAs are novel siRNA constructs which incorporate Unlocked Nucleobase Analogs (UNAs) within the oligonucleotide sequence. The survivin UsiRNA has been demonstrated to be highly potent, with an *in vitro* IC50 of 10 to 30 pM, which leads to caspase induction and apoptosis in cancer cells. A murine Hep3B-based orthotopic liver cancer model was used to assess the *in vivo* efficacy of the survivin UsiRNA DiLA2 liposomes with systemic administration. Dosing at 2 mg/kg (q3d x 6 doses; i.v.) resulted in approximately a 50% knockdown of survivin mRNA and 60% decrease in tumor weight. This result compared favorably with Avastin™ (bevacizumab)-treated mice which served as a positive control. A similar level of survivin mRNA knockdown was noted in a xenograft model of subcutaneously implanted liver tumors and systemic administration. In an orthotopic bladder cancer model, survivin UsiRNA DiLA2 liposomes were delivered topically (intravesical dosing) at up to 1.0 mg/kg (q2d x 4 doses). Greater than 90% inhibition of the survivin mRNA was observed, and there was a dose-dependent decrease in bioluminescence of up to approximately 90% in UsiRNA-treated mice which indicates reduced tumor growth. 5'-RACE analysis confirmed an RNAi-mediated mechanism of action for mRNA inhibition in each tumor model. Data for knockdown of multiple targets in tissues of normal mice and for a liver target in non-human primates has also been demonstrated with systemic administration of DiLA2-based liposomes. Taken together these data demonstrate that DiLA2 liposomes are an effective systemic and local delivery system for UsiRNAs, in both cancer and non-cancer indications.

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MicroRNA profiling of cutaneous T-cell lymphomas

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Abstract
MicroRNAs are a recently discovered class of naturally occurring short non-coding RNA molecules that regulate eukaryotic gene expression. There is emerging evidence to suggest that microRNAs are involved in the pathogenesis of many cancers including B cell lymphoma. There is however little data on the role of microRNAs in T cell lymphomas. Therefore we employed microarray analysis to investigate a possible role for microRNAs in the biology of cutaneous T-cell lymphomas; Sezary syndrome (SzS, n=21), mycosis fungoides (MF, n=26) and cutaneous anaplastic large cell lymphoma (cALCL, n=15) and relevant controls (CD4+ cells from healthy subjects (n=6), and skin biopsies from patients with inflammatory disorders (n=17). Using unsupervised cluster analysis we observed that the T-cell lymphomas have a distinct miRNA profile from their counterpart controls and have distinct profiles between diagnosis. We identified a number of miRNAs in common between the T-cell lymphomas and well as those specific for diagnosis. In summary, we have identified a number of Sz-associated microRNAs that may play a role in the pathogenesis of these diseases.

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Transposable elements as potential miRNA targets

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Abstract
The sequencing of the human genome revealed that 55% of its nucleotide sequence is composed of repetitive elements and that a large fraction of the "non-functional" DNA originated from mobile elements (1). They were considered until

recently as “selfish” or "Junk DNA" (2). For example, Alu elements comprise about 10% of the nucleotides of the human genome (1), with over one million inserted copies. There is evidence showing that the presence of repetitive elements had a great influence on the human genome and it has been shown that Alu repeats are mediators of recurrent chromosomal aberrations in tumors as in the case of the chromosomal translocation of intronic Alu elements (3). Recently, it was reported that some mammalian miRNAs are derived from genomic repeats and some of them show perfect complementarity with the MIR/LINE-2 class of repetitive elements, which are present within a large number of human mRNAs and EST transcripts (4). It was hypothesized that Alu elements within the 3’-UTRs are targeted specifically by certain miRNAs (5) and it has been proposed that a dual relationship exists between miRNAs and their Alu targets that may have evolved in the same time window. One hypothesis for this dual relationship is that these miRNAs could protect against excessively high rates of duplicative transposition, which would destroy the genome (6). Here we present the computational prediction of human miRNA/transposon interactions. We performed the analysis by using a combined approach based on thermodynamics and empirical constraints and found significant matches between miRNAs and human repetitive elements such as LINE, SINE, DNA transposon and ERV1. For example, over 30 miRNAs potentially target the L180_5 element (LINE1 family; 1883 nucleotides) and over 20 miRNAs are predicted to target the RICKSHA element (Non-autonomous DNA transposon fossil; 2030 nucleotides). We considered perfect seed complementarity only (7-mers or higher), with no G:U wobbles. The obtained results suggest a potential role of miRNAs in the regulation of repetitive elements and in particular transposons.

References

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